MICROBIAL ANALYSIS OF SHELLED EGGS AND

CHEMICAL AND FUNCTIONAL ANALYSIS OF

LIQUID EGGS

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MICROBIAL ANALYSIS OF SHELLED EGGS AND CHEMICAL AND FUNCTIONAL ANALYSIS OF LIQUID EGGS

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VITA

Vanessa Kristin Kretzschmar-McCluskey, daughter of Paula Kretzschmar and wife of Mitch McCluskey, was born June 25, 1981 in Cullman, Alabama. She graduated from Cleveland High School in Cleveland, Alabama in May 1999. She attended Auburn University in Auburn, Alabama majoring in Poultry Science with a minor in Spanish. She graduated in December 2003 with a Bachelor of Science in Poultry Science degree. In January 2003, she entered the Graduate School at Auburn University to pursue her Ph.D. under the direction of Patricia A Curtis, Ph.D.

DISSERTATION ABSTRACT

MICROBIAL ANALYSIS OF SHELLED EGGSAND

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Vanessa Kristin Kretzschmar-McCluskey

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Research has shown that the incidence of *Salmonella* Enteritidis (SE) in commercially processed table eggs is approximately 1 in 20,000. Although there has been great evolution of the egg industry, cases of SE infection continue to be problematic. It has been shown that through environmental maintenance and proper processing and storage practices, the number of reported SE outbreaks has declined

Although numerous studies have been conducted outlining possible vectors and modes of transmission through inoculation of laying hens, there is little information regarding the natural occurrence of SE in shell eggs.

From these experiments, it was concluded that hen age had a significant impact on the amount of microbial contamination, while hen strain had no effect. It was also determined that each of the three molting treatments contained significant amounts of microflora, with the non-fasted treatment exhibiting 4 interior samples that were positive for *Salmonella* Braenderup over the course of the final 12-month portion. There was no evidence of *Salmonella* Enteritidis discovered with any treatment during the course of the experiment, and none of the *Salmonella* spp. discovered in the environmental samples could be correlated to the species found in the shell eggs.

Because of the concern surrounding the bacterial content of shelled eggs, over 30% of those sold today are sold as pasteurized egg products. Pasteurization virtually eliminates bacterial pathogens from eggs by holding them at a high temperature for an amount of time which is specific to the particular product. However, previous research has shown that the pasteurization process can be detrimental to the functionality of eggs, causing problems for the baking industry.

The results of these experiments concluded that the theory of pasteurization being detrimental to the functionality of whole egg products was not applicable to this study. The albumen portion of this study utilized the same treatments with the exception of the addition of a triethyl citrate whipping agent instead of citric acid. The results indicated that the addition of whipping agent to pasteurized product greatly improved functionality while having no effects on chemical composition.

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This manuscript is dedicated to the author's family, Mitch McCluskey, Paula Kretzschmar, Henry & Madge Kretzschmar, and the rest of the Kretzschmar clan. Thanks for your love, support, much needed comic relief, and for not raising me to be a "damn dummy".

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I. INTRODUCTION

Over the years, the egg industry has seen its fair share of hardships. From microbial contamination issues, to bad press, the industry has been able to combat foodborne illness and help eggs regain their rightful place as one of the most healthy food choices consumers can make. However, there are always new and innovative ways to make the best better, and answers to lingering questions that have plagued the industry for decades. Through processes such as pasteurization, shell egg washing and grading, and on-farm control measures, eggs have proven to be a safe and wholesome food product, and their uses in other industries is limitless.

Egg-associated salmonellosis is one of the most important public health problems in the United States and Europe. SE shows no clinical signs in the adult hen and cannot be observed by the naked eye in the shell egg. Like many other species of *Salmonella*, SE can live in the intestinal tract of the hen and contaminate the egg through exposure to feces. However, due to the strict government regulations adopted in the 1970's, this type of transmission has become increasingly rare. It is possible, though, that the hens ovaries could become contaminated and the SE passed into the shell egg before the shell is formed (CDC, 2005).

The functional properties of shell eggs are well documented. Albumen, yolk, and whole egg play vital roles in creating food ingredients that consumers use on a daily basis. Meringues, salad dressings, and mayonnaise utilize the operative components of

yolk and egg white. The egg industry has recently been plagued with the problem of yolk contamination of the albumen. This occurs because of a weakening of the vitelline membrane, which allows lipids from the yolk to diffuse into the egg white. Research shows this may have an effect on functional properties such as whipping height, and may negatively effect the essentially lipid-free environment of the albumen. To remedy this situation, researchers must first know exactly how much fat has diffused through the vitelline membrane. With the use of proximate analysis measurements for calibration, it is possible to use rapid methods to obtain the same accurate results.

II. LITERATURE REVIEW

Food Safety Issues in the United States

Even though the United States has one of the safest food supplies in the world, foodborne illnesses continue to plague society, endangering public welfare and increasing the need for immediate action. Each year, foodborne infections cause millions of illnesses and hundreds of deaths; however, most go undiagnosed and unreported (Tauxe, 1997). Since the symptoms of an infection are commonly associated with many other illnesses, a proper diagnosis is rarely achieved. Nausea, diarrhea, abdominal cramps, fever, chills, and headaches can be easily associated with influenza or the common cold.

It is no surprise that the epidemiology of foodborne pathogens is dynamic. In recent years we have seen the emergence of new microorganisms and the re-emergence of old ones. Along with these changing infections, old procedures need to be updated or new ones created and implemented. What was once protocol for a certain foodborne illness may not be effective in treating the same illness today. Surveillance systems such as FoodNet and the Public Health Laboratory Information System allow physicians and the Centers for Disease Control and Prevention to monitor which infections are most reported and the number of cases and outbreaks associated with each.

There has been much progress in the prevention of foodborne diseases. Unfortunately there is no simple answer as to how total deterrence can occur. Proper

handling, not only from a consumer standpoint but from the industry as well, may hold the key to a safer food supply. It is important that all food producers make an effort to educate consumers about the proper handling and cooking procedures of products they have on the market. However, this is not the only avenue for success. Since very little of our food is processed at home, it is important to understand vehicles for the transmission of contamination in the chain of food production. Traditionally the food implicated in a foodborne outbreak was undercooked meat, poultry or seafood, or unpasteurized milk (CDC, 1997). However, foods that were once considered safe may now be hazardous if not properly handled. Thirty years ago, *Salmonella* Enteritidis (SE) was not a well known foodborne microorganism, but since the early 1980's it has become an increasing problem in shell eggs and egg products. The internal contents of eggs were once considered safe to be consumed raw, however, the emergence of SE as a major foodborne pathogen, we now know that this statement is false. Foods such as french toast, lasagna, pies and custards can pose a threat even though they are thought to be fully cooked (St. Louis *et al*, 1988).

It is important to human welfare that contamination is controlled at all levels of food production—from the farm to the table. Controlling foodborne pathogens throughout the food production chain enables producers to reassure consumers that the foods they are purchasing are safe and wholesome. Although foodborne illnesses have continued to be a problem, the United States has made substantial progress in identifying and controlling pathogens. As new technology emerges, we can expect to see innovative procedures and protocols pertaining to food safety becoming implemented.

Egg Related Salmonellosis

Over the past twenty years the incidence of foodborne salmonellosis has seen its share of rise and decline. The United States as well as other developed countries have been plagued with this illness as one of the leading causes of reported food related diseases. From 1988 to 1995, the number of cases of salmonellosis, excluding typhoid fever, varies between 40,000 and 50,000 (MMWR, 1996). The number of SE isolations have shown to be increasing (6-fold increase), with the main source of infection in the northeastern United States (FDA/CSFAN, 2004). However, there have been increasing cases in the south and west.

Human salmonellosis is usually contracted from the consumption of undercooked meat and poultry, raw eggs, and milk. Yet, many other foods have been implicated in the spread of salmonella organisms. Many times this illness occurs because of in-home or consumer contamination, not because the product was tainted during processing. Although consumers may argue that illness would not have occurred if the product was not initially contaminated, it is important to take into account food preparation surface cleanliness and personal hygiene practices, as they play key roles in the transfer of bacteria. This is just one of the reasons that education on proper handling and cooking procedures is so vital in consumer safety. In developed countries, human to human transfer is uncommon but can occur (FSRIO, 2005). This transfer would possibly be due to contact with fecal material.

Cross-contamination is a major factor in the occurrence of salmonellosis. Often, foods such as poultry and eggs are not prepared to the proper temperature and illness

results. It is easy to blame an inferior product as the source, but handling and preparation methods may be the real cause.

Throughout the 1990's the incidence of egg related illnesses was widely publicized. Luckily, by late 1998, the number of SE related foodborne salmonellosis was declining. However, many of these outbreaks were confined to the northeastern United States. In 1997, the District of Colombia Bureau of Epidemiology reported a rise in the number of incidences of gastroenteritis. In total, 75 people were afflicted with the illness, which was later discovered to have come from a brand of lasagna that was served at seven different events. The lasagna had become contaminated when a mixture of raw shell eggs and cheese used in the dish was improperly handled. Each batch implicated could be traced back to the same egg / cheese mixture. Upon further investigation, researchers discovered that two egg processors had obtained their eggs from farms that tested positive for SE. Because of this, the manufacturer of the lasagna switched to using pasteurized eggs and egg products in all of its dishes.

Earlier that year, contaminated raw egg whites and yolks were linked to a salmonellosis outbreak when members of a Girl Scout troop and their parents became ill after consuming tainted cheesecake. Upon further investigation, it was determined that the whites and yolk were prepared in a private residence and cooked in a double boiler until only slightly thickened. Although at first, this looked to be only a consumer handling issue, but tests of the farm that supplied the eggs revealed SE positive cultures in the feed, water, and feces of the laying hens. This finding led to the depopulation of layers on the farm, to prevent any further outbreaks of SE. In reality, there are two issues that need to be addressed. First, cooking whites and yolks until only slightly thickened

has not been scientifically shown to eliminate SE. Secondly, there was an obvious issue on the farm. Although there was no way for the consumer to know that the eggs were previously SE infected, it is important to be aware of proper cooking temperatures and techniques.

By the end of the 1990's, the number of reported cases of egg related salmonellosis was on the decline. By the year 2000, SE was still the second most common serotype reported to the CDC through the Public Health Laboratory Information System, but it only accounted for 6,000 isolates, a significant decrease when compared to previous years. By 2001, the CDC sited only 46 confirmed outbreaks, 28 less than the account a decade earlier (Department of Health and Human Services, 2003). However, this decline was not enough to prove that SE outbreaks were slowing down. The Department of Health and Human Services wanted desperately to see a dramatic decrease of 50% by the year 2005. In 2004, the U.S. Food and Drug Administration proposed a list of SE preventative measures to help meet the goal. The rule included a biosecurity program, provisions for procurement of chicks and pullets, record keeping, cleaning and disinfection, pest and rodent control, producer testing of the environment, and refrigerated storage of the eggs at the farm level.

Egg Associated *Salmonella* spp.

General Salmonella Bacteria History

Salmonella is one of the most frequently reported foodborne illnesses, not only in the United States, but worldwide. It was originally discovered and named for Dr. Daniel Salmon over 100 years ago. *Salmonella* spp. infect a wide range of hosts including humans and can cause diseases ranging from severe enteric fever to self-limiting

gastroenteritis that, in some individuals, can become systemic and life-threatening (Lacey, 1993). These microbes are among the most ubiquitous organisms that cause bacterial diarrhea and can cause paratyphoid or typhoid fever, depending upon the strain.

The *Salmonella* family includes over 2,300 serotypes of microscope bacteria which are one celled organisms (FSIS, 1998). It is interesting to note that if these microorganisms are present in food, there is no taste, smell, or overall appearance difference. Also, an animal infected with *Salmonella* will show no clinical signs, since this bacteria is commonly found in the intestinal tract and do not pose an immediate threat to health. However, strains that cause no symptoms in animals can and do make people sick, and vice versa.

The classification of subgroup or serovars of the *Salmonella* family have been widely discussed (Salmonella Information, 2005). The serovars that are most important in pathogenicity are those of the subgroup enterica, which contains over 2000 serotypes. These organisms are gram-negative, rod shaped, facultative, motile by means of flagella, and do not form spores. The exceptions to these rules are *S. gallinarum* and *S. pullorum* which are non-motile. Members of the genus *Salmonella* are usually catalase-positive, oxidase-negative organisms which ferment glucose and produce both acid and gas. *Salmonella* bacteria are within the family Enterobacteriaceae. Many strains are capable of growth on a chemically defined medium and can use citrate as a carbon source. Typical strains ferment neither lactose nor sucrose, and with a few exceptions, produce an abundant amount of hydrogen sulfide. *Salmonella* colonies produce characteristic reactions on selective media.

There are many ways to classify *Salmonella* species. Epidemiologically, strains can be classified according to their adaptation to human and animal hosts (FSIRO, 2005). Group1 includes those organisms which only cause disease to humans and higher primates (*S.* Typhi and *S.* Paratyphi). Group 2 contains *S. dublin* and *S. choleraesuis*, which cause infections in cattle and swine, but rarely infect humans. Group 3 includes two of the most important strains, *S.* Enteritidis and *S.* Typhimurium. These organisms transmit salmonellosis from humans to animals. Group 3 also includes other *Salmonella* serotypes that may cause gastroenteritis.

Salmonella organisms can also be characterized by their antigen groups. Antigen H or flagellar antigen, O or somatic antigen, and Vi antigen, which are possessed by only a few serovars, are the three main groups. H antigen may occur in either or both of two forms, phase 1 and phase 2. It is possible for these bacteria to change from one form to another (Giannella, 2005). O antigens occur on the surface of the outer membrane and are determined by specific sugar sequences on the cell surface (Rubin & Weinstein, 1977). Vi antigen is present in only a few serovars, most commonly *S.* Typhi. It is a superficial antigen that overlies the O antigen.

There are many factors that influence the survival of *Salmonella* organisms inside and outside their optimal environments. Those such as pH, temperature, redox potential, and available nutrients affect not only the endurance of specific organisms, but their growth as well. Temperature manipulation ranks among the most important environmental factors affecting bacterial growth, survival, and death.

Salmonella Enterica Serovar *Enteritidis*

Egg-associated salmonellosis is one of the most important public health problems in the United States and Europe. SE shows no clinical signs in the adult hen and cannot be observed by the naked eye in the shell egg. Like many other species of *Salmonella*, SE can live in the intestinal tract of the hen and contaminate the egg through exposure to feces. However, due to the strict government regulations adopted in the 1970's, this type of transmission has become increasingly rare. It is possible, though, that the hens ovaries could become contaminated and the SE passed into the shell egg before the shell is formed (CDC, 2005).

Colonization in Laying Hens

Understanding the mechanism that emphasizes the pathway that SE infection occurs in laying hens is crucial to reducing the number of outbreaks and human illnesses. Though many believe that the primary mode of transmission is though contamination of the shell egg surface as it passes through the cloaca and vagina, other suggestions such as contamination of the yolk, ovaries, and oviduct are also accepted as possible vectors for transmission. In 1990, Gast and Beard suggested that SE can frequently migrate into shell egg through the upper oviduct. However, there are not many studies that specifically target which part of the oviduct is more susceptible to colonization. In 2004, De Buck *et al* conducted a study that aimed to determine if the tubular gland cells of the isthmus or magnum were more heavily populated. Both sets of tissues were experimentally inoculated with SE ands monitored for 24 hours to determine growth populations and patterns. They discovered that the isthmus had higher levels of intracellular SE, and in turn, concluded that the evidence from the performed assays

favored that SE bacteria would more readily invade the tubular cells of the isthmus as compared to those of the magnum.

Migration and Penetration of Shell Eggs

Microbial contamination of the hen's egg has become a major cause of concern for consumers and public health officials. While the egg is naturally equipped with barriers that help keep microorganisms from penetrating into the interior shell and contents, it is unrealistic to believe that these defenses are not subject to failure. Exterior shell contamination is not an uncommon occurrence and has led to regulations that require washing and disinfection of table eggs before they are considered marketable to the public. On the other hand, it would be quite difficult to cleanse the interior shell, yolk, and albumen, making it important to ensure that motile salmonellae do not find their way into these components.

The Cuticle and Pores

The cuticle or "bloom" of shell eggs is a protein rich layer that is thinly deposited on the exterior surface of the shell. Since the shell egg exterior has several thousand pores that could potentially allow bacterial penetration, the cuticle acts as a protective barrier against contamination. In theory, the cuticle aids in ensuring the safety of shell eggs. In reality, however, the cuticle is removed or severely degraded when the eggs are washed or sanitized. A weak cuticle allows bacteria such as SE to enter the inner shell membranes and possibly the contents through the pores. However, a strong cuticle will add both strength and texture to the shell, which adds to the exterior shell quality.

The removal of the cuticle during washing has been a topic of controversy for many years. Although regulations for egg inspection have been in place since the 1928,

actual egg washing regulations were not strictly enforced until 1964. During this time, methods for washing mass quantities of eggs, via immersion washing, were developed. However, in the early 1960's outbreaks of *Salmonella* infection led to the implementation of shell egg temperature regulations. A study done by Brant and Starr (1966) determined that there were specific time-temperature requirements needed to de-contaminate the exterior shell of the egg. Due to the outcome of this research, the USDA established regulations governing immersion egg washing. In 1970, the Egg Products Inspection Act was passed; giving the USDA and FDA shared responsibility of egg safety regulations. Per this act, the FSIS would be responsible for inspecting the egg products to ensure proper labeling and distribution to consumers. AMS, on the other hand, would be in charge of restricted, or inedible, shell eggs (FSIS, Title 21, Chapter 15, 1031-1056). The role of the FDA was to govern the safety of all foods, including shell eggs under the FFDCA (212 USC 30, et seq.). However, in 1975 the use of immersion washing was banned in egg processing facilities. Among the reasons was the controversy of microbial contamination. It was argued that the removal of the cuticle by immersion washing and scrubbing would allow microbes found either on the exterior shell or in the wash water, the ability to penetrate into the egg through the pores. Because the cuticle provides some protection from microorganisms, some researchers believed that its removal would only help contaminate the interior portions of the egg. It was also determined that eggs should be washed in water that was 20 degrees warmer than the warmest egg. This would allow the interior contents to swell slightly, pushing dirt and microbes away from the egg, and in turn, aiding in the prevention of contamination (Clauer, 1997).

Although recent research has shown that due to the strict regulations utilized by the US government stating that shell eggs processed as table eggs are safe and virtually free of exterior microbial contamination after washing and sanitizing, the removal of the cuticle is still a hot topic. European egg processing regulations do not allow eggs to be washed prior to consumer distribution. EU regulations require that eggs sold directly to the public, defined as "Grade A", must not be washed or cleaned before or after grading. Producers also cannot chill or treat eggs for preservation. Grade B eggs, the second classification in the EU, may only be used by the food or non-food industries and can be washed or unwashed. In their view, the major disadvantage of egg washing is the potential damage to the physical barriers, such as the cuticle, which can occur during or after washing, for instance from incorrect operations, in particular washing eggs in cold water. It is also argued that even if eggs were washed, it would not prevent salmonellosis outbreaks from SE already deposited inside the egg.

The pores are located on the exterior surface of the egg shell. The average egg contains over 7500 pores, most of which are located on the large end of the egg. The concentrated numbers of pores at the large end of the egg serve a physiological purpose for hatching eggs by aiding in the transfer of oxygen and carbon dioxide through the air cell. Although this process is not necessary for unfertilized table eggs, the pores are a main area of focus when discussing interior egg quality. As the egg ages, carbon dioxide escapes through the pores, resulting in a decrease of interior quality, also, as the hen herself ages, she will lay eggs with larger pore sizes, creating a greater chance of motile *Salmonella* spp. entering through them. Investigators have suggested that the pores in

egg shells provide a means for entrance of microorganisms into the egg with subsequent spoilage as a result (Kraft *et al* , 1957).

Shell Membranes

In the 1960's the question of whether or not the contents were pathogen-free at the time of lay remained unanswered (Board, 1966). Earlier studies concluded that the shell was most susceptible to microbial penetration within a very short time after lay, based on the assumption that the yolk and albumen contract during cooling, causing organisms to be sucked through the pores (Ferdinandov, 1944). In 1964, Lifshitz *et al* determined that the inner shell membrane is a greater defense mechanism than the exterior membrane, not because of its chemical properties but because of its mechanical functions. While these scientists discovered that the outer membrane was thicker, heavier, and denser than the inner membrane, it did not prove to be an effective barrier against microbial penetration. They concluded that this is possibly due to the fact that the outer membrane is composed of a network of course fibers that allowed organisms through easily, while the inner membrane is composed of smaller fibers that create a more compact network, resulting in smaller pore size. This study did not, however, do any microbial analysis to support their claim and looked only at the physical properties of the egg. In 1962, Hartung and Stadelman concluded that there could potentially be a lag time of up to 20 days between penetration of the shells and the occurrence of a large interior bacterial load in the inner membranes. This was attributed to the mechanical structure of the shell membranes and their effectiveness in providing a mechanical barrier against contamination. However, it was also noted by Brooks and Taylor (1955) that it could be a combination of the protective membranes and the antimicrobial properties of

the albumen. Experiments performed a few years later indicated that the lag time is more of a reflection of the antimicrobials such as lysozyme, and that the shell membranes play only a minor role in restraining microbial contamination of the albumen (Board and Ayres, 1965).

According to Lifshitz and Baker (1964), the inner and outer shell membranes are 50-75 μ (inner membrane) and 15-17 μ (outer membrane), each being composed of a network of fibers consisting of keratin and mucin, with the interstitial fluid containing mainly albumen (Romanoff and Romanoff, 1949). However, as reported by Lifshitz *et al* (1964) the shell membranes do not always prove to be effective barriers against microbial penetration. In a study in which these researchers looked at the effectiveness of microbial invasion into the albumen through seeding of the air cell, they discovered that in order for microorganisms to pass into the albumen, they would have to penetrate through the inner shell membrane, which displays a much greater resistance to penetration than does the outer membrane. Subsequent investigations performed by Brooks (1960), Board (1964), and Board and Ayers (1965) determined that there was a greater possibility of invasion 4-6 days post inoculation, when large amounts of organisms were used and the eggs were held at a temperature of 37°C.

Albumen and Yolk

It has been determined that the yolk of shell eggs is a wonderful growth medium for certain bacteria, and *Salmonella* is at the forefront of concern. *Salmonella* serotype Enteritidis has been identified as being most commonly associated with eggs. Therefore, it is important to understand the mechanism by which these organisms are penetrating the exterior shell and migrating into and ultimately thriving in the contents. Studies have

shown that in order for the egg to effectively deter pathogens from entering it must possess a thick and even cuticular layer which will plug gas exchange pores without reducing their efficiency, and a multi-layered shell that is firmly attached to the underlying intact shell membranes (Nascimento *et al*, 1992). However, Nascimento *et al* found that the cuticular layer is rarely found as a uniform, thick covering, and that the integrity of the external and internal membranes is questionable when bacterial loads are high. In a study performed by these scientists, the relationship between shell structure and the movement of SE across the eggshell wall was researched. The results concluded that as shell quality decreases with age, a significant increase in bacterial penetration was observed. The occurrence of pitted areas, which also increased with hen age, was significantly correlated with penetration. The alignment of the mammillae was also significantly correlated. It was determined that no one factor could be singled out as a causative agent for bacterial invasion, but that the structure of the shell and membranes as a whole played a key role and that higher quality shells would be less susceptible to infection. It has also been determined that eggs from older laying flocks are more susceptible to bacterial infection. This is because, as flock or hen age increases and egg quality decreases, the yolks of eggs laid by those hens are placed closer to the shell in internal orientation. This is due to the fact that eggs from older flocks will have thinner albumen—the thick albumen will become extremely thin and the thin albumen will be almost water-like in consistency. Also, as this process takes place, the amount of active lysozyme in the albumen is decreased (Marsh, 1995). This allows a perfect environment for external penetration by motile *Salmonella.* As the bacteria enter through the pores,

they are not faced with the same effective barriers, and can therefore easily reach the yolk and rapidly multiply.

Egg shell quality has been a serious issue in the industry for quite some time. While *Pseudomonas* spp. have been shown to more readily migrate into the shells of poor quality eggs (Sauter and Petersen, 1969), they pose more of a spoilage than a food safety threat. *Salmonella* organisms, on the other hand, are dangerous to consumers because they can grow well in eggs and can cause infections in people of all ages. In a study done by Jones *et al* eggs from three control strains and one commercial laying strain were inoculated with either *Pseudomonas* flourescens, SE, or a combination of the two bacteria and were stored at 26°C. After storage, air cell, contents, exterior and interior shell samples were analyzed. They determined that there was more *Pseudomonas* found in the contents of the shell eggs than SE. This is precisely the opposite of what was determined to be present on the exterior shells. This study may suggest that *Pseudomonas* is more capable of penetrating the contents than is SE. However, this study also observed the differences in penetration rate as it relates to hen age. They discovered that both SE and *Pseudomonas* flourescens were more likely to contaminate the contents, air cells, and internal and external membranes of eggs from older birds than those of younger flocks. It was also determined that differences between the strains used in this study were significant. This may suggest that certain genetic strains are more capable of producing eggs that many be more susceptible to microbial penetration.

There are essentially two ways that egg contamination can occur. The first is through the exterior shell and the second is transovarian. An important fact to note is that most *Salmonella* species, including Enteritidis, are motile and can penetrate the shell and

migrate toward the yolk (Todd, 1996). In 2001, Grijspeerdt created a transport-reaction type model that incorporated a diffusion mechanism and linear transport due to motility and growth. The results of this simulation showed that bacterial infection of the shells of eggs can lead to very high concentrations in the yolk, even before the albumen is saturated with organisms. This could be due to albumen being an unfavorable growth medium because of lack of iron content and antimicrobials such as lysozyme. The concentrations of microorganism on the shell were not seen to play a key role in the numbers gathered in the yolk, because this model shows that SE can grow quite well on the outside of the shell. However, more studies are needed to ensure that this method was valid in correctly reporting bacterial movement.

A second study conducted by Grijspeerdt *et al* in 2004, researched individual-based models that would take into account the fact that contamination of SE usually occurs in very low numbers. It also looked at the transovarian method of infection and did not consider penetration through the shell. Several model parameters such as chemotaxis, growth rate, initial numbers, and linear swimming speed were used to determine the results. Bacteria were placed at several different points in the egg and their migration toward the yolk was monitored. The results of this experiment showed that the initial site of contamination was a key factor in the amount of contamination in the yolk. In some areas of the albumen, the number of SE cells inoculated was less than 100, making it difficult for many of the cells to survive. However, the ones that were able to travel to the yolk not only survived, but grew very rapidly. Chemotaxis also played an important role in migration. This is the phenomenon that motile bacteria can bias their random walk in the presence of attractants or repellents (Armitage, 1999). The

microorganisms in this study were attracted to the yolk, and made every effort of ultimately end up there.

It has been reported that SE may be deposited by infected hens in the yolk or albumen of their eggs (Gast *et al,* 2005). The higher the numbers of SE in the albumen, the more often the egg yolks are contaminated. This is of great concern not only to the egg industry in the United States, but also in Europe, where eggs are often not washed or refrigerated. Studies have shown that under certain conditions, such as unrefrigerated temperatures, the antimicrobials in the albumen are unable to control the growth of certain pathogens (Braun & Fehlhaber, 1995). SE is no exception. As a result of the unanswerable questions about SE contamination in shell eggs, the Food and Drug Administration defined shell eggs as a potentially hazardous food, which requires refrigeration during storage and care in preparation and handling.

In a study conducted by Conner *et al* (2006), experiments were conducted to determine the growth of SE in eggs as related to storage time and temperature, and vitelline membrane strength. Eggs were stored at 4, 10, 20, or 30°C for predetermined times and were inoculated with SE into the albumen of each egg with enumeration occurring 5 days after. The trial was replicated three times including the months of January, April, and August. They determined that eggs held for 9 weeks at 4°C showed no signs of SE growth and their vitelline membrane strength remained constant. However, the other storage times showed much variation, suggesting that flock age may have played a role. Although the rate at which the vitelline membrane deteriorates is important to determining the risk of SE in shell eggs, this study suggests that commercially produced eggs that are held at temperatures of ≤10°C for 7 weeks does not

increase the risk of SE growth. In 2000, Gast and Holt performed a similar study which compared different storage temperatures and inoculation levels on the growth of SE in experimentally inoculated eggs. They concluded that at 25°C storage for 2-3 days at an inoculation dose of 150 cells, the multiplication of SE was rapid, especially when the yolk was penetrated. However, at lower doses, temperatures, and storage times, SE contamination was less frequently observed and invasion into the albumen proved potentially fatal to the low dose bacteria. In 2001, Gast and Holt discussed the role of SE deposition on the yolk membrane and how this related to the contamination of the yolk contents. They determined that with proper refrigeration to inhibit the growth of SE, it was relatively infrequent and difficult for SE to contaminate the yolks.

Control Programs

Foodborne salmonellosis is a major public health concern in almost every country, and *S. enteritidis* has been reported as one of the most frequent serotypes found in the United States. Because of the increasing apprehension regarding the worldwide food supply, countries around the globe have been forced to implement regulations to ensure the wholesomeness of not only the products they produce, but the ones they import as well. Although the presence of any species of *Salmonella* can be detrimental to many food sources, the control of SE has become a source of interest for scientists. The objective of these control measures is to deliver *Salmonella*-free food to consumers.

Because of this, the United Egg Producers (UEP) developed a "5 star" total quality assurance program. The purpose of this program was to help ensure the safety of the nation's egg supply and provide consumers with the assurance that the egg industry is doing everything possible to produce a safe and wholesome product. This plan targets

five critical areas of poultry and egg safety. They include poultry house cleaning and disinfecting, rodent and pest elimination, proper egg washing, biosecurity, and refrigeration. Because cleaning and disinfecting is the first step in the removal of visible dirt and soil from eggs, it is listed at the top of the "5 star" list. By using proper steps and techniques, it is possible to remove from 99-99.99% of bacteria found on exterior egg shells (UEP, 2006).

If done properly, cleaning and disinfecting reduces the source and spread of bacteria, and will aid in the reduction of pest infestation. There are two types of cleaning that can be done, wet and dry. Wet cleaning includes the removal of all eggs, feed and other debris from the poultry house and the wetting down and soaking of all areas. It also includes high pressure washing of all surfaces and equipment. Dry cleaning, on the other hand, includes the removal of all eggs, feed, and other debris, but with the addition of sweeping and blowing all dusty areas. This can include the egg conveyor belt, feed troughs, and dropping boards. It is then necessary to run scraper blades to remove all manure and also to remove manure from the pit areas. After proper cleaning has been done, it is essential to disinfect the area. This can be done by applying the chosen disinfectant to surfaces as a spray foam. If areas cannot be reached with the foam, it may be necessary to use fumigation to ensure that bacteria are kept under control.

Rodent and pest control is another area targeted by the total quality assurance program. It is no surprise that volumes of research have been conducted on the relationship between rodent control and the spread of harmful bacteria. Quite simply, the best way to ensure that contamination does not occur from pests is to keep them out of the production area. This type of control can begin with a good sanitation program, but

the help of a pest control team may be needed to design a proper plan of attack for the specific producer.

The third step of the UEP's plan is to ensure proper washing of eggs. They determined that the best way to remove both visible and invisible soil and debris is to wash eggs at a temperature that is greater than 100°F and has a pH greater than 11. They also suggest that eggs be washed as soon after lay is possible and that a proper detergent can be used to soften the soil for easy removal. Wash water and sanitizers must be changed every 2-4 hours to prevent organic and microbiological build-up and the temperature, pH, and sanitizer concentration should be closely monitored.

For biosecurity purposes the UEP recommends that day old chicks be purchased from hatcheries that participate in the National Poultry Improvement Plan (NPIP) US Sanitation Monitoring Program. They also recommend that egg producers request a 9-3 NPIP form from the chick or pullet suppliers to ensure that they are in compliance, and that all feed ingredients be monitored for SE. This includes, but is not limited to, the proper labeling of all medications, pesticides, and feed additives that could present a potential hazard.

The final area of the "5 star" program is refrigeration. There have been numerous reports to support the theory that refrigeration helps prevent bacterial contamination. The UEP suggests that eggs should be refrigerated as soon as possible after washing and grading. They should be maintained at a temperature of 45°F and all transportation trucks should maintain this temperature. It is also important that all data recorders, thermometers, and recording devices be in good working order and condition. With the

implementation of this program producers will dramatically reduce their risk of having contaminated eggs and products. It is necessary, however, to have corrective action programs in place just in case something fails, and have records of every action performed as well as verification of all procedures. Although the idea of refrigeration has been proven to be a sufficient way of controlling potential bacterial hazards, it is often difficult to regulate. Government agencies have been unable to provide a cohesive strategy for regulation of refrigeration temperatures during transportation and storage, and often, the way eggs are processed means that they could potentially leave packing plants warmer than room temperature. They are washed in hot water, immediately placed in cartons, stacked in pallets of several dozen cartons, and then, frequently, shrink-wrapped in plastic. These industry practices make it difficult to cool eggs sufficiently, especially those eggs at the center of a pallet of cartons.

Since Europe does not allow washing of eggs before they are sold to consumers, the United States and Sweden are often looked upon with scrutiny when it comes to the safety of the egg supply. However, in recent years, the EU has begun to look more closely at these two programs in relation to their own reports of *Salmonella* outbreaks. The Swedish control of *Salmonella*, particularly SE, has allowed it to be one of the few countries who are not involved in the spread of different SE phage types. It has been reported that if any SE positives are found, the entire flock is destroyed.

One way that the Swedish government is controlling the risk of SE is by monitoring the grandparent flocks. Approximately 10 groups of grandparent flocks are imported into Sweden annually, and each of these flocks are brought in as day-old chicks

(Wierup *et al*, 1992). When these birds arrive, they are quarantined for 15 weeks. They are tested four times during this period, and any birds that arrived dead have their liver, yolk sac, and cecum tested for *Salmonella.* It is important to note, however, that SE has never been isolated from imported grandparent birds (Wahlström *et al*, 1993).

Another way that Sweden controls SE is by managing their laying hens. The National Food Administration, the National Veterinary Institute, and the National Board of Agriculture implemented policies to help regulate egg producers. In 1995, Wierup *et al* conducted a study that looked at the prevalence of *S.* Enteritidis in laying flocks. The first isolation of SE was discovered in 1987, when nine people became ill after visiting a restaurant, though none of the people ingested eggs or egg products. The second incidence of SE was found in 1990 during a routine visit to a slaughterhouse. The contaminated flock consisted of 60,000 layers. In 1991, the third outbreak of SE was reported when 167 people became ill from a flock that consisted of 19,000 birds. It was surmised that improperly heat-treated eggs were the cause. The fourth incident was limited to 2200 layers and was found at the control testing stage before slaughter. No human illnesses were reported. The fifth and final discovery occurred in 1993 when farm children became infected with SE. It was reported that the farm consisted of 20 hens and the children handled them regularly. Because of these outbreaks, the Swedish government implemented mandatory testing of all flocks and any flock that is found to be SE positive is euthanized. However, it is important to note that there was no correlation found between flock size and SE contamination.

The methods of production between the US and Sweden are similar in the fact that both countries are taking precautionary steps such as monitoring chicks and grandparent flocks for SE and testing birds to ensure that they are SE negative. Both countries have had similar SE contamination rates in commercial production situations, and with the implementation of specific control programs, both have been able to reduce the number of SE related foodborne illness.

It has become the objective of many countries to keep the incidence of *Salmonella* below 1% at the national level, and Finland is no exception. In order to be efficient, a control program must reach its targets effectively and cost efficiently (Maijala *et al*, 2005). When Finland joined the European Union, they were granted special permission to conduct their own *Salmonella* testing in meat and eggs, as well follow the procedures set by the EU Zoonoosis Directive. In egg production, *Salmonella* has never been detected in breeder flocks and in layer flocks, the prevalence has been well below the 1% target level. The most common serotype reported in *S.* typhimurium, however, SE has only been detected in layer flocks twice, in 1995 and 1999.

After the first year of the implementation of the Finnish *Salmonella* Control Program (FSCP) the target of 1% was reached with ease. This study showed that while the incidence of *Salmonella* was low in all facets of production, it could be kept under control or even lowered with the practice of certain control programs. The FSCP was concluded to be a necessary and successful program, which had been responsible for protecting public health in Finland.

While each country has its own ideas about regulatory actions, none has been at the forefront of the war on *Salmonella* Enteritidis like the United States. In addition to

government regulations, other actions are being taken to control the risk of SE in the US. Although many of these procedures are not being used commercially, they are still key players when it comes to future research. An example is the use of gamma irradiation. Research has shown that ionizing radiation at does up to 5 kGy can sufficiently reduce the numbers of non-spore forming organisms in food (Parsons and Stadelman, 1957). More recently a study conducted by Tellez *et al*, clean shell eggs were inoculated on the exterior shell with a solution of SE phage type 13a. The eggs were packaged into cartons and transported to Mexico, where they were irradiated within 2 hours of inoculation. The source of irradiation was Cobalt-60 gamma irradiation at levels of 1, 2, and 3 kGy. The results illustrated that at the level of 1 kGy, there was a significant reduction of SE in the shell and a greater reduction in the shell membranes. When levels of 2 and 3 kGy were used, the bacteria were reduced to non-detectable levels. However, while this experiment had good results for the microbial analysis, the effect of the irradiation process on egg quality and flavor was not as promising. The results also indicated that there was a 50% reduction in Haugh units and that yolk color was decreased dramatically. Researchers have found that not only had egg quality deteriorated during the irradiation process, but egg flavor was sacrificed as well. They determined that the dramatic changes in the flavor of both shell eggs and egg products made them unpalatable to consumers, often giving the impression of off flavors and smells. The results of these studies could account for the fact that there are no irradiated eggs sold in supermarkets today. However, this example proves that new and innovative methods for increasing egg safety are not readily accepted by consumers.

Because of the increased emergence of SE in the mid-1980's eggs and egg products were given a poor reputation, being labeled the primary source of infections (Rodrigue *et al*, 1990). Because of this, the egg industry suffered a huge blow to its revenue as the consumption and use of eggs declined. Researchers began working on procedures to enhance the microbial quality of shell eggs using ways other than those regulated by government. In a study conducted in 2001 by Barbour *et al,* research was done to produce a cleaner intact shell egg that were free from SE contamination by the application of pasteurized dry heat. This experiment used strains that were identified as very virulent by the WHO (World Health Organization). The eggs were inoculated using both high and low counts of SE cells and placed in a 57°C water bath for 25 minutes, followed by transfer to a hot oven at 55°C for 57 minutes. This process served as the pasteurization and dry heat treatment. The interior contents were tested for SE survival. The results indicated that the concentration of the SE would have to be low for the bacteria to be completely killed. It is likely that this would produce eggs that were free of other *Salmonellae* as well.

The use of natural antimicrobials has also been studied for their effectiveness in controlling SE in eggs. Products such as salad dressings and appetizers made with uncooked eggs have been listed as foods to be eaten with caution. However, many of these foods can be made with essential oils and other natural antimicrobials that may reduce the risk of SE infections. Another option may be to use pasteurized eggs, but more research is needed to determine how the functionality of these products differs from shelled eggs. Greek appetizers that were supplemented with oregano essential oils and lemon juice to adjust the pH, showed a significant decrease in the number of SE

organisms and even demonstrated bacterial death if the right combination of time, temperature, pH, and essential oil concentration was used (Koutsoumanis *et al*, 1999). However, this experiment did not detail whether the reduction and death of the microorganisms was due to the oregano oils or to the adjusted pH. It is highly possible that the decrease in pH was enough to lower the numbers of SE.

Researchers are always looking for ways to control the numbers of SE in eggs. These studies help the egg industry explore new discoveries in the war on foodborne illness. While there has been a decline in SE infections in recent years, the numbers can never be low enough. It is important to find a helpful balance between government regulations and innovative technologies.

Molting

Molting is a natural physiological process that occurs in chickens, fowl, and other avian species. It allows birds a pause in egg production and permits the reproductive tract and plumage time to regenerate. In commercial laying hens, this allows the body time to recuperate from a long laying cycle while resulting in a state of ovarian arrest leading to a second cycle of eggs. Molting is of vital economic importance to the egg industry. With proper procedures, it is possible to achieve a productive life from 70 to 105 weeks of age and for an additional 25 to 30 weeks if a second molt is used (Butcher & Miles, 2002). This is most important because these birds are bred specifically for egg production and the intent of the industry is to balance a profit through increased egg production, with the welfare of the birds.

The most common method of molting is induced molting. This is the practice chosen most often in the United States to synchronize the hens production cycle schedule.

Research has shown that several physiological changes occur during an induced molt. In a study done by Brake and Thaxton, hens were molted at 72 weeks of age and then again at 104 weeks of age. They utilized two successive seasons, winter and summer and a range of temperatures that varied from -6 to 10 C during the winter molt, and 18 to 35 C during the summer molt. In a similar trial utilizing commercial strains of Single Comb White Leghorn hens (Brake & Thaxton, 1979), it was shown that the body weights of the molted group were significantly less than the control group at day seven through day 35. The weights of the ovary, oviduct, and liver were also significantly less. The total body mass loss was approximately 25% with one-fourth of this loss being attributed to the liver, ovary, and oviduct.

In addition to the physiological changes that are common with an induced molting process, research has also shown that there is a relationship between body weight loss and post-molt egg production, shell quality and shell weight. In the early 1980s Baker *et al* conducted research that evaluated the post-molt egg production and shell characteristics provided by two strains of Single Comb White Leghorn hens that were induced into molt. In trial 1, the hens were returned to feed after a total body weight of 24, 27, 31, and 34% had been induced. In trial 2, feed was returned after a body weight loss of 20, 25, 30, and 35%. It was shown that the egg production was at its best when a weight loss of 27, 30, 31, and 35% had been achieved. The poorest egg producers were those who were returned to feed with 25% or less total loss. Shell quality in the groups that had higher egg production showed a value equal to or greater than that of the lower egg producers. It is important to note that these hens were of different strains, ages, pre-

molt treatment, and were reared differently (Baker *et al*, 1983). This could have played a significant role in the differences that were found between the groups.

There has been a great debate as to which method would attain the greatest production rate and quality of the eggs produced. The removal of specific nutrients such as sodium (Whitehead and Shannon, 1974), iodine (Arrington, *et al*, 1967), calcium (Gilbert and Blair, 1975), or zinc (Creger and Scott, 1977) will induce hens into a molt. It has been revealed that hens fed a calcium deficient diet of less than 0.3% will cease egg production in 7 to 10 days (Bell and Siller, 1962). Two experiments were conducted to determine if the addition of calcium during the beginning stages of the molt would increase the number of marketable eggs (Garlich and Parkhurst, 1982). The results of this study indicated that by adding a source of supplemental calcium, shell weight, percent shell and shell breaking strength did not show a significant decline. Also, the incidence of unmarketable shell eggs was reduced when oyster shell was available during the fast. This would suggest that calcium, while still allowing a decrease in overall body weight, may play an important role in shell quality and other shell characteristics.

It has also been proposed that the addition of certain water additives to hens on a forced molting program may increase the livability or post-molt laying performance. However, research has shown there is no evidence that the addition of vitamins or electrolytes will enhance post-molt production or mortality during molt. In a recent study, four groups of both molted and non-molted hens were given were given a treatment of distilled water, with the addition of 0.5% lactic acid and 0.5% acetic acid for two of the molted treatments (Kubena et al., 2005). The molted hens were feed restricted for 9 days, and seven days prior, all treatments were placed on 8 hours of light and 16 hours of

darkness per day. At the end of the study, the results concluded that there were higher levels of *Salmonella* Enteritidis in the crop and cecal contents in the molted group. However, there were no differences in the additions of the acids to the water. These studies show that the application of additives to the drinking water of laying hens has no effect on livability, post-molt performance, or SE positive samples after molting.

While molting remains the primary method of recycling a laying flock, other methodologies are becoming more prominent in the industry. Approaches that do not use complete feed withdrawal are becoming more desirable (Ruzler, 1998; Park et al., 2004; Anderson and Havenstein, 2007). According to Biggs *et.al.* (2003) non-feed removal techniques are not less effective in recovering post-molt egg production. Methods for non-feed removal may include feeding diets high in corn gluten, wheat middlings, corn, or a combination of 71% wheat middlings and 23% corn (Biggs et al. 2003, 2004). Additional alternatives may include a low sodium diet (Whitehead and Shannon, 1974), 10% and 15% sugar meal (Zimmerman et al., 1987), and grape pomace with added thyroxine (Keshavarz and Quimby, 2002). They also exhibit a greater skeletal integrity (Mazzuco et al., 2003). These results may suggest that an alternative method of molting may be more animal welfare friendly than the widely accepted feed withdrawal regimens. However, these unconventional practices have not proven to be consistent in halting egg production (Berry, 2003), and may in turn, cause a dramatic decrease in lay, but not allow a full rest period. This could mean that the flock would not reach it full production potential once the molt has concluded.

It has been noted that hens on an induced molting regimen show higher incidences of *Salmnella* Enteritidis (Holt and Porter, 1992) or a higher susceptibility to

SE infection (Holt, 1993) when compared to hens on a non-feed removal alternative. It is necessary to mention, though, that many experiments conducted for scientific research of SE as it relates to molting, use inoculated laying hens as their test subjects. Because of this fact, it is hard to determine if the results obtained are genuine reflections of the industry as a whole, or simply due to high levels of inoculum or commercial settings and breeds (Anderson et.al, 2001). When looking at the molting process from an unbiased point of view, it is necessary to take into account all options that may play a role in the acquired data. Factors such as age of hen, rearing conditions, levels of inoculum, and strain, may be a significant issue when choosing a molting procedure that best suits the research being conducted.

No matter which method of molting is chosen, there will always be both positive and detrimental effects. Critics of the molt deny that it is a necessary part of the life of a flock, and refute evidence to the contrary. Each side has their arguments, but the fact of the matter is that molting in the egg industry is a way of life. The primary goal is to generate as many eggs as possible while taking into account the welfare of the laying hens. In the future methods such as nutrient restricted diets may surpass induced or forced molting practices that utilize feed withdrawal. However, for now, the feed restriction method for molting is the technique that is most widely practiced in research studies as well as in the industry.

Molting is a natural physiological process that avian species use to aid in the regeneration of feathers, healing the reproductive tract, and the addition of body weight. Because of this process, producers are able to recycle laying flocks for many weeks past the first production cycle, and still maintain efficient egg production and quality. There

are many positive aspects to molting. According to Hester (2005), molting decreases the demand for chicks by 47% and reduces the need to process, render, or bury the same percentage of spent hens. In 2003, Bell discussed the fact that rejuvenation of flocks prevents the annual euthanasia of one hundred million male chicks. He also ascertained that molting improved both livability and egg quality when comparing the second production cycle to the first. In 1981, Brake *et al* concluded that molting resulted in the repopulation of the thymus gland with lymphocytes, thus aiding hen health. Another advantage is feather rejuvenation. Molting allows birds to re-grow feathers lost during production, thus improving thermoregulation.

However, with all of the positive aspects molting has to offer, there are negative effects as well. When feed, water, or lighting programs are changed, undoubtedly stress will occur in the laying flock. The most common molting program used is one in which feed is withdrawn in combination with light restriction. This can potentially lead to increased mortality within the first 2 weeks of the molt (Bell, 2003). It is also noted that on the first day of feed withdrawal, there was increased incidence of aggression. While this behavior seemed to subside within the first few days, other behaviors such as increased pecking, standing, and head movement were noted (Anderson *et al*, 2001). This increase could be indicative of foraging behaviors. It has also been noted that there is an increase in susceptibility to SE infection and increased numbers of SE organisms shed in the fecal material (Holt, 1993). This could possibly be due to the fact that in fasting hens, only a small number of SE bacteria need be present to cause infection (Holt and Porter, 1992).

Previous studies have shown the importance of molting to the economics of the egg industry, and the effects that molting has on the laying hen. However, because of those effects, there is some speculation that certain commonly used molting practices are not in the best interest of the birds. Although inducing a molt will recycle the laying flock, it is also important to ensure that post molt performance is not lost. Due to the ever-increasing public concern surrounding animal welfare, Keshavarz and Quimby (2002) conducted a study to evaluate a number of molting techniques that would be less stressful than 10 to 14 day feed withdrawal, while still attaining a practical sense of application and satisfactory post molt performance. They discovered that post molt egg production was highest in the treatments that had a continuous feed withdrawal and those who were subjected to one day of feed withdrawal followed by a diet containing 10 ppm thyroxine. They did not, however, find any differences in other post production factors such as egg quality. They discovered that the use of grape pomace along with the thyroxine treatment would yield similar results as those obtained by feed withdrawal, but the results did not indicate that this procedure was less stressful on the hens.

In 2003 and 2004, Biggs *et al* evaluated non-feed removal methods for molting programs. They utilized 60 week old white laying hens assigned to four treatments including those with restricted feed for 4 or 10 days, and those with no feed removal who had access to 95% corn or 95% wheat middlings molt diets, which contained additional vitamins and minerals. They discovered that both the feed restricted and wheat middlings treatments ceased to produce eggs within 8 days. The 95% corn diet group were still producing eggs at a rate of 3% at day 28, proving that this was not the best method to completely halt egg production. They also concluded that the hens feed the wheat

middlings diet, as well as those fasted for 10 days had higher post molt egg production and egg mass when compared to the 4 day fast and corn treatments. This study showed that a wheat middlings diet may be a more animal friendly way of inducing molt and that no drastic differences were seen when compared to the feed restricted treatment.

In 2001, the UEP commissioned 5 universities to conduct experiments to develop alternative molting programs that used non-withdrawal feeding programs to molt laying hens. Some of the studies conducted included those with non-nutritive additives, lower sodium, and low cost ingredients that would be low in energy and protein levels. The results of a study done by Koelkebeck *et al* (2006) concluded that it was possible to molt hens without removing feed, and do it successfully. They found that by feeding laying hens diets consisting of soybean hulls, wheat middlings, and corn they saw results that correlated with those produced by the feed withdrawal method. Because of these findings over the 5 year period, the UEP revised their recommendations to adopt these procedures and stated that only non-withdrawal methods for molting would be used after January 1, 2006. These guidelines apply to approximately 82% of the industry.

From animal welfare issues to bacterial contamination, eggs sold to consumers as table eggs have had to fight to regain their proper place on the list of most nutritious foods. However, one must consider that not all marketed eggs are sold in-shell. The liquid egg and products industry comprises a large percentage of eggs purchased. Restaurants, institutions, and hospitals all utilize liquid eggs to meet their needs, and because of this, it is important to look closely at the processes of creating liquid eggs and what measures are used to ensure safety.

Liquid Egg Processing

General Processes

Although shelled table eggs make up a large portion of the egg industry, pasteurized egg products are in high demand. According to the FSIS, approximately 30% of the 76 billion eggs consumed in 2007 were in the form of egg products, or eggs that have been removed from the shell and pasteurized. From the baking industry to specialty products, pasteurized products are developed utilizing whole egg mixtures, as well as separated yolk and albumen components. Many consumers favor these products because of the idea that pasteurization aids in the destruction of harmful bacteria and because they are perceived as "safer" than shelled eggs.

There are several processes that take an egg from its shelled form to packaged liquid product found in grocery stores. One of the first steps is holding. This includes choosing the eggs for breakout. Most breakers have their own flocks used to produced eggs for pasteurization; therefore they are able to utilize eggs produced throughout the life cycle of the hen. It is interesting to note that eggs from older hens may exhibit some of the same quality defects as older or aged eggs. This is important because, as the eggs age, the thick albumen becomes thin and watery and the vitelline membrane of the yolk can deteriorate and potentially pose problems with yolk breakage and contamination of the albumen. For this reason, egg products industry suggests that eggs be held for no longer than 7-10 days at refrigeration temperatures to prevent possible problems in breaking (AEB, 2007). This undesirable characteristic can also be observed in eggs from recycled hens or hens that are reaching the end of their life cycle.

The second step in processing liquid egg products is breaking. This is the point where it is determined if the egg will be separated into yolk and albumen components or if it will stay as a whole egg product throughout pasteurization. In many cases, liquid whole egg is created by combining albumen and yolk at the customer's desired ratio. It is important to note that not all egg processing facilities have their own breaking plants. Some may purchase broken out eggs from other companies, only to pasteurize and package them at their plant. However, it is not uncommon for an egg company to operate both the breaking and pasteurization facility. Before eggs are broken out, they are received at the plant, washed, sanitized, and sometimes candled to remove any eggs with imperfections that would render them unfit for human consumption. After this initial inspection, whole eggs are broken out and homogenized for uniformity. Finally the egg mixture is filtered to remove any residual shell fragments, membranes, and chalazae (AEB, 2007) and chilled before going through thermal pasteurization.

For pasteurization, certain specific times and temperatures must be used to maintain safety. According to the Code of Federal Regulations (Title 9, Volume 2 590.570, 2005) every portion of the product must be heated quickly to the required temperature and held at that respective temperature for at least the minimum holding time. In 2002, a revised edition of the International Egg Pasteurization Manual was published in cooperation with the United Egg Association and American Egg Board. This publication outlines the revised guidelines for the pasteurization of whole egg, as well as separate egg components, based on research in egg safety and quality. A table containing the USDA pasteurization requirements is listed below (Froning, *et al*, 2002).

These time-temperature combinations are effective in destroying *Salmonella* organisms at a level of 10^6 to 10^7 cells per gram of product (Stumbo, 1965).

Liquid Egg Product	Minimum Temperature (°C)	Minimum Holding Time (min)
Albumen (without chemicals)	56.7; 55.6	3.5; 6.2
Whole Egg	60.0	3.5
Whole Egg Blends	61.1; 60.0	3.5; 6.2
Fortified Whole Egg &Blends	62.2; 61.1	3.5; 6.2
Salted Whole Egg	63.3; 62.2	3.5; 6.2
Sugared Whole Egg	61.1; 60.0	3.5; 6.2
Plain Yolk	61.1; 60.0	3.5; 6.2
Sugared Yolk	63.3; 62.2	3.5; 6.2
Salted Yolk	63.3; 62.2	3.5; 6.2

It is important to note that products may be heated to temperatures higher than those listed above and can be held for longer periods of time. However, any lesser deviation from the required times and temperatures will render the product as not fully pasteurized and the egg processing company cannot guarantee safety. All pasteurized egg products are monitored for the presence of *Salmonella* and other microorganisms and are tested regularly by the processor and the FSIS.

Whole Egg Pasteurization

The subject of whole egg pasteurization procedures and their relationship to the destruction of *Salmonella* bacteria has been studied since the early 1940's. In 1946, Stewart *et al* conducted a study in which batches of *Salmonella*-free liquid whole egg

were artificially inoculated with 40,000 to 100,000 *Salmonella* organisms per mL. They wanted to determine which pasteurization procedures had the greatest detrimental effects to the microorganisms. Although at this time SE was not a prevalent species, *Salmonella* Pullorum had originated as the initial *Salmonella* species associated with eggs and egg products. This particular species has been essentially eradicated in today's industry; however, it posed a potential threat to the egg industry at the time of the 1964 study. These researchers discovered that at a temperature of 150°F most species of *Salmonella*, including Pullorum could be destroyed when held for 0.3 minutes, creating a 6-7 log reduction of the test organisms. Studies such as this helped pave the way for future research regarding pasteurization and eggs.

Although the present egg pasteurization guidelines were developed in 1969 to eliminate the threat of *Salmonella* contamination, there have been many proposed changes that challenge the effectiveness of the original guidelines. In 2004, Peters *et al* conducted a series of experiments that they suspected could bring about a change to the guidelines. They studied transovarian infection of eggs by SE, lower pH in egg whites due to rapid transport, and the addition of high amounts of sugar or salt to yolk and whole egg products, and new egg blends that could offer possible protection from SE. Their research had merit, but more research was needed to provide the most comprehensive information.

It is extremely important to maintain the functional properties of liquid whole egg because it is so commonly used as an ingredient in foods products. Studies such as those done by Baldwin (1986) have described how the application of heat can both positively and negatively impact the functionality of liquid whole egg. The challenge for the

industry is striking a balance between ensuring consumer safety and maintaining the functional integrity of the product. Because of this, Dawson and Martinez-Dawson (1998) conducted a study that discussed what effect specific pasteurization times / temperatures had on the functionality of liquid whole egg products. By using Response Surface Analysis (RSA), they were able to show the effects of a range of different pasteurization time- temperature combinations on quality and functional factors of liquid egg. They discovered that when measuring cake height volume, there was more of a time dependence than a temperature dependence. This is possibly due to the fact that when eggs are held at high temperatures for long periods of time, their proteins begin to denature and unfold, making them incapable of trapping the large amounts of air needed to create a greater cake volume. Solids content followed this same trend, being more time dependent. Viscosity, on the other hand, was found to be both time and temperature dependent. This is due to the fact that the proteins began to form a gel, making them more viscous.

Many times, citric acid is added to pasteurized whole egg products. There is little research discussing the reasoning behind this addition and its effect on cooked products in comparison to whole egg without the added citric acid. However, there is an obvious decrease in pH because of the acidic characteristics and one could conclude that this addition combined with pasteurization, could potentially have a positive impact on bacterial elimination. By definition, citric acid is a weak organic acid that is mostly associated with citrus fruits. It is a natural preservative and is often found in candies and soft drinks that require a sour flavor. Though it is widely used in the egg industry, there is not much research on the impact of this additive on the functionality of the liquid

whole eggs. When comparing the addition of citric acid to other acidic chelating agents in egg products, Gossett and Baker (1981) concluded that that although a sensory panel could see a decrease in flavor and color acceptability with other chelating agents, they could not detect a significant difference between the citric acid treatment and the control. This could signify that citric acid is a more reasonable choice for acid addition to egg products because, not only does it not change the flavor or appearance of the product, it is a natural preservative that aids in maintaining the yellow color of the eggs, preventing graying or greening.

Albumen Pasteurization

It is no surprise that the pasteurization of various egg components provides a safer product, essentially free of microbial contamination, to consumers. However, the positive aspects of pasteurization are counteracted with the negative impact it has on functionality, especially in the baking industry. Pasteurized liquid egg whites are used in many pre-packaged products and are sold in liquid form in grocery stores. Often, consumers will buy such products and expect them to perform in the same manner as a shelled egg in their cooked foods. However, this is not always the case.

One function of egg whites in food products is to produce foam. Foam is what is created when air is trapped between the protein matrices when whipping is applied. In shelled eggs, it is important to make sure that the eggs used for this function are produced from young flocks. As hen age increases, egg quality potentially decreases. This can cause the albumen component to become thin and watery, instead of thick and distinct, thus creating a less voluminous foam. This aspect can be detrimental to the baking

industry, with the fact being that eggs used from older flocks may produce cakes with less volume (Cunningham, *et al* 1960).

Water is the primary component of egg albumen (83%), containing protein and only trace amounts of fats and sugars. The main proteins are ovalbumin (54%), conalbumin (13%), ovamucoid (11%), lysozyme (3.5%) which also serves as a natural antimicrobial, globulins (8%), and ovomucin (1.5%) (Tanner, 2007). The protein partially unravels and forms a good foaming agent. A foam is formed by the protein forming a stable film around the included air. Studies show that the best foam is created when the protein is only partially unraveled. Over beating egg whites destabilizes the foam by fully unraveling the protein molecules. The protein is elastic, so when the egg white is cooked, and the air expands, the white stretches then sets in the expanded position. Although albumen is comprised of several different proteins, only a few are responsible for foam formation and stability. According to Cotterill and Winter (1955) the globulins create the actual foam formation, while the ovomucin-lysozyme complex aids in foam stability. Ovalbumin and conalbumin add heat-setting properties.

All proteins, including those in egg white, are made of long chains of amino acids. In a raw egg, these are raveled up in a tangled compact mass. Chemical bonds and interactions between the amino acids within each protein hold this mass in a specific shape and stop it from unraveling. As an egg cooks, the heat causes the bonds within the proteins to break, a process called denaturation. As these proteins strings unfold and entangle with other proteins, new bonds form between these amino acids and the amino acids of neighboring proteins, causing the texture change in a cooked egg. When egg albumen is pasteurized, the proteins it encompasses are partially denatured. When

pasteurized product is used for baking, many of the proteins needed to produce good, stable foam have already been partially destroyed. This, in turn, causes the bakery product to be less voluminous than if it were made with unpasteurized albumen from shelled eggs (Sahi, 2005).

Both dry and liquid egg albumen may sometimes contain additives that aid in foaming capabilities. A common additive is triethyl citrate or a form of citric acid. This is commonly used in pasteurized albumen products that have experienced some protein denaturation due to heat. Another additive is sodium lauryl sulfate, which is added to improve foaming properties. Triethyl citrate is used as a high boiling solvent and plasticizer for vinyl resins and cellulose acetates. It is a plasticizer permitted in the field of food additives, food contact material, medical, and pharmaceutical products. Sodium lauryl sulfate is a detergent and surfactant found in many personal care products (soaps, shampoos, toothpaste, etc.). It is an inexpensive and very effective foamer. These aids are capable of giving the product whipping properties that are comparable to, or even better than, the natural untreated product. The type of additive used is dependent on the type of product being produced. For instance, although not fully understood, triethyl citrate seems to be more preferable in liquid frozen or pasteurized whites, while sodium lauryl sulfate seems to work well with dried albumen.

Functionality

Eggs are very useful food ingredients and they possess a wide range of properties, including foaming, emulsification, gelation, color, and texturization. Egg proteins provide properties in confectionary products such as cakes and meringues, as well as improving the texture of baked goods, quiches, and pasta. According to Hasler (2000),

eggs are one of nature's most perfectly functional foods. Both albumen and yolk components have separate functional effects, and their combined whole egg product plays yet another role, producing different results.

Whole Egg Functionality

Although eggs provide many functional applications, one of the most common is the ability of eggs to coagulate or form a solid when heated (Stadelman, 1999). Unlike carbohydrate gels, which solidify upon cooling, protein gels such as the ones found in egg custard, exhibit the gelation process upon heating. In basic terms, a gel is an intermediate phase between a solid and a liquid. However, technically, it is defined as a substantially diluted system which exhibits no steady state flow. During the process of whole egg gelation, proteins are first denatured into an intermediate gel state, in which the proteins are unfolded to expose functional groups. This progression allows for the formation of the protein bonding network that eventually forms the solid state gel. Protein gels are irreversible, meaning that upon either re-heating or cooling, they will not return to their former liquid state. This is in contrast to gelatin gels, such as those seen in Jello® products, in which applying heat to the solid form will cause bond breakage, resulting in a return to the liquid state.

Another functional property of whole eggs is their ability to be used as both a leavening and humectant agent in bakery products. Researchers often use whole eggs as well as separate albumen components to study their functionality in sponge and angel food cakes. Although more research is needed to determine the functional effects of whole eggs in food products, various experiments have utilized sponge cake volume to determine leavening aspects. In recent studies, it was determined that increasing hen age

demonstrated an increase in sponge cake volume. In theory, the more volume a cake presents represents a better quality egg. Although this finding is in contrast to the hypothesis that eggs of older hens are lower in quality than those of younger birds, the effect on functionality could be due to the change in egg composition in correlation with increasing hen age. As hens age, the ratio of yolk to albumen differs from that of younger birds, showing an increase in yolk size and a decrease and thinning of the albumen. However, more research is needed to determine if this compositional change may explain the results.

Whole egg also contains a yolk portion which is essential in the emulsification of certain egg products such as mayonnaise and hollandaise sauce, salad dressings, cream puffs, and even cake batter containing shortening. Egg yolk, in itself is considered to be an emulsion. Researchers have discovered that the surface active agents in egg yolk help to form a film around oil globules and prevent their coalescence. This process helps to create a continuous phase of the emulsification and therefore a homogenous solution. Separate components of the egg yolk, lecithin and cholesterol also play vital yet opposite roles in emulsification. The compound cholesterol seems to favor a water-in-oil emulsion, whereas lecithin favors an oil-in-water emulsion (Corran and Lewis, 1924). It is important to note that for a stable emulsion, it is necessary to have just the right amount of emulsifier, because an excess will lead to instability.

Albumen Functionality

Proteins are made of long chains of amino acids. The proteins in an egg white are globular proteins, which means that the long protein molecule is twisted and folded and curled up into a more or less spherical shape. A variety of weak chemical bonds keep the

protein curled up tight as it drifts placidly in the water that surrounds it. When you beat raw egg whites to make a soufflé or a meringue, you incorporate air bubbles into the water-protein solution. Adding air bubbles to egg whites unfolds those egg proteins just as certainly as heating them.

Some amino acids are attracted to water; they're hydrophilic, or water-loving. Other amino acids are repelled by water; they're hydrophobic, or water-fearing. Egg-white proteins contain both hydrophilic and hydrophobic amino acids. When the protein is curled up, the hydrophobic amino acids are packed in the center away from the water and the hydrophilic ones are on the outside closer to the water. When an egg protein is up against an air bubble, part of that protein is exposed to air and part is still in water. The protein uncurls so that its water-loving parts can be immersed in the water, and its water-fearing parts can stick into the air. Once the proteins uncurl, they bond with each other, just as they did when heated, creating a network that can hold the air in place.

The foaming properties of albumen have long been praised for their role in confectionary functionality. Often, egg white foams are associated with soufflés, meringues, divinity, and angel food cakes. Because of its low fat content, angel food cake is frequently observed as being a healthier alternative to satisfying a person's sweet tooth. One of the most noticeable factors of this product is its light, airy texture, and its ability to produce a pleasantly sweet taste. The lightness of texture is due to the trapping of air by the proteins in the process mentioned above, while the sweet taste is due to the addition of sugar into the batter. However, this addition does more than just provide a delicious flavor. If added properly, it will help stabilize the foam. If sugar is added before foam formation occurs, it slows the denaturation of the proteins because it

competes with the other molecules for access to water. This does not allow the proteins to trap the air and create a stable foam. However, if the sugar is added to the mixture after the foam is formed, it helps to generate stability, by adding a touch of viscosity between the trapped air bubbles. If done properly, this should produce a stable foam with less weep.

Although the addition of sugar is one way to stabilize egg white foam, the addition of an acid, such as cream of tartar will also produce a stabilizing effect. The addition of acid in this form lowers the pH while increasing stability (Bailey, 1935; Barmore, 1934). The acidic supplement alters the net charge on the proteins, bringing them closer to the isoelectric point, and thus reducing the electrostatic repulsions. Because of this, the proteins pack more closely at the air-liquid interface, creating a more viscous foam (Oldham, *et al*, 2000). As previously mentioned, the globulins found in albumen are responsible for foam formation, with the ovomucin-lysozyme complex contributing to foam stability (Cotterill and Winter, 1955). It has also been revealed that ovomucin is the protein complex that is responsible for foam stability, while in 1981, Johnson and Zabik stated that conalbumin, lysozyme, ovomucin, or ovomucoid alone have very little or no ability to form a foam, but that the interactions of these play a vital role in the foaming process.

Proximate Analysis

Moisture

Taking measurements such as total solids analysis and moisture content of foods is imperative to the success of the foods in the industry. Moisture content can be a wonderful asset or a detrimental aspect of food products. If the moisture content is too

high, many adverse side effects, such as the unwanted growth of harmful bacteria may occur. However, if the moisture content is too low, you may have problems with staleness and poor mouthfeel.

There are three main forms of water in foods. Free water exists as free water within the intergranular spaces and pores of the food, and has properties that are similar to pure water. This type of water acts as a dispersing agent for colloids and the solvent for salts. Absorbed water, on the other hand, is water that is absorbed as a mono or poly molecular layer on the internal and external surfaces of the solid components by molecular forces. Finally, water of hydration is water that is chemically bound, such as lactose monohydrate. It can also include some salts.

Water is the most abundant compound in eggs. It comprises approximately 78% of whole egg composition and 88% of albumen composition. Analysis of this component of shell eggs can give detailed information about the amount of residual solids left in a sample after moisture extraction. With this knowledge, researchers can determine what impact bird strain and age has on solids content, and can therefore make suggestions to the egg industry as to what strains, ages, or combinations would best fit specific product needs. In a study done by Ahn *et al* (1997), utilizing hens of four different commercial strains of similar ages and fed consistent diets, eggs were collected, pooled, homogenized, and tested for differences in solid content in whole egg, yolk, and white components. It was determined that the total solids content of the whole egg component increased with hen age. The solids content of the albumen component was greatest in the younger birds (28 weeks of age), while the yolk: white ratio of eggs from hens ages 55-75 weeks was highest. Eggs from hens 97 weeks of age performed intermediately in all

categories. The results of this study concluded that both young and old birds produced eggs with low solids content, while middle-aged hens produced eggs with high solids content. They determined that that it may be more beneficial for processors and producers to utilize young and old hens for table egg production and birds of intermediate age for liquid egg production.

The primary method of moisture extraction involves the use of a heat source and time to draw out any unwanted liquid. Drying ovens provide good moisture removal in a controlled environment. Many scientific supply companies are manufacturing sample ovens which can be set to a specified temperature, and close tightly to ensure that no heat is lost or no moisture is allowed in. In experiments involving eggs and egg products, ovens are tempered to 60°C and samples are dried for a period of 24 hours. This ensures that no moisture, especially in the thick yolk component, is left in the sample.

Fat

The egg has often been considered one of nature's most perfect foods. It is the only system, outside of mammalian, which can support the self contained growth of an embryo. It provides a means of respiration, a high concentration of nutrients, as well as protection. However, eggs as a food source received a poor reputation several years ago because of their moderate fat and cholesterol content. Although consumer perception caused a decline in the 1980s and 1990s of the average number of table eggs consumed, the tide is now changing and eggs have fallen back into the public's good graces. This can partially be contributed to the fact that experimental research has shown what food scientists knew all along; that eggs have a high nutritional value, contain all 9 essential amino acids, and are low in both calories and saturated fat.

The average amount of total fat in a large egg whole egg (approximately 60 grams) is approximately 5 grams. However, the total saturated fat, the kind that has been linked to a higher serum cholesterol level, is only 1.6 grams. In albumen, the total fat is lowered to virtually 0 grams, with only trace amounts of lipids present. Eggs also contain less than 213 mg of cholesterol per egg, making eating an egg a day perfectly fine. The protein: lipid ratio in eggs is the highest of any naturally occurring food, and according to the American Heart Association, the amount of total fat in eggs is insignificant, and they are large part of many weight reducing diets. He also stated that high levels of serum cholesterol and a high risk of coronary heart disease have been correlated only in individuals who inherit high cholesterol levels from both parents.

Ash

The term "ash", with regards to food product analysis, refers to the inorganic residue that remains after total incineration of the organic matter in a particular food product. This method of analysis is important to food analysis because it is a good general indicator of product quality, can help to determine the type and distribution of minerals within plant products, can give an examination of food adulteration, and can also be indicative of the nutritional value of the food.

The ash content of food can and will vary within the foods selected for analysis. For example, the freshest foods will rarely have greater than 5% ash. Pure oils and fats will contain little or no ash, however dried meat products, such as dried beef can contain up to 11% ash on a wet weight basis. Foods that are mostly water, such as pasteurized egg products, will only contain approximately 1% ash.

Protein

Proteins are abundant in all cells and are important for specific biological functions and cellular structure. Amino acids, the building blocks of proteins, are linked together by peptide bonds. Proteins are composed of key elements, including hydrogen, carbon, oxygen, nitrogen, and sulfur, with nitrogen being the most characteristic feature of a protein's chemical structure. In this sense, protein analysis, and more specifically, nitrogen determination, is important in food analysis. However, protein analysis can be quite tricky because some food components possess similar physiochemical characteristics. For example, phospholipids, nucleic acids, and amino acids may contribute to non-protein nitrogen, which could cause incorrect measurements in the total protein content.

Ranges

Before performing any method of proximate analysis, it is important to understand what range of values is expected for eggs as to compare your results with the published data. For example, liquid or frozen albumen should contain approximately 12.1 grams solids, 10.2 grams protein, little to no lipids, and 0.68 grams ash per a 100 gram sample. Along the same lines as albumen, liquid or frozen whole egg should contain approximately 24.5 grams total solids, 12.0 grams protein, 10.9 grams total lipids, and 1.00 grams ash per a 100 gram sample.

Summary

There are many key components to helping the egg industry answer "how" and "why" questions. Although many studies have been conducted in the area of whipping performance and functionality, the problem continues to plague the industry. Factors

such as pressure, yolk contamination, freshness and processing temperatures negatively affect foaming properties. It is known that phosvitin and lipovitelline phospholipids wreak havoc whipping performance. This is a vital reason to keep unwanted elements out of egg whites. By comparing the whipping height data of unprocessed eggs to that of pasteurized albumen, conclusions may be drawn pertaining to foam stability and whipping peak height. Furthermore, findings suggest that as hen age increases, foaming quality and stability decreases. It is necessary to find a way to keep consistency at a maximum and utilize eggs rendered to the breaker. If eggs from young and old flocks are blended, it is possible to maintain an acceptable foam height and stability. Produced outcomes should include added angel food cake volumes and taller, more structured meringues. With further research investigators can help bridge the gap between information previously gained and that yet to be discovered.

The use of proximate analysis to calibrate cutting edge equipment is essential in the proper performance of such machines. Drying of the liquid portions creates the solid forms of albumen, whole egg, and yolk. As albumen is heated, for example, the protein is denatured building a solid matrix. When egg whites are added to tender products, it renders them easier to package and more convenient and palatable for the consumer. Comparisons of data show evidence of significant differences between unprocessed and pasteurized eggs. It is widely known that pasteurization will negatively impact the proteins in both whole egg and albumen that contribute creating and stabilizing foams. Although this was once thought to be detrimental to the baking industry, the utilization of certain additives such as sodium lauryl sulfate and triethyl citrate are heralded with aiding albumen products perform almost better than before they were treated. Although

whipping agent additives are not commonly added to whole egg products, the use of citric acid in pasteurized whole egg will aid in the preservation of the product, but further research is needed to determine what effects this additive has on the functionality of pasteurized whole egg. Further inspection of these observations will pinpoint where the disparities occur.

As previously mentioned, further investigation is needed to create fundamental links to pertinent industry questions. Because even trace amounts of yolk contamination can be detrimental to many aspects of functionality, it is imperative to determine rapid and accurate methods to determine the slightest amounts. Future funding will play a critical role in problem solving, not only for researchers, but for the egg industry as well.

Pasteurization is used as a way to create a seemingly sterile egg product, free from pathogens, which consumers view as safe. It is important to determine how these pathogen-free products perform functionally in food products. Does pasteurization have an effect on the performance of whole egg both with and without the addition of citric acid? How does this compare with the performance of unpasteurized product? Is the chemical make-up the same or altered? Do the same factors hold true for albumen products both with and without an added whipping agent? Each of these questions is pertinent to the egg industry. A look the different chemical and functional aspects of egg products, as well as related microbial testing may provide the needed answers.

MANUSCRIPT I

INFLUENCE OF HEN AGE ON SHELL EGG EXTERIOR, INTERIOR, AND CONTENTS MICROFLORA AND SALMONELLA PREVALENCE DURING A SINGLE PRODUCTION CYCLE

ABSTRACT

Salmonella Enteritidis (**SE**) infection in humans has been chiefly attributed to the consumption of raw or undercooked eggs or egg products. The objective of this study was to determine if increasing hen age influenced the total microflora counts or the prevalence of *Salmonella* spp. on the egg shell surface, within the shell, or in the contents. Eggs from Hy-Line W-98 and Bovans White layer strains were sampled approximately every 28 d from 17 to 66 wk of age from the 35th North Carolina Layer Performance and Management (NCLP&M) Test. The layers were managed under identical husbandry practices. This study utilized 45 eggs per hen population for a total of 90 eggs per period. Five replicates from each strain were represented within the samples, with the same hens being sampled each period. Pooled sets of 9 eggs were used for each strain. The exterior, interior shell, and contents were enumerated to obtain total aerobic counts and tested for the prevalence of SE.

There were 120 total egg pools taken, and, of these, there was one positive *Salmonella* sample for exterior and one for interior. For this study, essentially all eggs produced by the two strains during the 35th NCLP&M test were tested. Sampling of the egg began with rinsing the exterior shell to collect any microorganisms that may have

adhered to the surface. The pooled rinsate accounted for the exterior counts. The sampling of the interior shell included the shell membranes and matrix, as well as the sterilized exterior portion of the shell. Contents sampling included both the yolk and albumen components, as well as the chalazae. Hen age significantly ($P < 0.05$) affected the microbial loads on the egg components tested. Exterior counts increased in period 4. Interior counts were erratic ($P < 0.05$), increasing 2 log units over the 12-mo cycle. Contents data were not significantly different ($P < 0.05$) until period 12, when microbial loads increased from 0 to 1 log unit.

INTRODUCTION

Microbial contamination of the hen's egg is an ongoing concern for consumers and public health officials. While the egg is naturally equipped with barriers that help keep microorganisms from penetrating into the interior shell and contents, it has been shown that these barriers can fail. In 1964, Lifshitz and Baker determined that because of its density, the inner shell membrane is a greater defense mechanism than the exterior membrane, rather than because of its chemical properties. While these scientists determined that the outer membrane was thicker, heavier, and denser than the inner membrane, it proved to be an ineffective barrier against microbial penetration. Lifshitz and Baker (1964) also concluded that the outer membrane is composed of a network of course fibers that would allow organisms through easily, while the inner membrane is composed of smaller fibers creating a compact network, resulting in an effectively smaller pore size. This study did not, however, do any microbial analysis to support their claim and looked only at the physical properties of the egg. Board (1966) concluded that the shell was most susceptible to microbial penetration within a very short time after lay,

based on the assumption that the yolk and albumen contract during cooling, causing organisms to be sucked through the pores. However, he did not answer the question of whether or not the contents were pathogen-free at the time of lay.

It has been known that the yolk of shell eggs is a wonderful growth medium for certain bacteria, and *Salmonella* is at the forefront of concern. The *Salmonella* serotype Enteritidis (**SE**) has been commonly associated with eggs. Therefore understanding the mechanisms used by these organisms to penetrate the shell and colonize contents is extremely important. Nascimento (1990) found that the cuticular layer is rarely a uniform, thick covering and that the integrity of the external and internal membranes is questionable when bacterial loads are high. It was determined that the relationship between shell structure and the movement of SE across the eggshell wall was important. It was concluded that as shell quality decreases with hen age, a significant increase in bacterial penetration was observed that coincided with the occurrence of pitted areas, and was significantly correlated with the penetration. The alignment of the mammillae was also found to significantly correlate. It was concluded that no one factor could be singled out as a causative agent for bacterial invasion, but rather the structure of the shell membranes as a whole played a combined role and that higher quality shell would be less susceptible to infection. However, Nascimento et al. (1992) showed that a thick and even cuticular layer plugs the gas exchange pores without reducing their efficiency, and a multi-layered shell that is firmly attached to the underlying intact shell membranes was essential for the egg to effectively deter pathogens from penetrating the shell. In 2005, Jones and Musgrove examined clean eggs from an in-line operation which they inoculated by dipping into a solution of nalidixic-resistant SE, then measured for shell

strength and external and contents contamination. They showed only a weak negative correlation (r = -0.13) between external contamination of SE and shell strength, and no significant correlation was found between SE contamination in the contents and shell strength.

MATERIALS AND METHODS

Eggs from two commercial layer strains, Hy-Line W-98 (**HL**) and Bovans White (**BW**) were used as the source for monitoring the microbial population on and in shell eggs. The hens were managed under the guidelines outlined in the 35th North Carolina Layer Performance and Management **(NCLP&M)** Test (Anderson, 2005), under identical husbandry practices. Every 28 d, the previous 24 h egg production was collected from specific replicates, by staff wearing laboratory gloves, hairnets, and jumpsuits, to eliminate potential contamination by human hands, then placed in clean paper pulp flats and packed in a sterile plastic bag and then placed into a 30 dozen case. The eggs were stored in a 4˚C walk-in cooler until they could be picked up from the facility by an Auburn University technician the same day, and brought back to the laboratory where they were stored overnight in a 4°C cooler before undergoing microbiological analysis the following day.

Eggs for this experiment were collected from 3 replicates representing the HL and BW strains, with 20 eggs collected from each of the replicates. This was done to compensate for any eggs which may have become cracked during transport to the laboratory. Fifteen eggs from each replicate (45 eggs per respective strain) were randomized and the set of 45 eggs was broken down into groups of 9 egg pools for a total of 5 pooled sets per strain. Each individual egg was then placed into a small, sterile

rinsate bag with the addition of 25 mL of Buffered Peptone Water (**BPW;** Neogen Corp., Acumedia, Lansing, MI). The individual bags were then closed and shaken vigorously by hand for 1 min to ensure that the exterior shells were rinsed thoroughly. Rinsates were then pooled into another sterile bag with 9 rinses per pool. A small amount of rinsate was placed into sterile spiral plater cups and plated onto Plate Count Agar (**PCA;** Neogen Corp.) using a WASP spiral plater (Microbiology International, Frederick, MD). The additional rinsate was incubated at 37°C for 24 h for further *Salmonella* testing.

The eggs were then removed from the original rinsate bag with sterile tongs and flamed by dipping in 70% ethanol and passing through a Bunsen burner. Eggs were cracked onto sterile foil and the contents (both yolk and albumen) placed into a sterile stomacher bag, while the shells were placed into a sterile filter bag and gently crushed by hand. Pooled sets of 9 contents and shells were used just as with the exterior rinsates described above. The contents were placed in a stomacher bag and then homogenized in a Seward® 400 Circulator stomacher (Seward, Norfolk, UK) at 200 rpm for 1 min, and 10 mL was extracted and placed into another sterile stomacher bag with the addition of 90 mL of BPW to make a 1:10 dilution. The 100 mL of contents / BPW was then homogenized in a stomacher for 1 min at 200 rpm. A small amount of the contents was placed into a sterile spiral plater cup to be spiral plated onto PCA. The remaining mixture was placed into an incubator at 37°C for 24 h for further *Samonella* testing.

The bag containing the shells included the addition of 90 mL of BPW and was shaken by hand for 1 min to ensure proper washing of the interior shell and membranes. A small amount of this rinse was also placed into a sterile spiral plate cup for spiral plating on PCA and the remaining rinse was incubated for 24 h at 37°C incubator for

Salmonella testing. All spiral plated PCA plates from each of the three egg components (exterior rinse, contents, and interior rinse) were incubated at 37°C for 24 h to determine total aerobic counts.

After a 24 h incubation period, each set of 9 pooled rinses or contents for each of the three components was removed from the incubator and shaken by hand for one min to homogenize. Then 0.1 mL was removed from each bag, and placed into 9.9 mL of Rappaport-Vassiliadis R10 (**RV;** Neogen Corp) enrichment broth. Once inoculated, the RV tubes were then placed into a 42°C incubator for 24 h. The remaining egg rinses and contents were then discarded. The PCA plates that were inoculated the day before were removed from the incubator and read using a QCount® plate counter (Spiral Biotech, Inc., Norwood, MA), using the spiral plate function.

The RV tubes were removed from the 42°C incubator after 24 h and vortexed to ensure proper mixing. A 0.1 mL bioloop of inoculum was removed from the tube and streaked for isolation onto Brilliant Green Sulfapyridine agar (**BGS;** Neogen Corp.). Plates were then placed into a 37°C incubator for 24 h for the detection of *Salmonella* and *Salmonella*-like organisms. Positive colonies were indicated by a bright pink coloration of the media or individual colonies. If positive colonies were detected, they were transferred by the stab and streak method to Triple Sugar Iron agar (**TSI;** Neogen Corp.) and Lysine Iron agar (**LIA;** Neogen Corp.) for biochemical confirmation. A positive biochemical test was indicated by a red slant and yellow to yellow-black butt (bottom portion of the tube) for TSI and a purple slant and purple to purple-black butt for LIA. Positive samples on both TSI and LIA were then transferred to cryovials, with the

addition of 1 mL of Tryptic Soy Broth (**TSB;** Neogen Corp.) with 30% sterile glycerol for future analysis.

Upon completion of the 12-mo study, cryovialed samples were tested for *Salmonella* confirmation using latex agglutination. Samples were removed from the freezer and allowed to thaw at room temperature with 0.5 ml being extracted and placed into 9.5 ml TSB as a growth medium. After a 24 h incubation at 37°C, 1ml of sample was placed into 9 ml of TSB for another growth cycle. After an additional 24 h inclubation was complete, 0.1 ml of sample was transferred to PCA and stored at 37°C for 24 h. Individual colonies from the PCA were streaked for isolation onto BGS agar to ensure that the sample was positive for *Salmonella*-like species and that no contamination had occurred during storage, and incubated for 24 h at 37°C. Samples were then tested using Salmonella O antisera (BD Difco, Rockville, MD) and AntiserumVi groups Band D (BD Difco, Rockville, MD). Individual colonies from the non-selective media were placed onto disposable agglutination sheets, with the addition of the antisera. Both positive and negative controls were used to verify each sample. A clinical rotator (Fisher Scientific, Pittsburg, PA) was used to aid in the agglutination process, as the samples must be gently swirled for 1-2 min. Positive agglutinations were confirmed by the observance of coagulated areas within the sample. Negative agglutinations showed no signs of coagulation and the sample retained its initial integrity.

Statistical Analysis

The experimental design of this study was a 2 X 12 factorial arrangement of a completely randomized design, representing the two hen strains (Hy-Line W-98 and Bovans White) and the 12 mo study period. Plate counts were converted to base-10

logarithm values and subjected analysis of variance using PROC GLM and Fisher Protected LSD in SAS (SAS Institute, Cary, NC). Frequencies were obtained using the PROC FREQ of SAS. The values in Figure 1 represent the average of the 2 strains and replicates for a given period. Results indicating a zero count in bacteria were set at the lowest detectable limit of 10 cfu / mL, while results indicating a too numerous to count (TNTC) measurement were set to a limit that was 1 log unit higher than the highest detectable plate count (100,000 cfu / mL).

RESULTS AND DISCUSSION

As shown in Figure 1, hen age significantly ($P < 0.05$) affected the microbial loads on each of the egg components tested, however hen strain did not have a significant ($P > 0.05$) impact on bacterial counts. Interior shell APC counts were erratic ($P < 0.05$) and increased as much as 2 log units at points during the 12-mo period. This is evident by the decrease in interior shell counts in period 2, coupled with their highest peaks in periods 8, 10, and 12. Content counts remained minimal throughout the study period with only a slight increase in periods 5, 6, and 12. This may be attributed to a condition of Osteomalacia found in the laying flock during period 4, which may explain the slight increase in the exterior shell counts and some of the variability early in the experiment. However, the birds were treated and egg production returned to normal by period 5.

The frequency of *Salmonella*-positive samples, shown in Table 1, were low and are accounted for by a positive sample from the exterior shell in period 1, and a positive sample from the interior shell in period 6. Contents showed no positive *Salmonella* samples throughout the course of the 12 mo study. Each of these positive samples were determined to be so by testing on *Salmonella*-selective BGS agar and TSI and LIA

biochemical tests, followed by latex agglutination. The incidence of *Salmonella*-positives in either the interior shell or the exterior shell was 0.83% for 120 pooled sets. Both *Salmonella*-positive samples occurred in the Hy-Line W-98 layer strain.

Based on these findings, one can conclude that there is a relationship between the mechanical properties of the inner shell membrane and the contents of the shell egg being free of pathogens. Since no positive samples were discovered for the egg contents throughout the 12-mo study, it appears as though the shell's physical barriers and complex network of shell membrane fibers are effective against bacteria entering the contents. In essence, the interior membranes are able to trap the microorganisms before they reach the nutrient dense yolk. It is also possible that certain natural antimicrobials, such as lysozyme also play a key role in reducing the number of bacteria found in the albumen. One could also infer that the hens in the flock were negative for *Salmonella* colonization, since there was no transovarian deposition of *Salmonella* into the contents.

However, *Salmonella* samples were found on both the exterior and interior portions of the shell egg. The positive sample obtained on the exterior shell could possibly be explained by the egg coming into contact with contaminated material in the external environment. It is not uncommon for the bacterial colonies found in fecal material to adhere to the exterior portion of the shell and survive there. It is also possible that this could account for the positive sample that was obtained in the interior shell. One theory is that the yolk and albumen contract shortly after lay, causing microorganisms to be pulled through the pores of the shell (Board, 1966). If this is correct, one can assume that bacteria on the exterior shell could have theoretically moved into the inner shell

membranes and matrix. Also, since *Salmonella* bacteria are equipped with flagella, they have the ability to move to areas which are more conducive to survival.

Although it has been proven that as hen age increases, shell strength may become compromised, we did not find this to contribute to *Salmonella* contamination in our study. The positive samples occurred in periods 1 and 6, while no positive samples were found toward the end of the research.

Conclusion

In summary, the findings of this study suggest that environmental contamination in this facility may be more prevalent than colonization in the hens. More research is needed to determine whether the shell membranes or the antimicrobials in the albumen play the primary role in protecting the egg contents from environmentally transferred microbial contamination.

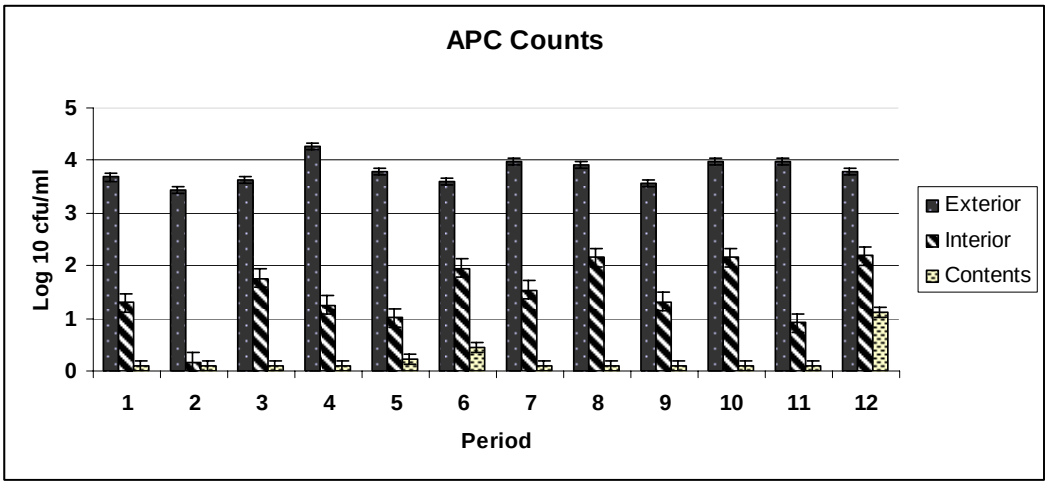
ACKNOWLEDGEMENTS

The authors would like to thank the North Carolina State University Piedmont Research Station staff for rearing the laying hens and helping to collect the eggs utilized in this study, the Auburn University Egg Quality Lab for their support and assistance, and the Poultry Products Safety and Quality Peaks of Excellence Program for funding this research.

Table 1. Frequency of *Salmonella* positives by strain, hen age, and sampling location

Hen Age	W- 98			Bovans White		
	Exterior Shell	Interior Shell	Egg Contents	Exterior Shell	Interior Shell	Egg Contents
20 wk	1/5	0/5	0/5	0/5	0/5	0/5
24	0/5	0/5	0/5	0/5	0/5	0/5
28	0/5	0/5	0/5	0/5	0/5	0/5
32	0/5	0/5	0/5	0/5	0/5	0/5
36	0/5	0/5	0/5	0/5	0/5	0/5
40	0/5	1/5	0/5	0/5	0/5	0/5
44	0/5	0/5	0/5	0/5	0/5	0/5
48	0/5	0/5	0/5	0/5	0/5	0/5
52	0/5	0/5	0/5	0/5	0/5	0/5
56	0/5	0/5	0/5	0/5	0/5	0/5
60	0/5	0/5	0/5	0/5	0/5	0/5
64	0/5	0/5	0/5	0/5	0/5	0/5

Figure 1. Aerobic plate counts of the exterior shell, interior shell, and contents.



MANUSCRIPT II

INFLUENCE OF HEN AGE AND THREE MOLTING TREATMENTS ON SHELL EGG EXTERIOR, INTERIOR, AND CONTENTS MICROFLORA AND SALMONELLA PREVALENCE DURING A SECOND PRODUCTION CYCLE

ABSTRACT

Salmonellosis is one of the most frequently reported foodborne illnesses in the world, and *Salmonella* Enteritidis (**SE**) is the serotype most associated with eggs. The objective of this study was to determine if increasing hen age and three different molting treatments influenced the total microflora counts or the prevalence of *Salmonella* spp. on the exterior of the egg shell, within the interior shell, or in the contents. Eggs from Hy-Line W-98 and Bovans White layer strains were sampled approximately every 28 d from 70 to 114 wk of age, with the molting period from 66 to 70 wk of age. Layers were utilized from the 35th North Carolina Layer Performance and Management Test (**NCLP&MT**), and managed under identical husbandry practices. This study consisted of non-fasted, non-molted, and feed restricted treatments with the use of 135 eggs per layer strain, for a total of 270 eggs sampled per period. The exterior, interior shell, and contents were spiral plated onto Plate Count agar (**PCA**) to calculate the total aerobic counts. Additional pre-enrichment, enrichment, conformational, and biochemical procedures were performed to test for the presence of *Salmonella* spp. Hen age and molting treatment significantly ($P<0.05$) affected the microbial loads on all three egg components.

Exterior, interior, yolk, and albumen counts increased during the molt period to as much as 1 log unit higher than the highest countable plate, which was $10^{5.}$ Exterior, interior, and contents counts rose ($P<0.05$) during period 15, with an increase ($P<0.05$) in the interior also in period 14, and in the contents in periods 14 and 17. There were a total of 360 egg pools and of those, 4 were positive *Salmonella* samples. Both the interior and exterior shell components and 2 of the 3 molting treatments had positive samples. Of these positives, 4 were confirmed as *Salmonella braenderup*. Three positives were associated with the interior component, while 1 positive was associated with the exterior shell component. Three of the 4 samples were related to the non-fasted treatment, while the remaining positive was found in the non-molted treatment.

INTRODUCTION

The relationship between the use of different molting procedures in laying hen flocks and *Salmonella* Enterica serovar *enteritidis* (**SE**) has been a topic of debate for many years. Although molting is an important economic tool for the egg industry, some may argue that it places undue stress on the flock, suppressing the immune system and creating an environment suitable for SE growth. In 2003, Holt reported that in a simulated flock situation, molted hens excreted higher SE numbers in feces, had increased counts in the internal organs, and were observed as having more intestinal inflammation. He also discovered that the hens in this study were 100- to 1000-fold more susceptible to SE organisms, making them potential vectors for transmission to uninfected birds in neighboring cages. In a study conducted by Butcher and Miles (1994), it was determined that no matter what factors are involved

in causing increased susceptibility to SE infection, laying hens subjected to a forced molt by feed removal are being stressed and are more likely to shed *Salmonella* bacteria as compared to non-molted hens.

Molting is a natural physiological process that occurs in chickens, fowl, and other avian species, which produces a cessation of egg production and permits the reproductive tract and plumage time to regenerate. According to Park *et al* (2004), this gives hens time to recuperate from a long laying cycle while causing a state of reproductive quiescence, leading to a second cycle of eggs. In 1994, Butcher and Miles reported that with proper procedures, it is possible to achieve a productive life from 70 to 105 wk of age and for an additional 25 to 30 wk if a second molt is used. This is important because laying hens are bred specifically for egg production and the intent of the egg industry is to make a profit. However, although increased egg production is a primary focus, the welfare of the birds is also of great importance (Anderson and Koelkebeck, 2007).

There are several methods used to induce birds into a molt. During a conventional induced molt, feed is removed or significantly limited, thereby inducing molt. Although there has been debate as to which method of induced molting will attain the greatest production rate and egg quality, the traditional induced molt procedure is still the comparative basis used, however, many poultry companies, to meet animal welfare guidelines, are now using non-fasting programs. It has also been reported that the removal of specific nutrients such as sodium (Whitehead and Shannon, 1974), iodine (Arrington *et al*, 1967), calcium (Gilbert and Blair, 1975), or zinc (Creger and Scott, 1977) will induce hens into a molt. In 1962, Bell and Siller

conducted an experiment which revealed that hens fed a calcium deficient diet of less than 0.3% would cease egg production within 7-10 d. Approaches that do not use complete feed withdrawal are becoming more desirable. According to Biggs *et al*, 2003, non-feed removal techniques are not less effective in recovering post-molt egg production. These methods may include feeding diets high in corn gluten, wheat middlings, or a combination of corn and wheat. These results appear to suggest that alternative molting methods may be more animal welfare friendly than the accepted feed withdrawal regimens. However, Berry (2003) reported that these unconventional practices have not proven to be as effective in ceasing egg production and could potentially cause a decrease in lay without allowing a full rest period. This could mean that the flock would not reach its full post-molt production potential once the molt has concluded. Although it has been noted that an induced molting regimen shows higher incidences of SE (Holt and Porter, 1992), it is important to mention that many experiments used to study the nature of SE in laying flocks use inoculated hens as their test subjects. Anderson *et al* (2001) questioned that because of the fact that it was difficult to determine if the results obtained in such studies were genuine reflections of the industry, or simply due to high levels of inoculum or commercial settings and breeds.

MATERIALS AND METHODS

Eggs from two commercial layer strains, Hy-Line W-98 (**HL**) and Bovans White (**BW**) were used as the source for monitoring the microbial population on an in shell eggs. The hens were managed under the guidelines outlined in the 35th North Carolina Layer Performance and Management Test (**NCLP&MT**) (Anderson, 2005),

under identical husbandry practices. Each period, on day 28, the previous 24 h of egg production was collected from five specific replicates of the utilized hen strains, by staff wearing laboratory gloves, hairnets, and jumpsuits, to eliminate potential human contamination, then placed in sterile plastic flats, packed in clean plastic bags, and positioned into a 30 dozen case. The eggs were stored in a 4°C walk-in cooler until they were removed from the Piedmont, North Carolina facility by an Auburn University technician on the day of collection, and transported to the Auburn University Poultry Science Building where they were stored overnight in a 4°C walk-in cooler before undergoing microbiological analyses the following day.

The eggs for this experiment were collected from five replicates from the HL and BW strains. A minimum of twelve eggs were collected from each of the 5 replicates-strain combinations. In order to ensure the minimum number of sound uncracked eggs, more eggs were collected than needed for analysis to compensate for any eggs that may have become cracked during transport to the laboratory. This was accomplished by hand candling to ensure all shells were intact upon arrival. During the course of this 12-mo experiment, three molting treatments, consisting of non-fasted (**NF**), non-molted (**NM control**), and feed restricted (**FR**) groups were utilized as described by Anderson and Havenstein (2007). Forty-five eggs from each replicate / strain / molt combination (135 eggs per respective strain) were randomized and the set of eggs was separated into groups of 9 egg pools for a total of 15 pooled sets per strain. The microbiological procedures used for this study were obtained from the USDA Food Safety and Inspection Service Laboratory Guidebook (2004). Each individual egg was then placed into a small sterile rinsate bag with the addition

of 25 mL of Buffered Peptone Water (**BPW**; Neogen Corp., Lansing, MI). The individual bags were then closed and shaken vigorously by hand for 1 min to ensure that the exterior shells were rinsed thoroughly. Rinsates from individual eggs were then pooled into another sterile bag with a total of 9 rinsates per pool. A small amount of the pooled rinsate was placed into sterile spiral plater cups and 50 µl plated onto Plate Count agar (**PCA**; Neogen Corp.) using a WASP spiral plater (Microbiology International, Frederick, MD). The additional rinsate was incubated at 37°C for 24 h for further *Salmonella* testing.

The eggs were then removed from the original rinsate bag with sterile tongs and flamed by dipping in 70% ethanol and passing through a Bunsen burner. With the exception of period 13 (molt period), eggs were cracked onto sterile foil and the contents (both yolk and albumen) were placed into a sterile stomacher bag, while the shells were placed into a sterile filter bag and gently crushed by hand. However, during period 13 the contents were separated into yolk and albumen pools to determine if microbial counts differed between yolk and albumen and what possible *Salmonella* organisms could be found in the separated components as opposed to the contents as a whole. The yolks were rolled on towels to remove any adhered albumen and each component was placed into a separate sterile bag. The results indicated that there was no significant difference ($P>0.05$) between the microbial counts of the separated components, so yolk and albumen contents were analyzed together as whole egg for the remaining 11 periods. Beginning in period 14 (one month post-molt), the contents were placed in a stomacher bag and homogenized in a Seward® 400 Circulator stomacher (Seward, Norfolk, UK) at 200 rpm for 1 min, and 10 mL of

contents was extracted and placed into another sterile stomacher bag with the addition of 90 mL of BPW to create a 1:10 dilution. The 100 mL of contents / BPW was then homogenized in the stomacher for an additional 1 min at 200 rpm. A small amount of the contents was placed into a sterile spiral plater cup to be spiral plated onto PCA. The remaining mixture was placed into an incubator at 37°C for 24 h for further *Salmonella* testing.

The filter bag containing the shells was gently crushed by hand and then 90 mL of BPW and the shells were shaken by hand for 1 min to ensure proper washing of the interior shells and membranes. A small amount of this rinsate was also placed into a sterile spiral plater cup for spiral plating onto PCA and the remaining rinsate was incubated for 24 h at 37°C for the first step in the enrichment process for *Salmonella* testing. All spiral plated PCA plates from each of the three egg components (exterior rinse, interior rinse, and contents, with the addition of separate yolk and albumen plates from period 13) were incubated at 37°C for 24 h to determine total aerobic counts.

After a 24 h incubation period, each set of 9 pooled exterior and interior rinses or contents for each of the components was removed from the incubator and shaken by hand for 1 min to homogenize. Then, 0.1 mL was removed from each bag and placed into 9.9 mL of Rappaport-Vassiliadis R10 (**RV**; Neogen Corp) enrichment broth. Simultaneously, 0.5 mL was also removed from the incubated rinsate bag and placed into 9.5 mL of Tetrathionate-Hajna (**TT**; Neogen Corp.) enrichment broth. Both the RV and TT tubes were placed into a 42°C incubator for 24 h. The remaining egg rinses and contents were then discarded. The PCA plates that were

inoculated the previous day were removed from the incubator and read using a QCount® plate counter (Spiral Biotech, Inc., Norwood, MA), using the spiral plate function.

The RV and TT tubes were removed from the incubator after 24 h and vortexed to ensure homogeneity. A 0.1 mL bioloop of inoculum was then removed from the tube and streaked for isolation onto Brilliant Green Sulfapyradine agar (**BGS**; Neogen Corp.) and Modified Lysine Iron agar (**mLIA**; Noegen Corp.). The plates were then placed into a 37°C incubator for 24 h for the detection of *Salmonella* and *Salmonella*- like organisms. Positive colonies were indicated by bright pink coloration of the media or colonies on BGS agar and purple to purple-black colonies on the mLIA agar. If positive colonies were detected, they were transferred by the stab and streak method to Triple Sugar Iron agar (**TSI**; Neogen Corp.) and Lysine Iron agar (**LIA**; Neogen Corp.) for biochemical conformation. A positive biochemical test was indicated by a red slant and yellow to yellow-black butt (bottom portion of the tube) for TSI and a purple slant and purple to purple-black butt for LIA. Samples that were positive on both TSI and LIA were then transferred to cryovials by removing colonies with a sterile loop, which contained 1 mL of Tryptic Soy Broth (**TSB**; Neogen Corp.), with 30% sterile glycerol for future analysis.

Upon completion of the 12 month study, cryovialed samples were tested for *Salmonella* conformation using latex agglutination. Samples were removed from the freezer and allowed to thaw at room temperature with 0.5 mL being extracted and placed into 9.5 mL TSB. After 24 h incubation at 37°C, 1 mL of sample was placed into 9 mL of TSB for another growth cycle. After an additional 24 h incubation was

complete, 0.1 mL bioloop of sample was transferred to PCA, streaked for isolation, and incubated at 37°C for 24 h. Individual colonies from PCA were selected and then streaked for isolation onto BGS agar and incubated for 24 h at 37°C, to ensure that the sample was positive for *Salmonella*-like species and that no contamination had occurred during storage, and incubated for 24 h at 37°C. Samples were then tested using *Salmonella* O antisera (BD Difco, Rockville, MD) and Antiserum Vi groups B and D (BD Difco). These two groups were chosen because they encompassed the *Salmonella* serotypes most commonly associated with eggs. Individual colonies from the non-selective PCA media were placed onto disposable agglutination test sheets, with the addition of the antisera. Both positive and negative controls were used to verify each sample. A clinical rotator (Fisher Scientific, Pittsburg, PA) was utilized to aid in the agglutination process, as the samples were gently swirled for 1-2 min. Positive agglutinations were confirmed by the observance of coagulated areas within the sample. Negative agglutinations showed no signs of coagulation and the sample retained its initial integrity. Samples that were agglutination positive for *Salmonella* were then sent to the National Veterinary Services Laboratories (**NVSL**, Ames, IA) for serotyping.

Statistical Analysis

The experimental design of the study was a 2 X 3 X 11 factorial arrangement of a completely randomized design, representing the two hen strains (HL and BW), the three molting treatments (NF, NM, and FR), and the 11 month study period in which the separated yolk and albumen components from the molt period were combined and analyzed as whole egg. For the molt period (period 13), microbial

counts were analyzed as exterior shell, interior shell, yolk, and albumen using both the PROC GLM and PROC FREQ in SAS version 9.0 (SAS Institute, Cary, NC). Plate counts were converted to base-10 logarithm values and subjected to analysis of variance using PROC GLM and Fisher Protected LSD in SAS version 9.0. Frequencies were obtained using the PROC FREQ procedure of SAS. Results indicating a zero count in bacteria were analyzed at the lowest detectable limit of 0.9 cfu / mL, while results indicating a too numerous to count (TNTC) measurement were set to a limit that was 1 log unit higher than the highest detectable plate count (100,000 cfu / mL).

RESULTS AND DISCUSSION

The values reported in figures 1-4 represent the average of the 2 strains, the three molting treatments, and replicates for a given period. As shown in Figure 1, exterior, interior, yolk, and albumen counts, when compared to exterior, interior, and contents counts shown in Figure 2, increased during the molt period by as much as 1 log unit higher than the highest countable plate, which exhibited a 10^5 value. In essence, the average interior counts for periods 14-24 indicate a 2 log decrease from those found during the molting period, while contents counts showed an approximate decrease of 3 logs, and exterior counts decreased by 2 logs. This indicates that bacterial shedding during the molt period was higher than all other periods. This is consistent with studies that suggest molting increases the frequency of bacterial shedding. Hen age significantly ($P<0.05$) affected the microbial loads on all three tested egg components (Figure 3), while molting treatment had a significant ($P<0.05$) effect on the frequency of *Salmonella* positive samples (Figure 4).

As shown in Figure 3, exterior, interior, and contents counts rose ($P<0.05$) during period 15, with an increase ($P<0.05$) in the interior counts in period 14, and in the contents in periods 14 and 17. The increase in counts during the first two periods post-molt were potentially due to stress caused by the molt, while the increase in counts in later periods could be related to hen age and the resumption of egg production post-molt. Although there was a significant difference ($P<0.05$) across periods, there were no differences ($P>0.05$) between populations of aerobic bacteria found within each molting treatment. This observation suggests that molted and non-molted birds shed the same amount of bacteria. This contradicts that theory that non-molted birds will shed fewer bacteria than non-fasted or feed-restricted birds.

However, the molting treatment, did have a significant ($P<0.05$) impact on the frequency of *Salmonella* positive samples. The results, shown in Table 1, indicated that the greatest number of *Salmonella* positive samples occurred in the non-fasted and non-molted treatments. This discovery contradicts the findings of pervious studies, which state that induced molting, including a feed restriction treatment, has been shown to increase shedding of *Salmonella* bacteria. However, this experiment showed that the feed-restricted treatment had the lowest frequency of *Salmonella*, when compared to the other two treatments. The discrepancy in results from previous studies could be due to the fact that this trial utilized the naturally occurring contamination and did not inoculate the hens in the beginning. We were simply looking for the natural occurrence of SE with no predisposed bias. Many studies similar to this one used hens infected with SE to ensure concentration in the gut, and therefore promote bacterial shedding. The results obtained in Figure 5 also indicate

that an increase in hen age had an effect on *Salmonella* frequency. It was discovered that the greatest occurrence was detected in period 21 when 3 interior and 1 exterior proved to be *Salmonella* positive.

All eggs produced by the two laying strains (BW and HL) during the collection period were utilized in this experiment. There were a total of 360 egg pools and of those, 4 were positive *Salmonella* samples. Two components and 2 molting treatments had positive samples. The four *Salmonella braenderup* samples were identified in period 21. Three positives were associated with the interior component, while 1 positive was associated with the exterior shell component. When comparing these results to the molting treatments used, it was revealed that 3 of the 4 positive samples were related to the NF treatment, while the remaining positive was found in the NM treatment. None of the Salmonella found in association with the shell eggs used for this study could be correlated to the samples taken of the environment. Although *Salmonella infantis* and *ohio* were discovered in the environment, these serotypes were not discovered in the eggs. This would suggest that the serotypes indicated with the shell eggs were not due to environmental contamination, but could be due to fecal contamination from the hens.

Conclusion

In summary, the findings of this study suggest that an induced feed-restricted molting treatment does not increase the incidence of *Salmonella* spp., including SE which has been documented to increase during molting. Of the *Salmonella* positives discovered in this trial, none were found to be SE. This is potentially due to the fact this experiment focused on the natural occurrence of SE, and no hens were inoculated

with bacteria in this study. The results of specific serotyping indicated that the strains found in the shell eggs did not match those of the environmental samples. Although 3 of the 4 *Salmonella braenderup* positives were linked to the interior shell, no *Salmonella* was found in the contents (yolk and albumen) of the eggs. This supports the theory that Salmonella infection could have occurred at some point through the duration of the study. This mode of transmission would provide a viable opportunity for organisms to enter into the shell eggs. Although bacteria can travel through the pores and into the interior shell membranes and matrices, the albumen does not provide a suitable growth medium, being deficient in nutrients and laden with antimicrobials.

ACKNOWLEDGEMENTS

The authors would like to thank the North Carolina Department of Agriculture and Consumer Services, Piedmont Research Station staff for rearing the laying hens and helping to collect the eggs utilized in this study, the Auburn University Egg Quality Lab for their support and assistance, and the Poultry Products Safety and Quality Peaks of Excellence Program for funding this research.

Figure 1. Aerobic bacteria plate counts for the molting period.

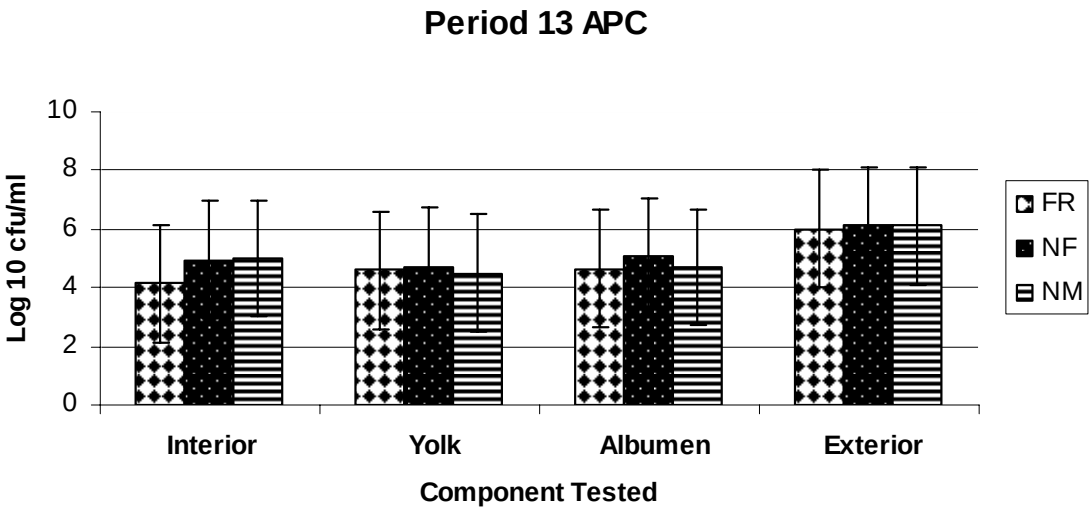


Figure 2. Aerobic bacteria plate counts for molting treatments from periods 14-24.

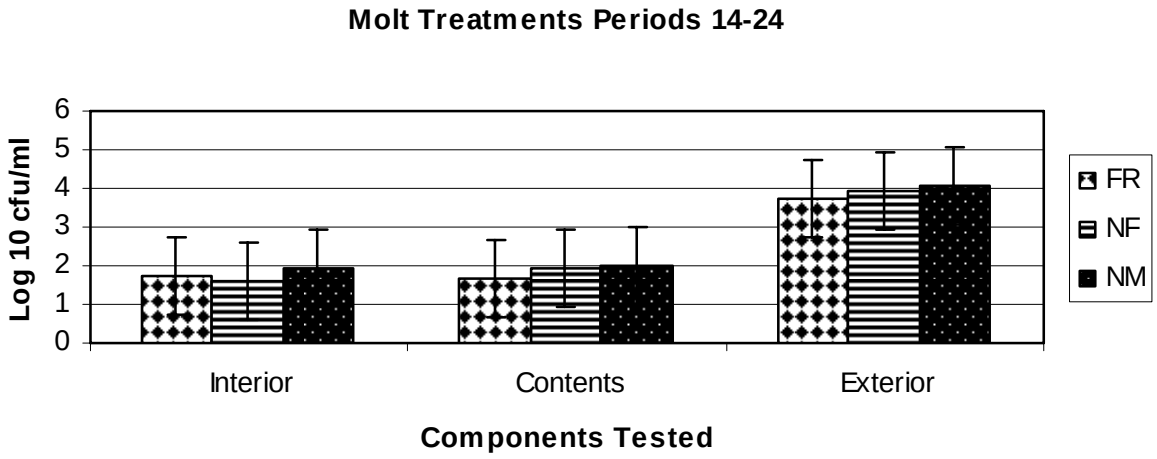


Figure 3. Aerobic bacteria counts shown per periods 14-24.

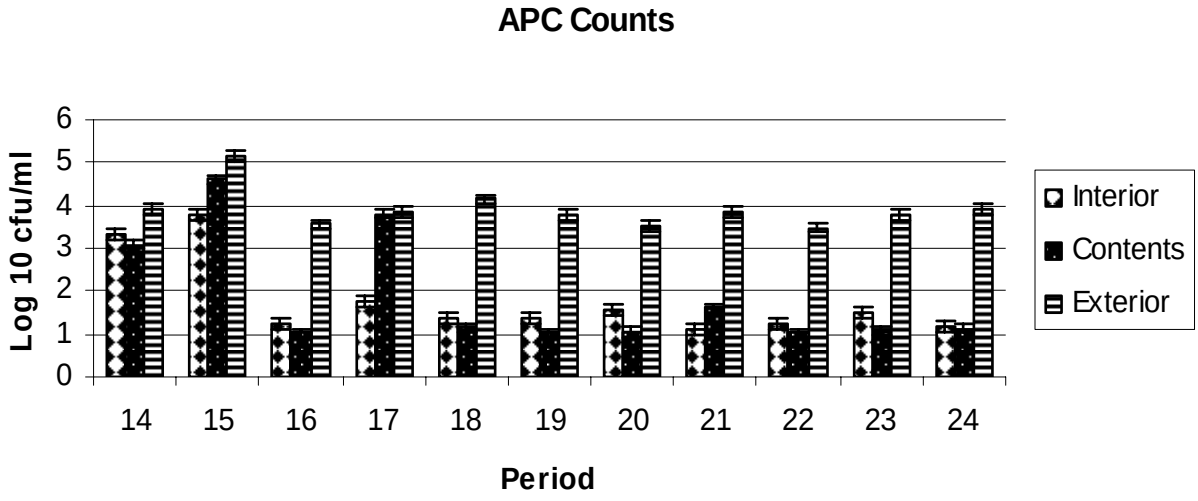


Figure 4. The frequency of *Salmonella* positive samples per molting treatment.

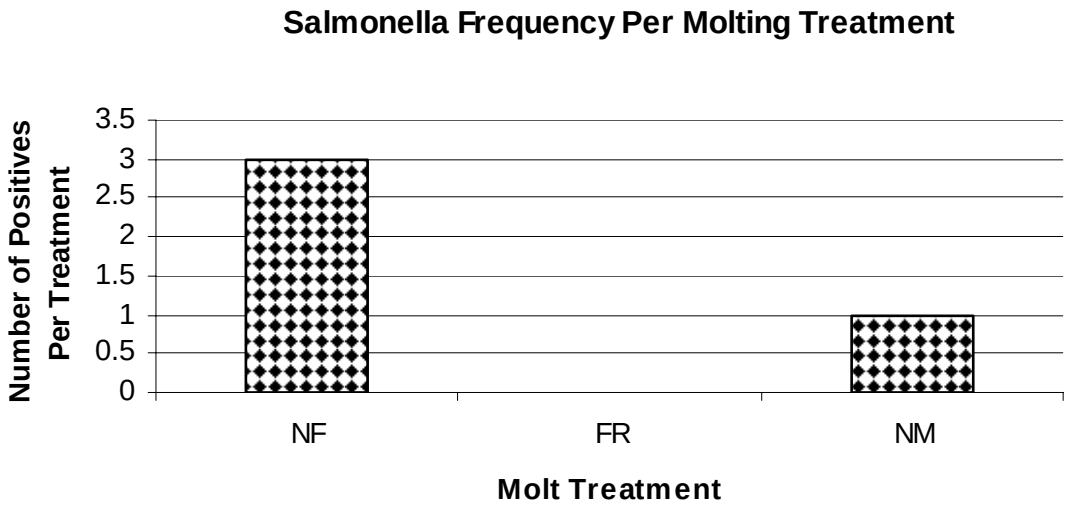


Figure 5. The frequency of Salmonella suspect positive samples by period.

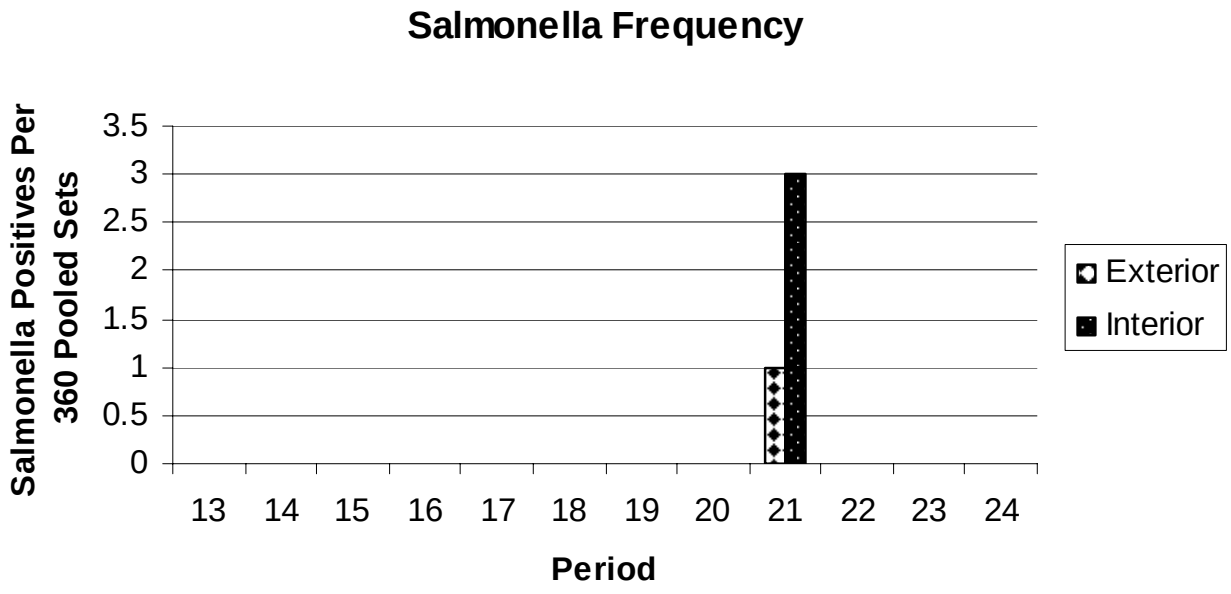


Table 1. Positive samples indicated by serotyping from the National Veterinary Services Laboratory.

Period	**Location**	**Molting Treatment**	**Serotype**
21	Exterior	NF	*S. braenderup*
21	Interior	NF	*S. braenderup*
21	Interior	NF	*S. braenderup*
21	Interior	NF	*S. braenderup*

MANUSCRIPT III

EFFECTS OF CITRIC ACID AND PASTEURIZATION ON THE FUNCTIONAL AND CHEMICAL ANALYSES OF LIQUID WHOLE EGG

ABSTRACT

Although a large portion of the eggs produced today are sold in the form of shelled eggs, approximately 30 percent of the eggs produced by the industry are broken out and marketed as pasteurized liquid egg products. The purpose of this research was to determine if the addition of citric acid to pasteurized whole egg, as well as the pasteurization process itself, influenced the results of functional experiments and chemical analyses. Liquid whole egg was obtained from egg processing facilities which supplemented a portion of its pasteurized product with citric acid. Four treatments consisting of unpasteurized, pasteurized, unpasteurized with citric acid, and pasteurized with citric acid liquid whole egg were extracted in 5 lb sample quantities. Samples were taken during three separate processing periods to ensure that egg lots were not duplicated. Each treatment was analyzed for pH, total moisture, total fat, total protein, sponge cake volume, sponge cake texture profile analysis **(TPA),** custard weep, and custard gel bloom. The results of this study show that neither pasteurization nor citric acid supplementation had a true, practical impact on the chemical composition of whole egg products. The use of the CEM provides rapid, accurate results for total moisture, fat, and protein of whole egg samples. Although further research is needed to achieve AOAC

approval, the results of this study indicate that this is a possible alternative to traditional proximate analysis procedures used for egg products.

The addition of citric acid to whole egg products created a more voluminous cake than those that were left un-supplemented. The theory that pasteurization is detrimental to whole egg functionality was not evident in this study. Both treatments that utilized the pasteurization process performed best in both custard weep and bloom, with the PC treatment also having a high sponge cake volume.

INTRODUCTION

Whole Egg Pasteurization

Although a large portion of the eggs produced today are sold in the form of shelled eggs, approximately 30 percent of the eggs produced by the industry are broken out and marketed as pasteurized liquid whole egg products. According to the FSIS (2007) approximately 30% of the 76 billion eggs consumed in 2007 were in the form of egg products, or eggs that have been removed from the shell and pasteurized. From the baking industry to specialty products, pasteurized products are developed utilizing whole egg mixtures, as well as separated yolk and albumen components. Many consumers favor these products because of the idea that pasteurization aids in the destruction of harmful bacteria and because they are perceived as "safer" than shelled eggs.

It is extremely important to maintain the functional properties of liquid whole egg because it is so commonly used as an ingredient in foods products. Studies such as those done by Baldwin (1986) have described how the application of heat can both positively and negatively impact the functionality of liquid whole egg. The challenge for the industry is striking a balance between ensuring consumer safety and maintaining the

functional integrity of the product. Because of this, Dawson and Martinez-Dawson (1998) conducted a study that evaluated what effect specific pasteurization times/temperatures had on the functionality of liquid whole egg products. By using Response Surface Analysis (RSA), they were able to show the effects of a range of different pasteurization time-temperature combinations on quality and functional factors of liquid egg. They discovered that when measuring cake height volume, there was more of time dependence than temperature dependence. This is possibly due to the fact that when eggs are held at high temperatures for long periods of time, their proteins begin to denature and unfold, making them incapable of trapping the large amounts of air needed to create a greater cake volume. Solids content followed this same trend, being more time dependent.

Many times, citric acid is added to pasteurized whole egg products. There is little research discussing the reasoning behind this addition and its effect on cooked products in comparison to whole egg without the added citric acid. However, there is an obvious decrease in pH because of the acidic characteristics and one could conclude that this addition combined with pasteurization, could potentially have a positive impact on bacterial elimination. By definition, citric acid is a weak organic acid that is mostly associated with citrus fruits. It is a natural preservative and is often found in candies and soft drinks that require a sour flavor. Though it is widely used in the egg industry, there is not much research on the impact of this additive on the functionality of the liquid whole eggs.

Whole Egg Functionality

Although eggs provide many functional applications, one of the most common is the ability of eggs to coagulate or form a solid when heated (Stadelman, 1999). Unlike carbohydrate gels, which solidify upon cooling, protein gels such as the ones found in egg custard, exhibit the gelation process upon heating. In basic terms, a gel is an intermediate phase between a solid and a liquid. However, technically, it is defined as a substantially diluted system which exhibits no steady state flow (Ferry, 1961). During the process of whole egg gelation, proteins are first denatured into an intermediate gel state, in which the proteins are unfolded to expose functional groups. This progression allows for the formation of the protein bonding network that eventually forms the solid state gel. Protein gels are irreversible, meaning that upon either re-heating or cooling, they will not return to their former liquid state. This is in contrast to gelatin gels, such as those seen in Jello® products, in which applying heat to the solid form will cause bond breakage, resulting in a return to the liquid state.

Another functional property of whole eggs is their ability to be used as both a leavening and humectant agent in bakery products. Researchers often use sponge and angel food cakes to study the functionality of whole egg and albumen. Although more research is needed to determine the functional effects of whole eggs in food products, various experiments have utilized sponge cake volume to determine leavening aspects. In a study conducted by Jones *et al* (1999), it was determined that increasing hen age demonstrated an increase in sponge cake volume. In theory, the more volume a cake presents represents a better quality egg. Although this finding is in contrast to the hypothesis that eggs of older hens are lower in quality than those of younger birds, the

effect on functionality could be due to the change in egg composition in correlation with increasing hen age.

MATERIALS AND METHODS

Four treatments of liquid whole egg, pasteurized (**P**), unpasteurized (**R**) (control), pasteurized with citric acid (**PC**), and unpasteurized with citric acid (**RC**) (control) were sampled from egg processing facilities during three non-consecutive processing periods. Eggs were collected on the day of process in sterile plastic bags with secure screw-top closures to prevent leakage. Samples were gathered by facility staff wearing sterile gloves, hairnets, and jumpsuits to prevent human contamination, and assembled into 5 lb quantities with three replicates (three 5 lb bags) representing each of the four treatments. The egg products were then shipped from the facility via overnight shipping in boxed coolers containing frozen cooler packs. They arrived at the Auburn University Poultry Science Building by 10:00 a.m. 1 day post process, and chemical analyses began immediately.

Beginning 1 day post process, the liquid egg products were measured for pH using an Accumet® excel XL 20 pH/Conductivity meter (Fisher Scientific, Pittsburg, PA). Total moisture and fat were measured using the CEM Microwave Technology Smart Trac® system in conjunction with the CEM Microwave Phoenix® system (CEM Corp., Matthews, NC). Protein content was calculated based on the results of CEM Smart Trac® and Phoenix® data.

Chemical Analysis

pH Measurement

Upon arrival at Auburn University, samples were tested for pH. This procedure was done with the use of an Accumet® excel XL 20 pH/Conductivity meter. Once calibrated, a small amount of egg sample was placed into a 50 mL beaker and the probe of the meter placed into the sample.

CEM Microwave Technology

Once whole egg samples had been tested using AOAC analytical methods, those results were used to calibrate the CEM Microwave Technology equipment. To test the total moisture content of the eggs, specially designed CEM sample pads were placed onto the internal balance using tongs, and the balance was tared and 2.0-2.5 grams of egg sample placed onto the pads. After the balance reached a stable weight, a reading was given as to the exact weight of the sample in units carried out to four decimal places. Simultaneously, the internal drying oven began to dry the weighed sample at 100°C until all moisture was removed. This process was 10 min in length. Once dry, the total moisture percentage reading appeared on the CEM results screen and total fat analysis could begin. Samples were removed from the internal balance with tongs and placed onto the CEM preparation station where they were gently rolled in a thin layer of CEM wrap. The rolled sample was then placed directly into the fat analysis tube and compressed to approximately .75 in before being placed into the Smart Trac® system. After the analysis tube was placed into the system, it provided a direct measurement of total fat, including both free and chemically bound fat in solids, liquids and slurries.

To test for the protein content of egg samples using the CEM, it was imperative to

employ the use of the CEM Phoenix Muffel Furnace®. To complete this analysis, disposable quartz ashing crucibles containing two small circular pads were placed onto an analytical balance using tongs. The disposable crucibles and pads were weighed and tared and 5.0-6.0 grams of sample placed inside. They were then removed from the balance with tongs and placed into the Phoenix Muffle Furnace® for ashing at 550°C for 35 min. Once ashing had occurred, the samples were removed and quickly reweighed to obtain the amount of ash. Once these three measurements (moisture, fat, and ash) were obtained, it was possible to calculate the percentage of protein in the samples using the formula:

100% - % total moisture - % total fat – % ash = % protein

Functional Analysis

Sponge Cake Volume Determination

To determine the volume of sponge cakes made with whole egg product, a Viking® (Viking Corp., Greenwood, MS) oven was pre-heated to 350°F approximately 20 min before the first cakes were added to it time to maintain a constant temperature. Using a 5 quart Kitchen Aid® (Kitchen Aid, Inc., St. Joseph, MI) mixing bowl, 138 grams of whole egg was added along with 89.4 grams of granulated sugar. The bowl was then placed onto a Kitchen Aid Artisan Series® (Kitchen Aid, Inc., St. Joseph, MI) mixer and a balloon whisk attachment attached. Samples were then mixed for 15 sec at speed 2 and for an additional 4 min at speed 10. Once complete, 2 mL of imitation vanilla flavoring was added to the mixture and homogenized for an additional 4 sec at speed 6. Once mixed, 66 grams of cake flour was added to the batter in four equal portions and whisked gently with a hand whisk for approximately 20 strokes per flour

addition. Cake pans with a pre-determined rapeseed volume were placed onto a balance and 75 grams of cake batter weighed into tared cake pans consisting of three sub-replicates per replicate. The cakes were then placed into the pre-heated ovens and baked for 17 min, removed after cooking and placed inverted onto cooling racks for at least 2 hr post bake. After cooling, the cakes were turned upright and the tops lightly dusted with cake flour to ensure that the rapeseed did not stick to the cakes. The remaining volume of the pan was filled with rapeseed and leveled off using a small metal spatula until the rapeseed was flush with top of the pan. The pre-determined volume of the cake pan was then recorded, and the rapeseed was measured into a graduated cylinder using a funnel. The volume of the rapeseed was recorded and the cakes were saved for TPA analysis. The cake volume was determined using the following formula:

Pre-determined pan volume – rapeseed volume = cake volume.

Sponge Cake TPA Analysis

The texture profile analysis of the sponge cake samples was done using a Stable Micro System's TA.XT Plus Texture Analyzer (Texture Technologies Corp., Marietta, GA). The TPA function of this equipment measures hardness, sponginess, gumminess, resilience, chewiness, cohesiveness, and force, graphically displaying the results onto the computer to which it is attached. Once selecting the TPA function from the project list, cake samples were placed under a cylindrical Texture Analyzer attachment to ensure an even measurement. The force at which the cakes will be compressed is then calibrated using a 5 kg load cell and a 2 kg weight. Once completed, the cakes are then compressed twice, with the probe taking both the first and second peak measurements at 4 cm.

Custard Weep

Custards are a product of the gelation of a protein based product that gels upon heating and displays an irreversible process, unlike sugar based gels. The egg custard samples were prepared by first pre-heating Viking ovens to 350°F. In a mixing bowl, 250 mL of whole egg, 0.5 c of sugar, 1.5 tsp vanilla, and 0.25 tsp salt was added. The ingredients were then placed on the Kitchen Aid mixer and a balloon whisk attached. Custard samples were blended for 1 min at speed 4, and 3 c of hot whole milk added immediately following the 1 min blend. The custard mixture was then measured to 100 mL and poured into 4 oz ramekins that were lightly sprayed with cooking spray. Simultaneously, 60 grams of the custard mixture was placed into 100 mL beakers that would be used to measure the gel force. Three sub-replicates of each replicate were taken to test for product variance. The samples were then placed into a pan of hot water measuring 0.5 in from the top of the ramekins and placed into the ovens where they were baked for 40 min. Once baked, a small metal spatula was inserted into the center of the custard to ensure that it was properly cooked. Samples were then placed onto cooling racks and allowed to come to room temperature (approximately 1.5 hr.). While the samples cooled, sample containers were prepared for weep measurement. The lid of the plastic container was placed onto a balance. Using gloves to prevent the addition of moisture and oils from human hands, 5 Whatman weighing papers (110 mm) were added to the lid to ensure that any residual moisture from the custards would be absorbed. The containers were then labeled with the weight of the Whatman papers. Once the custards had finished cooling, the ramekins were inverted to release the sample onto the weighing papers in the custard container. The custards prepared in the beakers were covered with

Fisher Scientific Parafilm® wrap and both sets of custards were placed into a 4°C walk-in cooler for 18-24 hr.

Once the samples were chilled for the appropriate time, the weep samples were removed from the cooler and gently removed from the Whatman paper. The lid of the container, the weighing papers, and the residual weep was weighed on a balance and the weight recorded. The amount of custard weep was then calculated using the formula: Final weight of lid, weep, and paper – initial weight of lid and paper.

Custard Bloom

The custard samples prepared in the 100 mL beakers were removed from the cooler and taken to the lab for force analysis using the Texture Analyzer. Using a 5 kg load cell and a 2 kg weight, the force of compression was calibrated. As the custard was compressed 4 cm, the force results were displayed on the screen.

Statistical Analysis

The experimental design of this study was a Completely Randomized design (CRD). Statistical analysis was done using the SAS® Statistical Package version 9.0 (SAS Institute, Cary, NC). Averages of the samples taken from the sub-replicates were analyzed using the General Linear Model (PROC GLM) in SAS and means found to be significantly different were analyzed using Fisher's Protected LSD.

RESULTS AND DISCUSSION

Although the treatments containing citric acid (PC and RC) were slightly lower in pH, this was not enough to create a statistical difference when compared to the non-citric treatments. Egg processors are choosing to provide companies and consumers with a slightly more acidic product because those with a lower acidity have been shown to have

a longer shelf-life post-process. Although the citric products perform as well as non-citric chemically, there are issues concerning functionally, especially in bakery products, which needs to be addressed through further research.

Once the pH measurements were taken, chemical analyses were performed on each of the treatments. This was done using the CEM Microwave Technology Smart Trac® System, which performs both total moisture and total fat much more quickly than conventional methods. There were no statistical differences ($P>0.05$) in total moisture, fat, or protein between any of the treatments.

It was discovered that the treatments with citric acid supplementation were more effective in producing a high volume sponge cake and exhibited a positive impact during TPA analysis. As shown in Figures 1 and 2, the treatment that contained citric acid was significantly different ($P<0.05$) from the P treatment. This does not concur with the theory that citric acid can be detrimental to baking products. Although the exact reason for this needs further research, it can be speculated that citric acid acts as a mechanical barrier which protects certain proteins from denaturation during the pasteurization process. Although the P treatment was observed to be a softer cake, displaying lower force measurements, this is not necessarily a positive aspect. The P treatment cakes were stickier upon visual observation and exhibited aspects of being gummy in texture and having a potentially chewy mouthfeel. These observations were verified by the results of TPA analysis, indicating that the PC treatment possessed the qualities of a more ideal sponge cake.

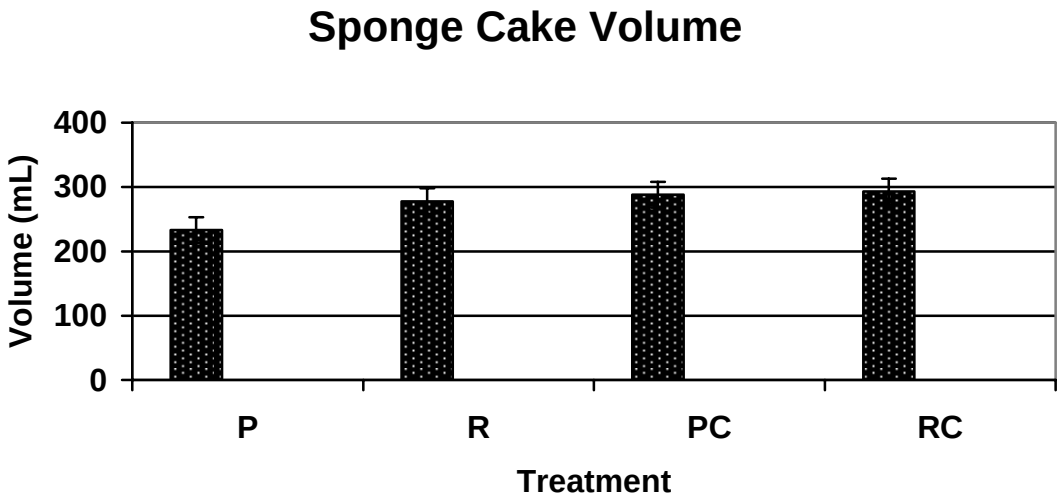
Although citric acid improved the functionality of whole egg products in a baking sense, it seemed that pasteurization had a greater impact on the functionality of egg based

gels. The results of custard bloom and weep, shown in Figures 3 and 4, indicated that the two pasteurized treatments (P and PC, respectively) had a more positive impact on gel strength and formation. As the figures show, the P and PC treatments displayed a higher bloom force, meaning that the custard was more stable. In contrast, the non-pasteurized treatments (RC and R) showed weaker gels with more weep.

Conclusions

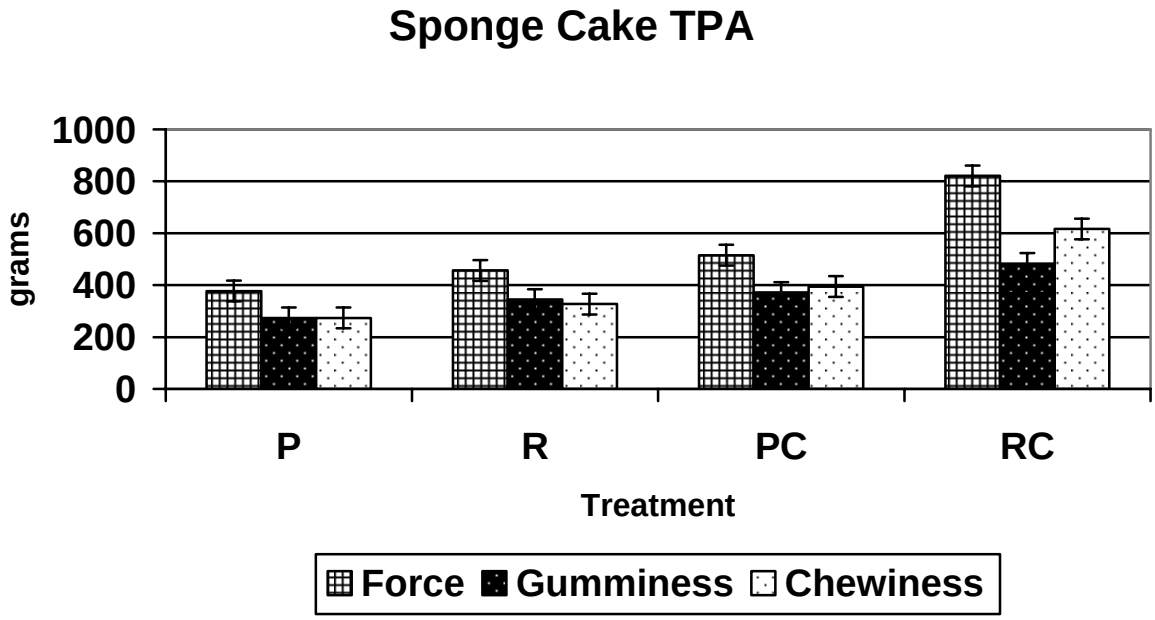
The results of this study show that neither pasteurization nor citric acid supplementation had a true, practical impact on the chemical composition of whole egg products. The results of the functional portion of this experiment revealed that the addition of citric acid to whole egg products created a more voluminous cake than those that were left un-supplemented. It seems that the addition of certain chemical agents such as citric acid, provide a mechanical barrier that protect whole egg proteins from the damaging effects of pasteurization, in turn, protecting their functional properties. Although more research is needed to discover the chemical mechanisms that drive the reactions between egg proteins and citric acid, the results of this study conclude that supplementation with this product may improve the functionality of whole egg products. It was also discovered that the pasteurization process in general improved the stability of custards. The results of analysis of the P and PC treatments indicated that the egg-based gels were stronger and more stable than those that did not utilize pasteurization.

Figure 1. Sponge cake volume



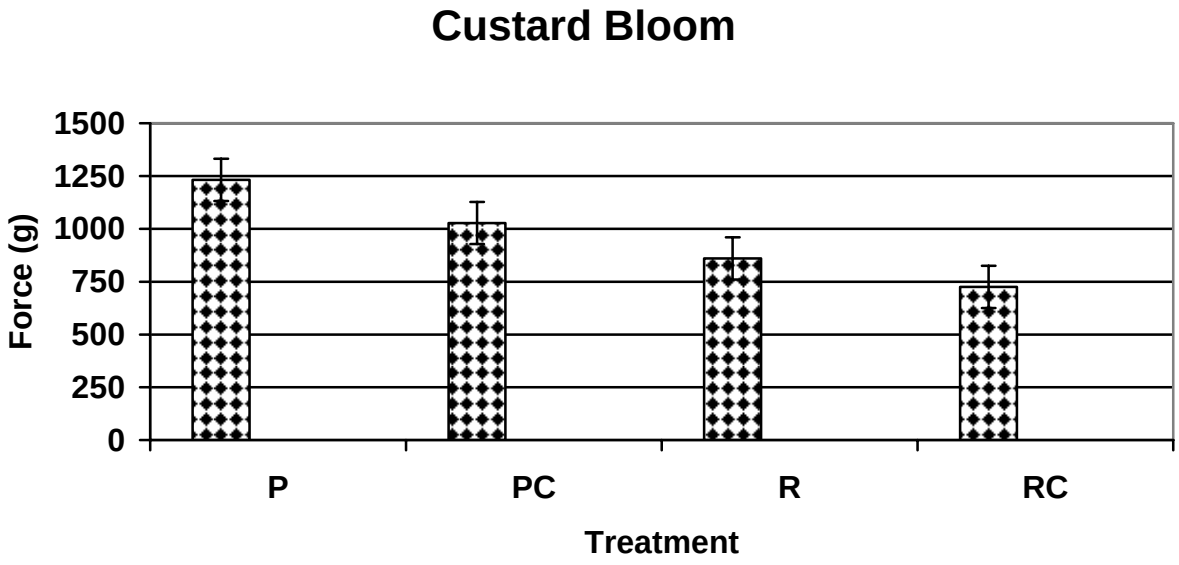
P<0.05

Figure 2. Sponge cake texture profile analysis for force (hardness), gumminess, and chewiness



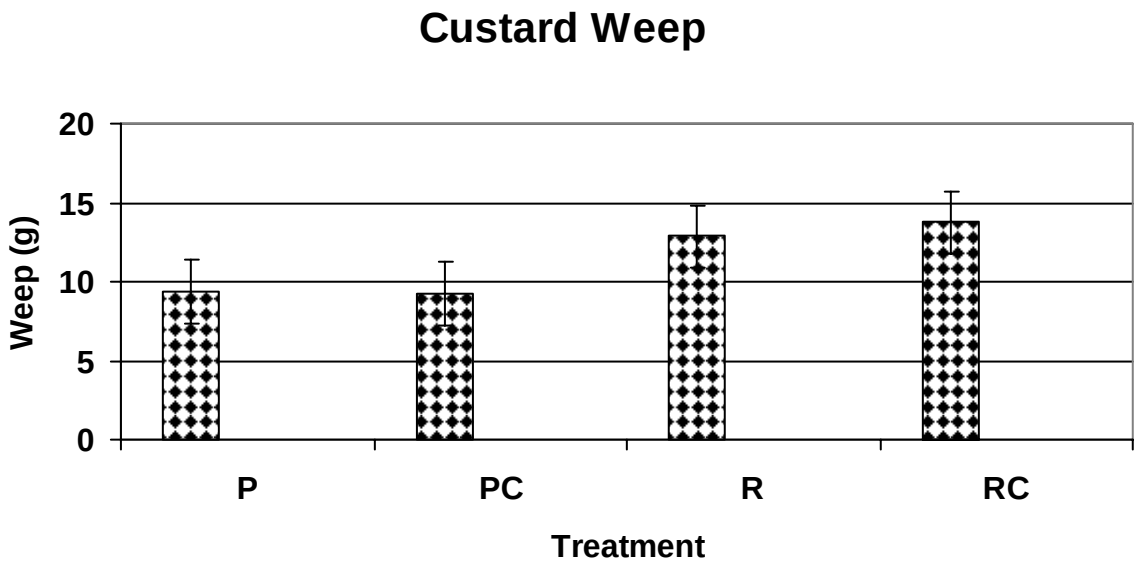
P<0.05

Figure 3. Custard bloom as analyzed using the Texture Technologies Texture Analyzer



P<0.05

Figure 4. Custard weep measurements



P<0.05

MANUSCRIPT IV

EFFECTS OF TRIETHYL CITRATE WHIPPING AGENT AND PASTEURIZATION ON THE FUNCTIONAL AND CHEMICAL ANALYSES OF LIQUID ALBUMEN

ABSTRACT

Egg albumen plays many roles in the creation of bakery products. It is used to make angel food cakes, meringues, candies, and many other confections. Because of this, egg processors are marketing pasteurized egg albumen with added whipping agents. However, there have been questions as to whether it performs as well as fresh albumen in functionality studies. The purpose of this research was to determine if the addition of triethyl citrate whipping agent to pasteurized albumen, as well as the pasteurization process itself, influenced the results of functional experiments and chemical analyses. Liquid albumen was obtained from egg processing facilities which supplemented a portion of its pasteurized product with triethyl citrate. Four treatments consisting of unpasteurized, pasteurized, unpasteurized with whipping agent, and pasteurized with whipping agent liquid albumen were extracted in 5 lb sample quantities. Samples were taken during three separate processing periods. Each treatment was analyzed for pH, total moisture, total fat, total protein, angel food cake volume, angel food cake texture profile analysis **(TPA),** and whipping height. There were no statistical differences ($P>0.05$) observed for the pH measurements of each of the four treatments, as well as no differences ($P>0.05$) in total moisture, total

fat, or total protein. While no differences ($P>0.05$) were found in whipping height measurements, there was a difference ($P<0.05$) observed in the angel food cake volume. The pasteurized **(P)** treatment had the poorest performance overall, with the pasteurized with whipping agent **(PW)** showing the greatest angel food cake volume. These results show that the addition of triethyl citrate whipping agent has a positive impact on angel food cake volume. The same results hold true for TPA analysis, where the PW treatment displayed the most positive and desirable characteristics.

INTRODUCTION

Albumen Pasteurization

It is no surprise that the pasteurization of various egg components provides a safer product, essentially free of microbial contamination, to consumers. However, the positive aspects of pasteurization are counteracted with the negative impact it has on functionality, especially in the baking industry. Pasteurized liquid egg whites are used in many pre-packaged products and are sold in liquid form in grocery stores. Often, consumers will buy such products and expect them to perform in the same manner as a shelled egg in their cooked foods. However, this is not always the case.

One function of egg whites in food products is to produce foam. Foam is created when air is trapped between the protein matrices when whipping is applied. In shelled eggs, it is important to make sure that the eggs used for this function are produced from young flocks. As hen age increases, egg quality potentially decreases. This can cause the albumen component to become thin and watery, instead of thick and distinct, thus creating less voluminous foam. This aspect can be detrimental to

the baking industry, with the fact being that eggs used from older flocks may produce cakes with less volume (Cunningham, *et al* 1960).

Water is the primary component of egg albumen (83%), while it also contains protein and only trace amounts of fats and sugars. The main proteins found are ovalbumin (54%), conalbumin (13%), ovamucoid (11%), lysozyme (3.5%) which also serves as a natural antimicrobial, globulins (8%), and ovomucin (1.5%) (Tanner, 2007). The protein partially unravels and forms a good foaming agent. Foam is created when the protein forms a stable film around the included air. Studies show that the best foam is created when the protein is only partially unraveled. Over beating egg whites destabilizes the foam by fully unraveling the protein molecules. The protein is elastic, so when the egg white is cooked, and the air expands, the white stretches then sets in the expanded position. Although albumen is comprised of several different proteins, only a few are responsible for foam formation and stability. According to Cotterill and Winter (1955) the globulins create the actual foam formation, while the ovomucin-lysozyme complex aids in foam stability. Ovalbumin and conalbumin add heat-setting properties.

Both dry and liquid egg albumen may sometimes contain additives that aid in foaming capabilities. A common additive is triethyl citrate, a form of citric acid. This is commonly used in pasteurized albumen products that have experienced some protein denaturation due to heat. Another additive is sodium lauryl sulfate, which is added to improve foaming properties. Triethyl citrate is used as a high boiling solvent and plasticizer for vinyl resins and cellulose acetates. It is a plasticizer permitted in the field of food additives, food contact material, medical, and

pharmaceutical products. Sodium lauryl sulfate is a detergent and surfactant found in many personal care products (toothpaste, shampoo, etc.). It is an inexpensive and very effective foamer. These aids are capable of giving the product whipping properties that are comparable to, or even better than, the natural untreated product. The type of additive used is dependent on the type of product being produced. For instance, although no fully understood, triethyl citrate seems to be more preferable in liquid frozen or pasteurized whites, while sodium lauryl sulfate seems to work well with dried albumen.

Albumen Functionality

Proteins are made of long chains of amino acids. The proteins in an egg white are globular proteins, which means that the long protein molecule is twisted and folded and curled up into a more or less spherical shape. A variety of weak chemical bonds keep the protein curled up tight as it drifts placidly in the water that surrounds it. When you beat raw egg whites to make a soufflé or a meringue, you incorporate air bubbles into the water-protein solution. Adding air bubbles to egg whites unfolds those egg proteins just as certainly as heating them.

Some amino acids are attracted to water; they're hydrophilic, or water-loving. Other amino acids are repelled by water; they are hydrophobic, or water-fearing. Egg-white proteins contain both hydrophilic and hydrophobic amino acids. When the protein is curled up, the hydrophobic amino acids are packed in the center away from the water and the hydrophilic ones are on the outside closer to the water. When an egg protein is up against an air bubble, part of that protein is exposed to air and part is still in water. The protein uncurls so that its hydrophilic parts can be immersed in the

water, and its hydrophobic parts can stick into the air. Once the proteins uncurl, they bond with each other, just as they did when heated, creating a network that can hold the air in place.

The foaming properties of albumen have long been praised for their role in confectionary functionality. Often, egg white foams are associated with soufflés, meringues, divinity, and angel food cakes. Because of its low fat content, angel food cake is frequently observed as being a healthier alternative to satisfying a person's sweet tooth. One of the most noticeable factors of this product is its light, airy texture, and its ability to produce a pleasantly sweet taste. The lightness of texture is due to the trapping of air by the proteins in the process mentioned above, while the sweet taste is due to the addition of sugar into the batter. However, this addition does more than just provide a delicious flavor. If added properly, it will help stabilize the foam. If sugar is added before foam formation occurs, it slows the denaturation of the proteins because it competes with the other molecules for access to water. This does not allow the proteins to trap the air and create a stable foam. However, if the sugar is added to the mixture after the foam is formed, it helps to generate stability, by adding a touch of viscosity between the trapped air bubbles. If done properly, this should produce a stable foam with less weep.

MATERIALS AND METHODS

Four treatments of liquid albumen consisting of pasteurized **(PA)**, unpasteurized **(RA)**, pasteurized with whipping agent **(PW)** and unpasteurized with whipping agent **(RW)** were obtained from egg processing facilities during three non-consecutive processing periods. Research samples were collected on-site at the

processing facility by employees wearing laboratory gloves, jumpsuits, and hairnets to prevent contamination, and divided into three 5 lb shipment bags per respective treatment. The albumen samples were shipped overnight to the Auburn University Egg Quality and Safety Laboratory on the same day they were processed. They arrived to Auburn 1 d post-process in tightly sealed shipment bags placed in sealed shipment coolers containing freezer packs. The cooler containing the albumen was then placed into a labeled box for shipping.

Upon arrival, the shipments were opened and the bags of albumen separated into sets of 3 bags per treatment, for a total of 12 bags during each processing period. Each bag was considered to be a replicate, creating a total of 3 replicates per treatment per processing period, and a total of 9 replicates per treatment throughout the course of the study. Each replicate (bag) was then further broken into 3 sub-replicates to ensure that a large enough population of the replicate was sampled. Three samples were taken from each sub-replicate, for a total of 9 samples per replicate per processing period. Throughout the study, a total of 324 albumen samples were analyzed for chemical composition, whipping height, and angel food cake volume.

Chemical Analysis

Beginning 1 day post process the liquid albumen was measured for pH readings using an Accumet® excel XL 20 pH / Conductivity meter (Fisher Scientific, Pittsburg, PA). The meter was first calibrated using solutions of a known pH. A small amount of sample, approximately 50 mL was placed into a beaker and the probe of the pH meter inserted into the sample. Total moisture, fat, and protein were

measured using the CEM Microwave Technology Smart Trac® system in conjunction with the CEM Microwave Phoenix® system (CEM Corp., Matthews, NC). To test the total moisture content of the albumen, specially designed CEM sample pads were placed onto the internal balance using tongs. Once placed, the balance was tared and 2.0-2.5 grams of albumen placed onto the pads. Once the balance was stabilized, a reading was given as to the exact weight of the sample in units carried out to four decimal places. Simultaneously, the internal drying oven began to dry the weighed sample at 100°C until all moisture was removed. This process was 5 min in length. Once dry, the total moisture percentage reading was transferred on the CEM to the results database and total fat analysis could begin. Samples were removed from the internal balance with tongs and placed onto the CEM preparation station where they were gently rolled in a thin layer of CEM wrap. The rolled sample was then placed directly into the fat analysis tube and compressed to approximately .75 in before being placed into the Smart Trac® system. Once the analysis tube was placed into the system, it provided a direct measurement of total fat, including both free and chemically bound fat in solids, liquids and slurries.

To test for the protein content of albumen using the CEM, it was imperative to employ the use of the CEM Phoenix Muffel Furnace. To complete this analysis, quartz ashing crucibles containing two small circular pads, were placed onto an analytical balance using tongs. The quartz crucibles and pads were weighed and tared and 5.0-6.0 grams of albumen placed inside. They were then removed from the balance with tongs and placed into the Phoenix Muffle Furnace® for ashing at 550°C for 35 min. Once ashed, the samples were removed and quickly reweighed to obtain

the amount of ash. Once these three measurements (moisture, fat, and ash) were obtained, it was possible to obtain the amount of protein in the samples, using the formula for percentage of protein:

100% - % total moisture - % total fat – % ash = % protein

Functionality

Angel Food Cake Volume

To determine the volume of angel food cakes made with albumen products, a Viking® (Viking Corp., Greenwood, MS) oven was pre-heated to 350°F approximately 20 min before the first cakes were added, to give it time to maintain a constant temperature. Using a 5 quart mixing bowl, 180 mL of albumen was added along with 2.70 grams of cream of tartar and 0.90 grams of NaCl. The bowl was then placed onto a Kitchen Aid Artisan Series® (Kitchen Aid, Inc., St. Joseph, MI) mixer and a balloon whisk attachment attached. Samples were then mixed for 45 sec at speed 10. Once complete, 2 mL of imitation vanilla flavoring was added to the mixture and homogenized for an additional 4 sec at speed 6. After mixing, 66 grams of cake flour with the addition of 46 grams of granulated sugar was twice sifted, and added to the batter in 3 equal portions, gently folding it into the batter with a hand whisk for approximately 20 strokes per flour addition. This process ensured that the foam which formed was not disrupted. Cake pans with a pre-determined rapeseed volume were placed onto a balance and 75 grams of batter weighed into the tared pans, in a ratio of three samples per sub-replicate. The cakes were then placed into the pre-heated ovens and baked for 17 min, removed after cooking and placed inverted onto cooling racks for at least 2 hr post bake. After cooling, the cakes were

turned upright and the tops lightly dusted with cake flour to ensure that the rapeseed used to determine cake volume did not stick to the cake tops. The remaining volume of the cake pan was filled with rapeseed and leveled off using a small metal spatula until the rapeseed was flush with top of the pan. The pre-determined volume of the pan was then recorded, and the rapeseed was slowly measured into a graduated cylinder using a funnel. The volume of the rapeseed was recorded and the cakes were saved for TPA analysis. The cake volume was determined using the formula: Pre-determined pan volume – rapeseed volume = cake volume.

Whipping Height

Whipping height measurements of each of the four treatments were obtained by measuring 200 mL of liquid albumen sample into a graduated cylinder and placing it into a 5 quart mixing bowl. The bowl was then attached to a Kitchen Aid Artisan Series® mixer with a balloon whisk attachment. The liquid albumen was whipped for 45 s a speed 6, followed by an additional 45 s at speed 10. Once foam had formed, the bowl containing the albumen was removed from the mixture and the foam gently leveled off with a spatula to obtain a proper whipping height measurement. A clear plastic ruler was inserted into the foam approximately 0.5 in from the center of the bowl and the measurement recorded in millimeters.

Angel Food Cake TPA

The texture profile analysis of the sponge cake samples was done using a Stable Micro System's TA.XT Plus Texture Analyzer (Texture Technologies Corp., Marietta, GA). The TPA function of this equipment measures hardness, sponginess, gumminess, resilience, chewiness, cohesiveness, and force. Once selecting the TPA

function from the project list, cake samples were placed under a probe attached to a cylindrical attachment to ensure an even measurement. The force at which the cakes were compressed is then calibrated using a 5 kg load cell and a 2 kg weight. Once completed, the cakes were compressed twice, taking both the first and second peak measurements at 4 cm.

Statistical Analysis

The experimental design of this study was a Completely Randomized design (CRD). Statistical analysis was done using the SAS® Statistical Package version 9.0 (SAS Institute, Cary, NC). Averages of the samples taken from the sub-replicates were analyzed using the General Linear Model (PROC GLM) in SAS and means found to be significantly different were analyzed using Fisher's Protected LSD.

RESULTS AND DISCUSSION

Although the treatments containing triethyl citrate (PW and RW) were slightly lower in pH, there was no statistical difference ($P<0.05$). Although the triethyl citrate products perform well chemically, there are issues concerning functionally, which were addressed in this research. There were no statistical differences ($P>0.05$) in total moisture, fat, or protein between any of the treatments.

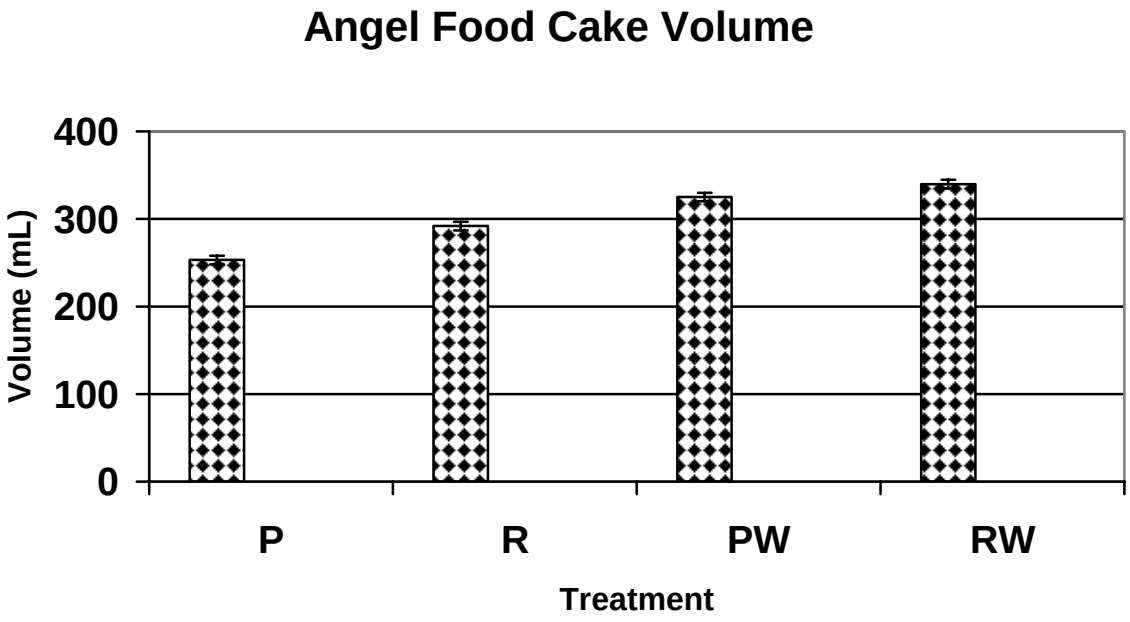
It was discovered that the treatments with triethyl citrate whipping agent supplementation were more effective in producing a high volume angel food cake and exhibited a positive impact during TPA analysis. As shown in Figures 1 and 2, the two treatments that contained whipping agent were significantly different ($P<0.05$) from the P treatment. Although there were no significant differences in whipping height, it was determined that once baked, the treatments containing the whipping

agent produced an angel food cake with a greater volume. It was also determined that the PW treatment was a softer cake, having a less gummy and chewy texture. The P treatment produced a gummy, chewy cake that displayed a thicker crust on the top, in turn, creating a less desirable angel food cake. This concurs with the theory that pasteurization of albumen, without the addition of an additive to act as a barrier, can be detrimental to baking products. Although the exact reason for this needs further research, it can be speculated that triethyl citrate acts as a mechanical barrier which protects certain proteins from denaturation during the pasteurization process.

Conclusions

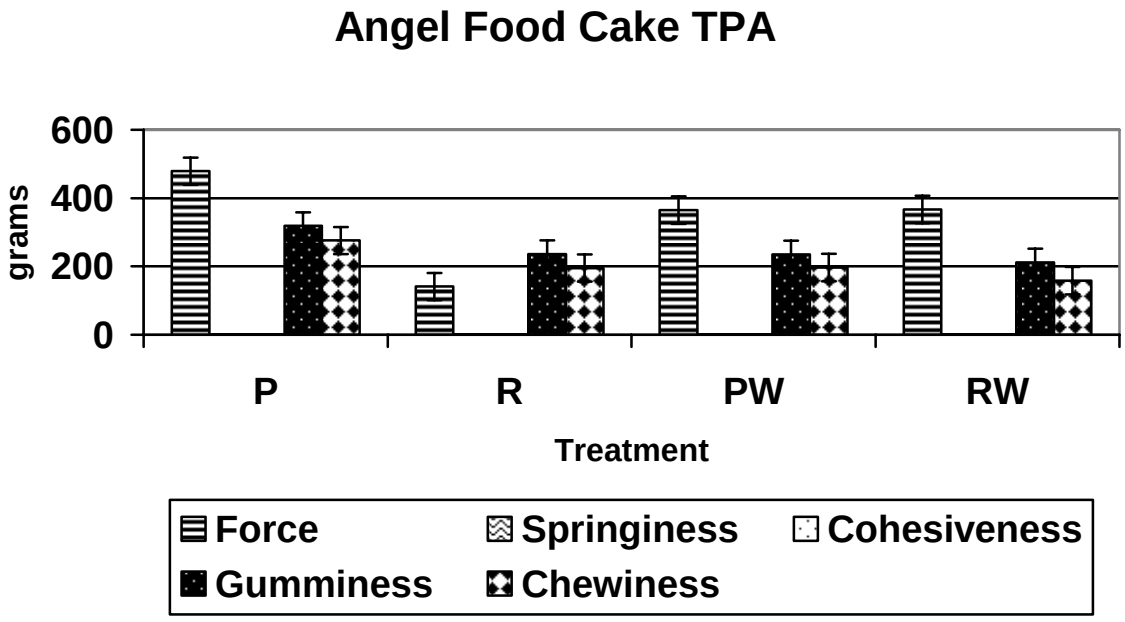
The results of this study show that neither pasteurization nor triethyl citrate whipping agent supplementation had a true, practical impact on the chemical composition of albumen products. The results of the functional portion of this experiment revealed that although there were no statistical differences observed in the whipping heights of the treatments, the addition of triethyl citrate to albumen products created a more voluminous cake post-bake, which exhibited a more desirable TPA analysis than those that were left un-supplemented. More research is needed to discover the chemical mechanisms that occur between egg proteins and triethyl citrate. However, it was evident by the results obtained from the P treatment, that pasteurization without the supplementation of a whipping agent has a negative impact on the functionality of albumen.

Figure 1. Angel Food cake volume



($P<0.05$)

Figure 2. Angel Food cake texture profile analysis



($P<0.05$)

SUMMARY OF RESULTS

Although it may seem that the sets of experiments conducted in this dissertation are not related, in reality they are not distant relatives. The microbiological research, which comprises the first two experiments, studied the natural occurrence of the organism, *Salmonella* Enteritidis. This is the pathogen most commonly associated with eggs and egg products. Although we tested for other bacteria, the recovery of this specific organism was the primary target.

The study was conducted over a two year period in which the first 12 months acted as the pre-molt period and the second 12 months served as the molt and post molt portion of the research. One of the goals was to observe whether an increase in hen age had an effect on the total microflora counts, as well as the incidence of SE. For each of the experiments, the exterior shell, interior shell including membranes and matrices, and contents containing both yolk and albumen portions were tested. Although we utilized two different hen strains for this research, it was discovered that only hen age affected the total microflora counts of each of the egg components. Also, there was no incidence of SE observed throughout the entire study of the shell eggs, and also none found in the environmental samples. During the molt and post molt portion of the study, three molting treatments were used. No *Salmonella* samples were discovered in the feed-restricted treatment, with the non-molted and non-fasted treatments showing the only signs of *Salmonella* spp., none of which were

SE. The second set of experiments dealt with pasteurized liquid products. Over 30% of the eggs marked today are sold in this form, mainly because of their relationship to the previous study. When eggs are pasteurized, they are virtually bacteria free. This creates a safer product for the consumers, but leaves questions as to how they function in food products. The results of this study indicated that although an acidic supplement was added to both the whole egg and albumen products, there were no significant differences ($P>0.05$) in the pH between any of the treatments analyzed. This was also the case for the chemical analyses performed on both products. We discovered no differences in total fat, total moisture, or total protein. It was also observed that those products that were supplemented with the acidic products (citric acid and triethyl citrate) created more voluminous sponge and angel food cakes, which exhibited the best overall texture profile analysis. The whole egg pasteurized and whole egg pasteurized with citric acid products also exhibited a stronger gel bloom and created custard products with less weep.

These results indicate that although eggs are pasteurized to protect consumers from possible contact with bacteria, the actual chance of contracting SE from shelled eggs is very low, as none was discovered over the course of our two year study. However, if consumers are choosing to buy pasteurized products over shelled eggs, it would be better to choose those products supplemented with either citric acid or triethyl citrate whipping agent, as they provide a more functional use in baking than do un-supplemented products

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APPENDICES

Ashing (Proteins)

Supplies Needed

Phoenix System by CEM
189 Quartz fiber ashing crucibles
378 Crucible liners (disks)
1 set large tongs to transfer crucibles to and from oven
1 set small tongs or tweezers
Multiple pairs of Nitrile Laboratory Gloves
1 set of Phoenix System Gloves provided by CEM
Scale that measures to 0.0001 grams
Eggs for sampling
30 small Turkey Basters
1 small plastic lunch tray

Procedure

1. Program the Phoenix System for 20-35 minutes and 550°C and allow the ashing furnace to pre-heat to 550°C.

2. With Nitrile gloves on and using small tongs, weigh a crucible with 1 disk to the nearest 0.0001 mg and record the weight.

3. Tare the scale.

4. Weigh 5.0 grams of sample to the nearest 0.0001 mg into the tared crucible and record the weight of the sample.

5. Wearing the Phoenix System gloves, place the crucible with sample and disks in the furnace using the large tongs and ash for 20-35 minutes.

6. Remove the crucible from the furnace using the large tongs and place them onto the small lunch tray immediately after removal. The samples will be cooled by the time all the samples are removed from the furnace.

7. Immediately re-weigh the crucible containing the ash and the disks to the nearest 0.0001 mg and record the weight. DO NOT leave the crucibles on the counter to cool; they must be weighed immediately to avoid any uptake of moisture from the environment.

8. Calculate the % ash in an Excel spreadsheet by using the formula:

$$\% \text{ Ash} = \frac{\text{Post ashing weight} - \text{crucible and disk weight}}{\text{Sample weight}} \times 100$$

Moisture and Fat

Supplies Needed

Multiple pairs of Nitrile Gloves
1 set of small tweezers
189 CEM weigh pads
Eggs for Sampling
30 small Turkey Basters
189 CEM plastic sheets

Procedure

1. Press enter to get the Smart Trac out of sleep mode.

2. Press # 3 for "Load Method"

3. Choose either #1 for Albumen samples or # 2 for Whole Egg or Yolk samples.

4. Once a method is chosen, select "Ready" to run a test.

5. Choose the "Sample ID" option and type the name of the sample to be run, pressing "Ready" when finished typing.

6. Wearing a pair of Nitrile gloves, with tweezers, place 2 CEM weigh pads on the CEM balance inside the machine and close the lid.

7. Press "Tare" to tare the pads on the balance.

8. Once tared, place 2.0 and 2.5 grams of sample evenly onto the pads and record the weight onto the prepared spreadsheet. Close the lid and allow the test to run (approx 5 min for albumen samples and 10 min for whole egg or yolk).

9. Once test is complete, the screen will read the moisture content of the sample. Record this % Moisture reading onto the spreadsheet.

10. Remove the sample from the CEM with tweezers and roll out 1 sheet of plastic wrap from the CEM station.

11. Place the sample on the plastic wrapper and roll the sample length wise until you have a tube shaped sample.

12. Place the sample and plastic wrap into the holding tubes and compress using the compression tool provided by CEM. Be careful not to push the sample out of the end of the holding tube.

13. Remove the oil standard from the Smart Trac and replace with the holding tube containing the sample.

14. Press “continue” to run the test.

15. Once test is complete the screen will give you the fat %. Record the % Fat onto the spreadsheet and discard the sample by pushing it out of the holding tube with the instrument provided with the machine.

Albumen Whipping Height

Supplies Needed

6 5 quart Artisan style Kitchen Aid mixers
12 5 quart Kitchen Aid mixer bowls
12 Kitchen Aid balloon whisks
6 clear plastic 12 inch rulers
6 timers
12 250 mL graduated cylinders
6 rubber tipped spatulas
16.2 liters of liquid, room temperature albumen (10.8 liters of unpasteurized albumen, 5.4 liters of pasteurized albumen, 5.4 liters of pasteurized albumen with whipping agent.)

Procedure

1. Before adding sample, allow the Kitchen Aid mixer with balloon whisk attachment to run for 1 min. at speed 10.

2. Measure 200 mL of room temperature albumen sample into a graduated cylinder and place in the mixer bowl.

3. Whip the albumen for 45 seconds at speed 6, followed by 45 seconds at speed 10 to get a stiff peak foam.

4. Remove the whisk and mixer bowl from the mixer.

5. Level the foam with the rubber tipped spatula, making sure not to crush or disrupt the foam.

6. Insert a 12 inch clear ruler into the center of the bowl and measure the foam height to the nearest millimeter.

7. Record the measurement on the prepared Excel spreadsheet.

Angel Food Cakes

Supplies Needed

108 Teflon coated cake pans with volume inscribed on side
6 Artisan style 5 quart Kitchen Aid Mixers
12 5 quart Kitchen Aid Mixer bowls
12 Kitchen Aid balloon whisks
6-12 hand whisks
1 sharpie marker
1 roll colored scientific tape
1 package post-it notes
6 rubber tipped spatulas
6.48 liters of liquid albumen each week
32.4 grams salt each week
97.2 grams cream of tartar each week
6624 grams (approx **14.6 lbs**) sugar each week
2376 grams (approx **5.2 lbs**) cake flour each week
72 mL Vanilla each week
2 sifters
4 bowls for sifted materials
20 weigh boats for measuring
12 250 mL graduated cylinders
1 10 mL graduated cylinder

Ingredient List

Homogenized Albumen	180 mL
NaCl	0.9 grams
Cream of Tartar	2.7 grams
Fine Sugar	138 grams
Vanilla	2 mL
Granulated Sugar	46 grams
Cake Flour	66 grams

Procedure

**** Pre-Heat the Oven to 350°F before beginning procedure.

1. Gently hand shake the bags containing the albumen samples for 30 seconds.

2. Sift the cream of tartar and NaCl together.

3. Place 180 mL of room temperature albumen into the mixer bowl and add the sifter NaCl and cream of tartar mixture.
4. Mix at speed 10 for 45 seconds.

5. Add the fine sugar to the batter in 3 increasingly larger portions, mixing at speed 6 for 4 seconds after each addition.

6. Add 2 mL vanilla while mixing at speed 6 for 4 seconds.

7. Sift together the granulated sugar and cake flour **twice**.

8. Add the sifted cake flour and sugar mixture to the batter in 3 equal portions. After each addition, fold the mixture into the batter using the hand whisk with less than 20 strokes for each addition.

9. Weigh 75 grams of batter into a tared cake pan and label the pan using the scientific tape to denote which sample it is. Batter will make 3 cakes.

10. Place pans into the pre-heated oven and bake for 17 min.

11. Remove cakes from the oven and invert onto a cooling rack for at least 2 hours.

12. After cooling turn the pans upright and dust the tops of the cakes lightly with cake flour.

13. Record the volume in mL that is engraved on the side of the cake pan on the prepared Excel spreadsheet.

14. Fill the pan with rapeseed until the top is completely covered and level the pan using a ruler or spatula until the rapeseed is flush with the top of the pan.

15. Measure the rapeseed into a graduated cylinder with a funnel, and record the volume in mL on the spreadsheet.

16. **DO NOT** discard cakes after seed volume is taken.

TPA Measurement for Angel Food and Sponge Cakes

Supplies Needed

Texture Analyzer
Spatula
Garbage Can

Procedure

1. Turn on the Texture Analyzer by flipping the switch on the back of the base of the machine.

2. Open the Texture Analyzer program icon located on the desktop.

3. Login to the program using MCKEE as the username and MCKEE as the password.

4. Click the “Project List” icon

5. Choose the “Personal Projects” option from the list.

6. Click the “TPA” option.

7. Next, choose the “TA” option from the menu at the top of the screen.

8. Scroll down that list and choose “calibrate force”. *** Make sure the proper load cell is in the machine (5 kg).

9. After calibration, choose the “Run a Test” option.

10. On the test screen, choose “Path”→ Browse → My Documents → and create a new folder with the date of the test → open the folder.

11. Make sure auto save is checked to ensure that your data will be saved.

12. Place the cake on the machine, and run the test.

13. After the test is complete, the cake can be discarded.

Custards

Supplies Needed

81 4 oz ramekins
81 100 mL glass beakers
6 Kitchen Aid Mixers
12 mixer bowls
12 balloon whisks
6 timers
1 roll parafilm
4 large baking pans
81 plastic storage containers
5 boxes of 100 whatman papers
1 sharpie marker
Post-it notes
Baking racks
Saucepans
6.75 liters of whole egg
13.5 cups sugar
40.5 teaspoons vanilla
6.75 teaspoons salt
81 cups milk (approx. **6 gallons** milk)
1 can Pam Cooking Spray
6-12 graduated cylinders
Measuring cups

Procedure

1. Pre-heat oven to 350°C.

2. In a mixer bowl, add 250 mL of whole egg, .5 cups sugar, 1.5 teaspoons vanilla, and .25 teaspoon of salt. Blend for 1 min at speed 4 with the Kitchen Aid mixer.

3. Heat milk in a saucepan until very hot, stirring occasionally so milk does not stick to the pan.

4. With the mixer on speed 4, slowly add 3 cups of heated milk, turning off the mixer once all milk has been added.

5. Lightly spray 3 ramekins with Pam spray and label using a sharpie marker.

6. Add 100 mL of the mixture into the ramekin.

7. Using 3 100 mL beakers, place 60 grams of the mixture into the beakers.

8. Place beakers and ramekins into a large baking pan and fill with very hot water to within ½ inch of the top of the ramekins.

9. Bake at 350°C for 40 min.

10. While custards are baking, place 5 whatman papers on a tared container lid and record the weight to the nearest 0.0001 gram. Place a post-it note with the weight of the papers on the top of the lid of the plastic container.

11. Remove the beakers and ramekins from the oven and place on a wire cooling rack until they reach room temperature.

12. Once the beakers reach room temperature, cover the tops of the beakers with parafilm and place in the walk-in cooler (4°C) for 18 hours.

13. When the ramekins reach room temperature, gently run a knife around the edge of the ramekin to loosen the custard. Invert the ramekin over the whatman papers until it drops in the center of the paper. Place the lid over the container with the custard and place in the cooler for 18 hours.

14. After 18 hours, remove the custards in the plastic containers from the cooler and transport to the lab. Gently remove the lid and discard the custard in the garbage can, making sure not to tear the paper. Weigh the paper to the nearest 0.001 gram to determine the amount of weep from the custard.

15. Remove the beakers from the cooler and perform Bloom test using texture analyzer.

Bloom for Custards

Supplies Needed

Texture Analyzer
Spatula
Garbage Can

Procedure

1. Turn on the Texture Analyzer by flipping the switch on the back of the base of the machine.

2. Open the Texture Analyzer program icon located on the desktop.

3. Login to the program using MCKEE as the username and MCKEE as the password.

4. Click the “Project List” icon

5. Choose the “Personal Projects” option from the list.

6. Click the “Bloom” option.

7. Next, choose the “TA” option from the menu at the top of the screen.

8. Scroll down that list and choose “calibrate force”. *** Make sure the proper load cell is in the machine (5 kg). Follow directions on screen.

9. After calibration, choose the “Run a Test” option.

10. On the test screen, choose “Path”→ Browse → My Documents → and create a new folder with the date of the test → open the folder.

11. Make sure auto save is checked to ensure that your data will be saved.

12. Place the custard on the machine, and run the test.

13. After the test is complete, the custard can be discarded.

Sponge Cakes

Supplies Needed

6 Kitchen Aid Mixers
12 mixer bowls
12 balloon whisks
6 250 mL glass beakers for weighing egg
6 hand whisks
12 graduated cylinders
1 10 mL graduated cylinder
81 Teflon coated cake pans
1 roll scientific tape
Sharpie markers
Post-it-notes
3726 grams whole egg (approx **8.2 lbs**)
2413.8 grams sugar (approx **5.3 lbs** sugar)
54 mL vanilla
1387.8 grams cake flour (approx. **3.05 lbs**)

Procedure

1. Pre-heat oven to 350°C.

2. Stomach whole egg for 30 seconds at 200 rpm to homogenize.

3. Place 138 grams whole egg and 89.4 grams sugar into the mixer bowl.

4. Mix for 15 seconds at speed 2, then for 4 min. at speed 10.

5. Add 2 mL vanilla and mix at speed 6 for 4 seconds.

6. Add the cake flour to the batter in 4 equal portions, folding in after each addition with less than 20 strokes.

7. Weigh 75 grams of batter into a tared cake pan.

8. Place pans in the oven and bake for 17 min.

9. Remove the pans from the oven and invert on a cooling rack for at least 2 hours.

10. After cooling, turn the pans upright and lightly dust the tops with flour.

11. Fill the pan with rapeseed until the top is mounded and then level off until the seed is flush with the top of the cake pan.

12. Record the volume on the side of the pan.

13. Measure the rapeseed into a graduated cylinder by using a funnel and record the volume.

14. DO NOT remove the cakes from the pans after rapeseeding.

15. Save cakes for TPA analysis on the texture analyzer.