VALIDATION OF COOKING METHODS USING SHELL EGGS

INOCULATED WITH *SALMONELLA* SEROTYPES

ENTERITIDIS AND HEIDELBERG

Except where reference is made to the work of others, the work described in this thesis is my own or was done in collaboration with my advisory committee. This thesis does not include proprietary or classified information.

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VALIDATION OF COOKING METHODS USING SHELL EGGS INOCULATED WITH *SALMONELLA* SEROTYPES ENTERITIDIS AND HEIDELBERG

Alexis Larrisa Davis

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Alexis Larrisa Davis, daughter of Danny and Geneva Sanders, was born August 24, 1981 in Indianapolis, Indiana. She graduated from Jacksonville High School in Jacksonville, Alabama in May 1999. She attended Auburn University in Auburn, Alabama majoring in Animal Sciences in the Muscle Foods Option. She graduated in May 2004 with a Bachelor of Science in Agriculture degree. In August 2004, she entered Graduate School at Auburn University under the direction of Patricia Curtis, Ph.D.

THESIS ABSTRACT

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Salmonella Enteritidis has been associated with eggs and egg products for a long time. However, *Salmonella* Heidelberg is now also being associated with eggs and egg products. Experiments were therefore done to determine which cooking methods were considered to be safe. The following American Egg Board cooking methods were chosen: hard and soft cooked, scrambled, over-easy, sunny-side-up, and poached using a pan insert and the free flowing method.

Shell eggs were purchased from the grocery store and candled for cracks. The eggs were then inoculated with a *Salmonella* cocktail and cooked. The microbiological testing was done using the USDA approved methods for *Salmonella* recovery.

Findings indicate that the AEB hard cooked, soft cooked, and poaching methods are safe. The same is not true for the over-easy and scrambled egg cooking methods, in which SE or SH were recovered from cooking product. The over-easy came back positive for SH, and scrambled came back positive for SE PT4 and PT30. The free poached egg cooking method came back positive for SH and SE PT4 in two of the 30 cooked samples. Given this low frequency of SE and SH recovery, the over-easy and scrambled cooking methods can be considered reasonably safe. The free poached method could be considered safe if cooked to the maximum of five min. The sunny-side-up cooking method had a total of 19 of 30 samples that were *Salmonella* positive after cooking, with some samples having a combination of multiple salmonellas. The sunny-side-up method should be considered unsafe.

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I. INTRODUCTION

Salmonella enterica serotype Enteritidis is one of the most common *Salmonella* serotypes worldwide, particularly in developed countries (Patrick, et al., 2004). Case control studies of sporadic human *Salmonella* outbreaks and infections showed that shell eggs were a major risk factor for disease (Mishu, et al.1994; St. Louis, et al.1988; Passaro, et al.1996). However when examining the natural occurrence of *Salmonella* in laying hens, it was found that the incidence of *Salmonella* Enteritidis (SE) is low in eggs. It was found by Poppe et al. (1992), a Canadian researcher, that less than 0.065% of eggs tested were positive for SE (roughly two positives from a sample size of 16,000 eggs). In fact, in studies done by a European researcher Humphrey et al. in 1989a and 1991 *Salmonella* serotypes other than SE were also isolated from eggs.

Salmonella Heidelberg (SH) like other nontyphoidal salmonellae appears to be mostly associated with food. An average of 2,180 cases of SH infection were reported annually in the US between 1993 through 1997, which accounted for ~6% of all *Salmonella* culture confirmed infections (CDC, 1998). A study done by Schoeni et al. (1995) showed growth and penetration of SH in the egg, and SH can also been found on eggshells (Jones et al., 1995). In an infection study conducted by Gast et al. (2004), SH was shown to invade reproductive tissues of laying hens which could then be deposited inside the developing egg similar to that seen with SE.

It has been difficult to determine if SH infections are a result of a contaminated eggshell or eating intact eggs contaminated transovarian. Because of the potential presence of *Salmonella* in shell eggs, numerous reported outbreaks have been associated with eating raw or undercooked eggs (Anonymous, 1990; Anonymous, 1996a, 1996b). Several cooking studies done by Humphrey et al.(1989b), Baker (1990), and Tharrington et al. (2003) show that some cooking methods will destroy *Salmonella* if present while others will not.

The seven cooking methods: hard and soft cooked, scrambled, over-easy, sunny-side-up, poached and free poached were chosen to determine if they were adequate for the destruction of SE and SH. The cooking methods used came from the American Egg Board (AEB) website (http:www.aeb.org).

Microbiological tests were evaluated when determining whether a cooking method could be considered safe, reasonably safe, or unsafe.

II. LITERATURE REVIEW

Salmonella

Salmonella, a member of the family Enterobacteriaceae, is a gram negative facultative rod. These microorganisms are relatively small (about 0.5µm by 2 to 3 µm) in size with the motile strains having peritrichous flagella. A lack of lactose fermentation, fermentation of glucose with the gas production, and the production of hydrogen sulfide (H_2S) from thiosulfate are characteristics usually used to differentiate *Salmonella* from other family members. Even though *Salmonella* can grow in a wide range of temperatures it has been found that the optimum growing temperature is 37°C (Bierer et al., 1961).

Salmonella Enteritidis

Salmonella Enteritidis (SE), *Salmonella* Typhimurium (ST), and *Salmonella* Heidelberg (SH) has accounted for most of the human salmonellosis cases in the United States (Altekruse et al., 1997). *Salmonella enterica* serotype Enteritidis is one of the most common *Salmonella* serotypes worldwide, particularly in developed countries (Patrick, et al., 2004). Due to its presence in eggs and its heat resistance, *Salmonella* is tested for in both shell eggs and pasteurized egg products (Froning et al., 2002).

Case control studies of sporadic *Salmonella* outbreaks and infections showed that shell eggs were a major risk factor for human foodborne disease (Mishu, et al., 1994; St. Louis, et al., 1988; Passaro, et al., 1996). However, when examining the natural occurrence of *Salmonella* in laying hens, it was found that the incidence of SE is low in eggs.

Poppe et al. (1992) reported less than 0.065% of eggs tested were SE positive (roughly two positives from a sample size of 16,000 Canadian eggs). In December 1996, in response to the increasing occurrences of human illnesses associated with the consumption of shell eggs the Food Safety and Inspection Service (FSIS) began a comprehensive risk assessment of SE. The objective of this risk assessment was to establish the complete risk of foodborne illness from SE, then to evaluate and identify potential strategies for reduction of the risk, after which needs and data were identified, and then lastly prioritize efforts for future data collection (USDA, 1998). In studies done by Humphrey et al. (1989a; 1991), other *Salmonella* strains, ST and SH, not just SE were found in eggs.

Salmonella Heidelberg

SH like other nontyphoidal salmonellae appear to be mostly associated with food products containing eggs and poultry was identified as the cause of most of the outbreaks (Chittick et al., 2005). An average of 2,180 cases of SH infection were reported annually between 1993 and 1997, which accounted for about six percent of all *Salmonella* culture confirmed infections (CDC, 1998). A study done by Schoeni et al. (1995) demonstrated growth and penetration of SH in the egg; and Jones et al. (1995) found SH on eggshells.

It has been difficult to determine if SH infections are a result of a contaminated eggshell or eating intact eggs internally contaminated prior to oviposition. SH has been found in the ovaries and peritoneum of chickens used for egg laying and in chicken manure (Snoeyenbos et al., 1969; Gast et al. 2004; Keller et al., 1997; Okamura et al., 2001). In an infection study conducted by Gast et al. (2004), SH was shown to invade reproductive tissues of laying hens which could then be deposited inside the developing egg similar to that seen with SE.

Presence of *Salmonella* in laying hens

In a study done by Henzler et al. (1994) some egg associated SE outbreaks were traced back to the layer houses. When sampled, the same phage types of SE from the outbreak matched the culture taken from the eggs on the farm. Gordon and Tucker (1965) were able to demonstrate that the *Salmonella* spp. has the ability to pass from the alimentary canal, via the blood, to the ovaries. This phenomenon shows that contamination of SE can occur in the reproductive tract, with the outside of vitelline membrane, and the surrounding albumen being the most important sites of contamination (Humphrey 1994; Humphrey and Whitehead, 1993).

While the occurrence of SE can happen in the reproductive tract naturally, Miyamoto et al (1997) did a study where hens were inoculated via different routes to examine where contamination in the oviduct was occurring. When inoculation was done intravenously, colonization of the ovary occurred causing the eggs that were forming in the oviduct to become contaminated. After the intravaginal inoculation, it was shown that colonization of the lower portions of the oviduct took place which still resulted in the

internal contamination of the egg. Therefore, contamination may be occurring due to the penetration of the shell in the oviduct and not just the colonization of the ovary. The occurrence of contamination of eggs as they entered the lower oviduct was also reported by Keller et al. (1995). Another study was done where contaminated hens and noncontaminated hens were penmates, as result the noncontaminated hens became contaminated and laid infected eggs (Gast and Beard, 1990). SE in the environment can possibly lead to SE in the hens and then in the eggs produced by those hens.

Presence of *Salmonella* in eggs

It has been shown that SE and SH can be isolated from the ovaries and fecal matter of chickens (Padron, 1990; Humphrey et al., 1991; Gast and Beard, 1992). Therefore, the contamination of shell eggs from the hen is possible. A study by Schoeni et al. (1995) was done to show the growth and penetration of SE, SH, and *Salmonella* Typhimurium (ST) in eggs. The serotypes were inoculated into the yolk or albumen and monitored at temperatures 4, 10 and 25°C. In this study, inoculation site did not affect bacterial growth, and populations of all strains showed an increase of 10^3 cfu/g (3 logs) or more in just one day regardless of inoculation site when incubated at 25°C. Moreover, these researchers demonstrate the potential for penetration through the shell by all strains when they were applied via feces to shell. Upon penetration of the shell an increase of three to five $\log_{10}$ to cfu/g was observed for SE, ST and SH in the yolk and the albumen when eggs were held at 25°C for 24h, and growth continued with additional storage time (Schoeni et al., 1995). However, it has also been shown that when SE is located outside of the vitelline membrane in the surrounding albumen (Gast and Beard, 1990; Humphrey,

et al., 1991; Conner, et. al., 2003), the SE has remained dormant until storage related changes in the vitelline membrane induce rapid growth (Humphrey, 1994).

It has been shown that factors in the albumen have an inhibition effect on *Salmonella*. This may explain why low numbers of cells observed in eggs laid both by natural and artificially inoculated hens did not reach the high growth levels exhibited in artificially inoculated eggs (Bradshaw et al., 1990; Humphrey, 1990; Humphrey et al., 1991; Stephenson et al., 1991). Movement of *Salmonella* through the albumen into the yolk has an increased chance of occurring over an extended amount of time as the integrity of the albumen viscosity and vitelline membrane decreases. This can occur faster if temperatures are elevated (Humphrey and Whitehead, 1993; Hara-Kudo et al., 2001; Latimer et al., 2002; Messens et al., 2004).

A recent study done by Gast et al., demonstrates that when SE and SH are placed directly on the yolk and stored at 20°C and 30°C for 36h that these organisms will penetrate into the yolk (Gast et al., 2007). In many of the outbreaks associated with SE, holding eggs at room temperature over a period of several hours have been an implicating factor (Lin et al., 1988). It has been shown that temperature has a great impact on the microbial growth of SE on the outside and in the contents of shell eggs (Curtis et al., 1994; Frazier 1967; Hillerman, 1955; Humphrey, 1990; Kim et al., 1989; Kinner et al., 1981; Thornton, 1991).

Salmonellosis

Because of the potential presence of *Salmonella* in shell eggs, numerous reported outbreaks have been associated with eating raw or undercooked eggs (Anonymous, 1990;

Anonymous, 1996a, 1996b). The association of salmonellosis with eggs is not a new problem by any means. The Egg Products Inspection Act of 1970 (Sanders et al., 1963; CDC, 1964) was passed as a requirement that bulk egg products would be pasteurized and the shell eggs would be federally supervised for the inspection of cracks before they left the plant (Anonymous, 1987).

A *Salmonella* infection associated with a foodborne outbreak usually leads to gastroenteritis. When one to ten cells of a disease causing strain are ingested, penetration of the small intestine epithelial lining can occur. Destruction of the epithelial lining is caused by the growth of *Salmonella* in the underlying tissues. Salmonellosis has even been linked to reactive arthritis. *Salmonella* usually needs an incubation period ranging from 8 to 72 hours after which the victim will suffer from abdominal pain and diarrhea accompanied sometimes with a fever. In healthy adults, this is a self limiting disease. Since this infection usually resolves itself in five to seven days, treatment is not given in most cases, unless the patient becomes severely dehydrated or the infection moves to the intestines. If rehydration is needed, it is usually done so by a treatment of intravenous fluids. If the infection does spread to the intestines, antibiotics such as ampicillin, gentamicin, trimethoprim/sulfamethoxazole or ciprofloxacin can be used (CDC, 2005). However, it can be life threatening in the elderly and small children (Zwadyk, 1992; D'Aoust, 1997; Levine et al., 1991).

Heat Resistance of *Salmonella*

When using some cooking methods such as frying (over-easy and sunny-side-up), poached, and free poached it is important to make sure that the white is completely

cooked with the yolk being firm, but not hard, also scrambled eggs should not be runny when cooked (AEB, 2007). However the facts sheet found on USDA.gov (USDA, 2006) suggest that if a recipe requires the use of raw eggs, such as homemade ice cream and eggnog that a cooked egg-milk mixture should be used instead. The mixture should be heated gently to temperature of 70°C (160°F). Another alternative to the cooked egg milk mixture is to use a pasteurized egg product when using a recipe that requires the use of raw unpasteurized eggs. All other recipes containing eggs should also be cooked to 70°C (160°F).

The standard temperature for the inactivation of Salmonella is 63°C. All cooked eggs and dishes containing eggs should be served immediately after cooking, or placed into shallow containers for quick cooling and refrigerate at once for later use. These eggs should then be used within 3 to 4 days. It has also been mentioned here and several other places that employees and anyone that handles food should wash there hands, utensils, equipment and work areas with hot, soapy water before and after contact with eggs.

Cooking Methods and Effects on *Salmonella*

Cooking is achieved via heating. The energy used for heating is transferred by conduction. The conduction heating method is best described by the heat moving from one particle to another by contact (Potter, 1986). This would include the fried, boiled, and poached eggs methods. The fried and poached methods use direct heat from the pan to cook, while the hard and soft cooked and free poached methods get their heat for cooking from the boiling water.

Hard and Soft Cooked: The hard and soft cooked method is often done by placing a single layer of eggs in a sauce pan. Enough tap water is then added so that there is at least 2.54 cm (1 in) of water above the eggs. The sauce pan is then covered while the contents come to a boil. After the water has begun to boil the heat is turned off and the sauce pan is removed from the burner to prevent further boiling and over cooking of the egg. The eggs should stand covered, in the hot water for 15 min for large eggs, the soft cooked method the eggs should stand for 5 min. Cold water should be run over the eggs immediately or the eggs should be placed in ice water until cooled, this is used to stop the cooking process of the egg.

If the cooking process is not stopped when cooking hard cooked eggs a greenish ring can appear on the yolk. This reaction is not harmful, but merely undesired. The greenish ring is caused by a natural reaction on the surface of the yolk by sulfur and iron compounds; which occurs when the egg is either cooked too long or when there is a high iron content in the water used to boil the eggs, because soft cooked eggs are not in the water as long as hard cooked the greening of the yolk is not a problem. To remove the shell, the egg should be tapped gently all over, and then rolled in between hands or on a hard surface to loosen the shell.

When peeling start at the large end of the egg. Holding the egg under running cold water or dipping it in a bowl of water will help aid in shell removal. Hard cooked eggs that are still in the shell maybe kept in the refrigerator for up to one week, if the eggs have been shelled they should be used immediately (AEB, 2007). In a study done by Humphrey et al. (1989b) survival of SE Phage Types (PT) 4, 8, and 13a, ST PT 141 and 110, and S. Seftenberg 775W was examined in inoculated eggs using the boiling,

frying and scrambled cooking methods. When the egg was boiled for 4 min, all four of the test strains survived, with the S. Seftenberg 775W being the most recoverable from the cooked yolk. However, when the eggs (inoculated at 10^5cfu/g) were boiled for nine min (until the yolk solidified) no *Salmonella* were recovered. This was also true for when the eggs were boiled for ten min and inoculated at 10^7cfu/g.

Chantarapanont et al. (2001) compared the effectiveness of the killing of SE in the yolk of eggs that were hard-cooked using the methods recommended by the American Egg Board (AEB) to that of the cooking method used by Humphrey in his 1989 study. U.S. Grade A medium and extra large eggs were stored at 5°C for no more than five days. When using the AEB method, six eggs were brought to a boil in a 3-quart (2.84 liter) saucepan on an electric stove within approximately eight min. The pan was then removed and allowed to set at ambient temperature (23 ± 2°C) by immersion into an ice-water bath for no more than 15 min. The yolk was analyzed for SE at 0-15 min at 3 min increments after removal from the burner.

In comparison, Humphrey et al. (1989b) placed six eggs into boiling water and analyzed after 3-15 min in 3 min intervals, and the pan and water stayed on the burner throughout the 15 min. The eggs from both methods were cooled by immersion into ice-water slurry and held for no longer than 15 min once they were removed from the hot water. In medium eggs cooked by AEB method, regardless of the initial egg temperature, SE PT34 or 4 were not recovered. However, in the extra large eggs, SE PT34 was found in three of the six eggs cooked with the initial temperature of 10°C when eggs were removed after three min holding time. SE PT 4 was recovered from four of six extra large eggs that were held at three min and from two of six extra large eggs that were held

at six min. All of these eggs had an initial temperature of 10°C. For the eggs that were cooked using the Humphrey et al. (1989b) method and only placed into boiling water for three min regardless of initial temperature were found to be positive for both SE PT 34 and PT 4. Only one medium egg of six with the initial temperature of 10°C came back positive for PT34. SE PT4 was recovered when the eggs were placed into the boiling water for six min.

When looking at the extra large eggs that were placed into boiling water for six min, six of the six cooked with an initial temperature of 10°C came yielded SE PT's 34 and 4. Only two of the six eggs with the initial inoculation temperature of 21°C were positive for SE PT 34. For the extra large eggs with the initial temperature of 21°C three of the six were positive for SE PT 4. When held in the boiling water for nine min only one of the six eggs with the initial temperature of 10°C was positive for PT4, while all other samples at this temperature were negative.

All other eggs, regardless of inoculation temperature, that were held in the boiling water for 12 or 15 min came back negative for both phage types of *Salmonella*. When looking at the results of both cooking methods, it appears SE PT 4 was the most heat resistant. It is evident that the size of the egg and initial temperature have an inverse relationship and both play an important role in determining the death rate of SE in the egg yolk of shelled eggs. It also demonstrated that bringing the eggs and water to a boil together is more effective than placing the eggs in water that is already boiling.

In 2003, Tharrington et al. (2003) evaluated the safety of egg consumption based on various cooking methods using heating curves and the standard of a inactivation of *Salmonella* at 63°C. When looking at the hard and soft cooking methods, it was

determined that both methods reached 63°C, which makes them acceptable for the destruction of *Salmonella* should it be present.

In a study performed by Licciardello et al.(1965), the hard-boiled cooking method was executed by placing eggs in water at 20°C (68°F) bringing them to a boil and then allowing them to simmer. It took 15 min for the eggs to reach the recommended *Salmonella* inactivation temperature of 61°C (which is 142°F), demonstrating that for large sized eggs the recommended cooking times of 15-20 min was more than adequate for the inactivation of *Salmonella* using the hard-boiled method. In another study done by Baker (1990), seven min were need for the complete killing of the *Salmonella* from inoculated (10^8 cfu/egg) eggs.

Scrambled: For the preparation of scrambled eggs, it is suggested that two eggs and 30mLs milk be beaten together until blended. To get fluffy eggs you should beat them vigorously to incorporate air. Eggs should be cooked in a 17.78 cm (7 in) to 20.32 cm (8 in) omelet pan by heating one teaspoon of butter or cooking spray over medium heat, until a drop of water sizzles. The egg mixture should be poured into the ready pan. As mixture sets, an inverted spatula may be used to gently draw mixture across the bottom and sides of the pan, as large soft curds begin to form.

Eggs should be cooked until they have thickened with no visible liquid egg remaining. When cooked in large batches scrambled eggs may have a chemical reaction (from the formation of sulfur in the egg white and ferrous sulfide from the iron in the yolk) that may occur when the eggs are cooked at high temperatures or cooked in an cast iron skillet, or the combination of the two. This reaction is harmless and causes the eggs

to take on a greenish hue which may be undesirable to the consumer. This discoloration can also be prevented by serving eggs as soon as possible after they have been prepared. Holding eggs over a direct heat source may also contribute to the greening. However, eggs may be stored for a short period of time by placing the pan of eggs on top of a pan of hot water.

When scrambled eggs are held too long they may become dry, should this occur the cooked eggs should be discarded and new ones cooked. Raw eggs should not be added to eggs that have been previously cooked to "refresh" them (AEB, 2007). Humphrey et al. (1989b) studied the survival of SE, ST, (egg associated salmonellas) and S. Seftenberg during scrambled egg cooking method. Eggs were cooked in a saucepan with melted margarine or vegetable oil. The eggs were prepared using two methods. The rapid cooking method (over a high flame) or the slow cooking method (over a low to moderate flame) in a Panasonic 600 w microwave oven until no visible liquid remained.

The decision of which cooking method to use was dependent on personal preference. The time and temperatures for this cooking method were measured in the center of the egg. It was determined from this study that a rapid method of cooking was more effective than a slow method. Of the 15 eggs that were inoculated with the egg associated salmonellas, none came back positive. For those inoculated with S. Seftenberg, two of the three samples came back positive. When the slow method was used, 29 of the 30 eggs cooked tested positive for *Salmonella*. The rapid cooking method was much more effective at destroying the salmonellas then the slow cooking method (Humphrey et al., 1989b).

In a 1990 study done by Baker, eggs inoculated in the yolk with 10^8 cfu SE/egg were scrambled in an electric frying pan at 121°C. Under these conditions, scrambled eggs considered *Salmonella* free required cooking for one min. However Tharrington et al. (2003) determined that after one minute of cooking the coolest surface temperature recorded was 48°C, which is not adequate to destroy *Salmonella*, but after two min most of the liquid egg portions had reached 67°C which would be considered safe. After three min with no visible liquid and the production of soft curds the final temperature ranges were 74-80°C. This would make scrambled eggs that were cooked between two and three min safe for consumption.

Over-easy: For preparation of the over-easy cooking method, a 17.78 cm (7 in) to 20.32 cm (8 in) omelet pan should be heated over medium high heat with 5mL to 30mL butter, cooking oil, or cooking spray until a drop of water sizzles. The eggs should be broken out and slipped into the pan. It is common for two eggs to be cooked at the same time. For this cooking method, fresh eggs will produce the best shape. According to the USDA Egg-Grading Manual, fresh eggs will have an egg white that has an elongated oval shaped with a cloudy white color and a thick viscous texture. The white should stay close around a tall standing yolk (USDA Egg-Grading Manual, 2000). The heat should be immediately reduced to prevent the burning of the egg white. Brown lacey edges around the egg white signify that the protein in the white has been overcooked.

While cooking eggs, the pan should be covered tightly with a lid. The eggs should be cooked slowly until the whites are completely set and the yolks have begun to thicken but are not hard. The eggs should be gently turned over using a spatula to cook

the second side. To prevent the sticking of the egg, a nonstick and cooking spray can be very helpful (AEB, 2007). In Humphrey's (1989b) study for the over-easy cooking method, eggs were broken out into sterile Petri dishes and inoculated into the center of the yolk with a syringe and needle. The egg was then cooked in a frying pan containing vegetable oil heated to a temperature of approximately 120°C. The cookers were asked to make sure that the whites were solid and opaque which took about 1.5 min to 2 min. The eggs were then turned over and cooked for up to 1 minute longer. Cooking temperatures and times were measured in the center of the eggs. ST PT 141 was not recovered in any eggs. Phage Type 110 was recovered from four of the nine eggs. SE PT 4 was recovered in five of the nine eggs and S. Seftenberg was recovered in eight of the nine eggs cooked. For the salmonellas that were recovered from this method, a further enrichment had to be done to recover the organisms. This indicated that the organisms present had ten cells per gram left after cooking.

In the Baker (1990) study, 46 commercial egg farms were sampled by gathering 100 eggs from facility. The eggs were inoculated (10^8 cfu/g) in the yolk through the shell, and incubated at 12°C for 24 h, and cooked over-easy in an electric frying pan was set at 121°C. The eggs were cooked until they appeared to be visually done. Under these conditions a total of five min cooking time to produce an SE-free egg. The five min total time describes a cooking method where the egg is cooked for three min and then turned over and cooked for an additional two min. When Tharrington et al. (2003) performed this cooking method, the eggs were fried for five min on one side before they were turned over. It was determined that typical consumers fry eggs between one to five min which has been determined inadequate for destruction of *Salmonella*.

Sunny-side-up: For preparation of the sunny-side-up cooking method, a 17.78 cm (7 in) to 20.32 cm (8 in) omelet pan should be heated over medium high heat with 5mL to 30mL butter, cooking oil, or cooking spray until a drop of water sizzles. The eggs should be broken out and slipped into the pan. It is common for two eggs to be cooked at the same time. For this cooking method, fresh eggs will produce the best shape. The heat should be immediately reduced to prevent the burning of the egg white. Brown lacey edges around the egg white signify that the protein in the white has been overcooked. While cooking, the pan should be covered tightly with a lid. The eggs should be cooked slowly until the whites are completely set and the yolks have begun to thicken but are not hard. The egg should then be gently lifted out of the pan using a spatula. To prevent the sticking of the egg, a nonstick and cooking spray can be very helpful (AEB, 2007).

In the 1989b Humphrey study, eggs were broken out into sterile Petri dishes and inoculated into the center of the yolk. The egg was then cooked in a frying pan containing vegetable oil heated to a temperature of approximately 120°C. The cookers were asked to make sure that the whites were solid and opaque which took about one and a half to two min. Cooking temperatures and times were recorded in the center of the eggs. All *Salmonella* serotypes and phage types survived after the sunny-side-up cooking treatment. In Baker's 1990 study, eggs were inoculated in the yolk through the shell at 10^8cfu/egg. The eggs were then incubated at 12°C for 24 hours prior to being cooked. The sunny-side-up cooking method was done with an electric frying pan set at 121°C. Eggs were cooked until they appeared to be visually, and the time needed for complete kill was seven min (which is not considered a sunny-side-up egg). Tharrington et al. (2003) determined that eggs cooked in this manner without the use of the basting

technique or a lid used to cover the cooking pan, did not reach 63°C in the amount of time that consumers use for this cooking method; thus it is concluded that temperatures adequate for SE inactivation were not achieved. Furthermore, there is no guarantee that the consumer will use the basting method or a lid when using this method.

Poached: The poaching method is usually done using a specifically designed poaching pan, which usually comes with an egg insert pan that should be removed so that the water can be poured into the pan. After the water has been brought to a boil, the heat should be reduced to keep the water gently simmering. The egg insert should then be sprayed with cooking spray and placed back into the pan. A cold egg should be broken into the egg insert and then the lid should be placed back on the poaching pan. The egg should be cooked for about three to five min until white is completely set and yolk begins to thicken but not harden. Using a small spatula to run along the edge of the egg insert to release the egg from the sides, the pan should then be inverted to remove egg (AEB, 2007).

Tharrington et al. (2003) used heating curves to determine that cooking times ranging between six to seven min produced the acceptable temperature of 63°C. As that temperature was reached, the albumen was set yet still soft and the yolk was a dark yellow color with about half of it gelling with the remainder still a liquid. When the egg was held for eight min the internal temperature reached 78°C and the yolk was solid with no remaining liquid. The yolk also began to turn light yellow, as seen in hard cooked eggs.

Free Poached: Free poached eggs may be prepared by bringing 5.1 to 7.6 cm of water, milk, broth, wine, tomato juice or any preferable liquid to a boil in a saucepan. Using a liquid other than water tends to stain the egg (tomato juice will tint the eggs red). The heat should be reduced to keep water gently simmering. Cold eggs should be broken into a custard cup, saucer or bowl. Fresh eggs are recommended for this cooking method as they hold their shape better. While holding the dish close to the surface of the water, the egg(s) is carefully slipped into the simmering water. It is easier to produce a nicely shaped egg when the water is left alone (stirring of the water is not needed). The egg(s) should be cooked until the whites are completely set and yolk begins to thicken but not harden. According to AEB (2007) this should take three to five min. A slotted spoon may be used to lift out the egg(s) out of the water. Egg(s) can be drained in the slotted spoon or on a paper towel. The rough edges can be trimmed if it is desired (AEB, 2007).

In the Baker (1990) study, for free poached method, the eggs were inoculated in the yolk through the shell with a 0.5 ml inoculum size containing 10^8cfu/egg. The eggs were then incubated at 12°C for 24 hours prior to being cooked. The egg temperature was taken every 15 seconds the eggs were cooked until they appeared to be visually done. When this boiling water method was used, it took five min to achieve the complete killing of the *Salmonella*.

Some of the studies used reference date back as far as 1989, because there have been changes in how eggs should be stored and cooked an update on cooking treatments was needed. The study done by Tharrington et al. (2003) was designed to investigate time-temperature relationship that would ensure the destruction of SE based on certain

cooking methods. However that study did not have an inoculation aspect to aid in proving the destruction of SE.

Statement of Research Objectives

The objective of this study was to test the effectiveness of American Egg Board (AEB) approved cooking methods (hard and soft cooked, scrambled, over-easy, sunny-side-up, poached, and free poached) on shell eggs inoculated with *Salmonella* Enteritidis and Heidelberg.

III. MATERIALS AND METHODS

The *Salmonella enterica* serotype Enteritidis (SE) phage types: 3, 4, 8, 9a, 22, 23, 24, 28 and *Salmonella enterica* Heidelberg (SH) phage type 11 used in the project were obtained from Dr. Richard Gast at the Egg Safety and Quality Research Unit, USDA Agricultural Research Service Russell Research Center in Athens, Ga., and from Dr. Charles Benson Professor of Microbiology Chief of Clinical Veterinary Microbiology at New Bolton Center at Pennsylvania State University. The original samples were both cryovialed (a loopful of sample was placed into a mixture of 70% TSB and 30% glycerol and added to a vial) and streaked onto tryptic soy agar (TSA) (Acumedia Manufacturers Inc., Lansing, Michigan) slants using a sterile loop. The cryovials were stored at -80°C; the slants were incubated for 24h a 37°C. To culture an experimented inoculum, a loop full was used to remove a small amount of *Salmonella* from the slant and placed into a test tube containing 10mL of sterile tryptic soy broth (TSB) (Hardy Diagnostics, Santa Maria, CA) and incubated at 37°C for 24h.

Preparation of Inoculum

For each SE and SH tested a 1/1,000,000 dilution of a 24h culture as described above was made using TSB as the diluent. A 3mL aliquot from each diluted culture were

combined in a sterile glass bottle to make the *Salmonella* suspension used for inoculation purposes of tested eggs. Confirmation of the bacteria level in the SE and SH suspension, was obtained by spiral plating technique (Microbiology International, Frederick, MD) using plate count agar and brilliant green sulfa agar (Hardy Diagnostics, Santa Maria, CA) that were incubated for 24 h at 37°C before counting colonies. After incubation, the plates were counted using the spiral function of the Q count plate reader (Microbiology International, Bethesda, MD).

Collection and Handling of Egg Samples

The eggs that were used in this study were purchased from a local grocery store on each Monday morning and stored in a refrigerator until they were needed later in the week. Eggs were candled with a Lyon Hi-Intensity Egg Candler (Lyon Electric Company Inc., Chula Vista, CA) to exclude any cracked and leaking eggs were discarded. Acceptable candled eggs were held at 4°C no more than five days prior to cooking for later use.

Cooking Methods

All cooking methods used in this study were taken from the American Egg Board (AEB) website (http://www.aeb.org). The time and temperatures were recorded for each method using a type K thermocouple (Digi-Sense via Cole-Parmer, Vernon Hills, IL) or infrared thermometer (Infrared/ Type K thermometer, Fisher Scientific, Pittsburgh, PA) attached to a Tempest Data Logger (Tangent Systems Inc., Charlotte, N.C.)

Hard and soft cooked eggs were prepared by placing a single egg into an empty sterile sauce pan (1.4 L) and adding sterile water until the egg was covered. Eggs were then cooked on a single hot plate (Toastmaster inc., Macon, MO). The water was brought to a boil, which took about 10 to 12 min, after which the pan was removed from the hot plate and allowed to sit for 15 min for hard cooked and five min for soft cooked.

Scrambled eggs were scrambled in a frying pan by intermittently scraping the bottom surface toward the center of the skillet using a sterile spatula. This was continued until no visible liquid remained (this took about two min) as recommended by the AEB.

The over-easy and sunny-side-up eggs were cracked into a preheated sterile 20.32cm (8 in) frying pan (heated to at least 121°C) and covered with a glass lid. The hot plate temperature was then switched from high to low. The eggs were cooked until the thick and thin albumen were completely set and the yolk began to thicken (~1.5min). After this took place, the lid was removed the egg was then turned over and cooked in a covered frying pan. The lid was then replaced for an additional thirty seconds of cooking. Sunny-side-up eggs were not turned after the original 1.5 min.

Poached and free poached eggs were cooked differently. For the poached method a sterile poaching pan with insert was put onto a hot plate and brought to a rolling boil (about 10-12 min). An egg was then removed from the refrigerator and cracked into the poaching insert (the insert was sprayed with a no-stick cook spray (Pam) to prevent egg from sticking and to simulate cooking at home). Immediately after cracking the egg into the poaching insert, the lid was placed on the pan. The hot plate temperature was changed from high to low. The egg was cooked for five min, which was the time it took for the thick and thin albumen to set and the yolk to thicken. Free poached eggs were

cracked and dropped into boiling water (Figure 1). The hot plate temperature was reduced from high to low. The egg was cooked until the thick and thin albumen was set (about three min). This method was modified from the original three to five min cooking time because at three min the egg has the characteristic described by the AEB to indicate doneness.

Temperature Measurement and Inoculation Procedures

For hard and soft cooked eggs a hypodermic type K thermocouple (Digi-Sense via Cole-Parmer, Vernon Hills, IL) attached to a Tempest Data Logger (Tangent Systems Inc., Charlotte, N.C.) was inserted through the egg shell in the large end of the egg through the air cell into the yolk of the egg with the aid of a Lyon Hi-Intensity Candler. Super glue was then used around the thermocouple to seal the hole in the eggshell. A sterile syringe was used to deposit 0.1mL of the *Salmonella* suspension through the large end of the egg, and air cell onto the vitelline membrane placement of the inoculums was confirmed with the aid of a Lyon Hi-Intensity Candler. After injection, the hole was sealed with super glue.

Scrambled eggs were aseptically cracked into a preheated sterile frying pan (heated to at least 121°C). Immediately after cracking the eggs into the frying pan an Eppendorf pipetter was used to deposit 0.1mL of the S*almonella* cocktail onto the vitelline membrane of each test eggs (two eggs were used). Temperature was measured using an infrared thermometer (Infrared/ Type K thermometer, Fisher Scientific, Pittsburgh, PA) every 5s. After two min. the frying pan was removed from the hot plate (Toastmaster Inc., Macon, MO) and the eggs were scraped into a pile about 5.08 cm (~2

in) tall. A thermometer was immediately inserted into the thickest portion of the scrambled eggs to get the final temperature.

After cracking the over-easy and sunny-side-up eggs into the frying pan, an Eppendorf pipette was used to deposit 0.1mL of the *Salmonella* suspension onto the vitelline membrane on the surface of the egg yolk. A wire type K thermocouple (Tangent Systems Inc., Charlotte, N.C.) attached to a Tempest data logger (Tangent Systems Inc., Charlotte, NC) was then placed into the vitelline membrane on top of the yolk and a glass lid was placed on the frying pan. When the egg was ready to be turned the lid was removed and the thermocouple gently removed so the egg could be turned. The thermocouple was placed back into the yolk through the cooked albumen, and the lid was then replaced for an additional thirty sec. The eggs cooked to sunny-side-up doneness were not turned.

Immediately after cracking the egg into the poaching insert, an Eppendorf pipette was used to deposit 0.1mL of the *Salmonella* suspension onto the vitelline membrane surface of the egg yolk. A wire type K thermocouple (Tangent Systems Inc., Charlotte, N.C.) was attached to a Tempest data logger (Tangent Systems Inc., Charlotte, NC) was then inserted into the vitelline membrane on top of the yolk and the lid was placed on the pan. For the free poached method a temperature curve was made separately from the set of eggs that were inoculated. For the temperature curve, 30 eggs were used. The eggs were cracked into a ladle and the wire type K wire thermocouple (Tangent Systems Inc., Charlotte, N.C.) attached to a Tempest data logger (Tangent Systems Inc., Charlotte, N.C.) was then inserted into the yolk to record the temperature (Figure 2). The ladle was then lowered and submerged into the boiling water for three min (Figure 3). For the

inoculated eggs a sterile syringe pre filled with the inoculum was inserted into the large end of the egg through the air cell. After injection the hole sealed with super glue. The eggs were inoculated in shell because once you cracked the egg into the boiling water there was no way to put the inoculums on the egg surface. The lid was then placed on the pan and allowed to cook as stated in the cooking section.

In-Shell Controls

An in-shell control was used for the hard and soft cooked and free poached cooking methods. A sterile syringe pre filled with the *Salmonella* suspension or sterile distilled water was inserted into the large end of the egg through the air cell onto the vitelline membrane of the yolk, using a Lyon Hi-Intensity Candler (Lyon Electric Company Inc., Chula Vista, CA) to confirm placement of the *Salmonella* suspension or distilled water. After injection the hole sealed with super glue. Eggs were then cracked onto sterile foil and into a sterile 1000mL beaker. The beaker was then covered with a piece of sterile foil and reweighed to get the egg weight.

Broken Out Controls

This control was used to represent the scrambled, over-easy, sunny-side-up, and poached methods. Immediately after cracking the egg into a 1000mL beaker, an Eppendorf pipette was used to deposit 0.1mL of the *Salmonella* suspension or sterile distilled water onto the vitelline membrane on the surface of the egg yolk. The beaker was then covered in a piece of sterile foil, reweighed to get egg weight, and set aside until needed for enumeration.

Enumeration and *Salmonella* Detection

For each cooking method an aerobic plate count (APC) was conducted, to determine the number of bacteria, if any, which were present after cooking. The lowest detectable level of APC using 3M Petri Films™ is 10cfu/g. Each cooking method also had microbiological testing to determine if the samples were positive or negative for SE or SH.

A dilution of one to ten was used based on egg weight to determine the amount of buffered peptone water (BPW) (Biotrace, Muncie, IN) to add before mixing. A large mixer (Talboys/Troemner, Thorofare, NJ) with sterile paddles was used to mix the egg for two min. A sample (0.1mL) of the diluted egg was removed and plated onto Aerobic Plate Count Petri-film (3M Microbiology Product, St. Paul, MN), which was incubated for 48h at 37°C. The films were read by counting the red colonies (the controls were not taken past the enumeration step).

Another 0.1mL was removed from the original diluted egg, and placed into 10mL of Rapport-Vassiliadis R 10 (RV) broth (Hardy Diagnostics, Santa Maria, CA). A 0.5mL sample was removed from the diluted egg and added to 10mL of tetrathionate hajna broth (TT) (Hardy Diagnostics, Santa Maria, CA). Both tubes were then vortexed and placed in a 42°C incubator for 24h. After 24h the tubes were removed and vortexed again. A 10µL loop full was removed from reach tube and streaked for isolation onto brilliant green sulfa agar (BGS) (Hardy Diagnostics, Santa Maria, CA) and modified lysine iron agar (MLIA) (Hardy Diagnostics, Santa Maria, CA).

The four plates (each TT tube equals one BGS plate and one MLIA plate; each RV tube equals one BGS and one MLIA plate) were then inverted and placed into a 37°C

incubator and incubated for 24h. All positive plates (bright pink with colonies for BGS and black colonies for MLIA) were then stabbed and streaked with a sterile needle by stabbing the butt and streaking the slant onto triple sugar iron agar (TSI) and lysine iron agar (LIA) slants and incubated for 24h at 37°C. Positive tubes for LIA have a purple slant with a purple butt with or without hydrogen sulfide (H_2S) production. Positive TSI tubes had a red slant and a yellow butt with or without H_2S production. H_2S production produces a black butt with a sulfur smell. A 10µL loop full was removed from the positives tubes, and transferred into a cryovial; mixture of 70% TSB and 30% glycerol was added to the vial before it was placed into a -80°C for confirmation at the end of the three weeks.

The positive samples were confirmed using *Salmonella* O Antisera, H Antisera, Antiserum Vi group D and group B (BD Difco, Rockville, MD) to determine if the positive samples belong to group D or B *Salmonella*. The positive cryovialed samples were regrown using a 10µL loop from each cryovialed sample and placed into a 10mL TSB tube (Hardy Diagnostics, Santa Maria, CA) to incubate for 24h at 37°C. This was done two more times after which a 10µL loop full is removed and plated onto an APC plate. The agglutination was performed using glass microscope slides. Two boxes were drawn on each slide using a wax pencil one box for the negative control and the other for the sample. In one box on the slide a sterile drop of the negative control was placed (0.85% NaCl solution), and in the other a drop of the *Salmonella* antisera was placed. Using a sterile plastic needle a uniform colony was picked off of an APC plate and mixed with the *Salmonella* antisera. The slide is then rocked for one min using a Fisher clinical

rotator (Fisher Scientific, Pittsburgh, PA). A test was considered positive if the background of the slide was slightly cloudy

Serotyping

All positive samples were transferred to TSA slants and incubated for 24h at 37°C. The samples were then shipped to the USDA National Veterinary Services Laboratory in Ames, Iowa for phage typing.

Statistical Analysis

A completely randomized design was used. An egg represented one experimental unit except in the case of scrambled egg, where it was two eggs. For each of the seven cooking treatments and controls 30 eggs were utilized. The within treatment effects on the percentage of *Salmonella* positive samples were determined with a chi-square analysis using the PROC FREQ procedure of SAS (SAS Institute, Cary, NC). While the total aerobic plate counts were analyzed using PROC GLM. The PROC GLM was used to determine the p values of each treatment to determine if there was a significant difference within the weeks (3) of each treatment.

IV. RESULTS AND DISCUSSIONS

The starting inoculation level of all cooking methods was 4.3 $\log_{10}$ cfu/g, 3.5 $\log_{10}$ cfu/g was recovered from raw eggs. The hard cooked method had a reduction of 2.5 $\log_{10}$ cfu's/g, soft cooked and poached methods had a reduction level was also $\leq$ 2.5 $\log_{10}$ cfu's/g. While the scrambled and over-easy methods had a reduction of 2.4 $\log_{10}$ cfu's/g. The free poached method had a reduction level of 2.1 $\log_{10}$ cfu's/g. The sunny-side-up method had a reduction level of 0.7 $\log_{10}$ cfu's/g. Sunny-side-up had the lowest reduction level of all the cooking methods (Table 1).

When the aerobic plate counts (APCs) were done using 3M Petri Films™, hard and soft cooked yielded $\leq$ 10cfu/g (Table1) and reached 63°C after nine min of cooking (Figure 1, and Figure 2). The findings in previous studies done by Licciardello et al., (1965), Humphrey et al. (1989), Baker (1990), and Chantarapanont et al. (2001), supported the recommendation of the AEB for the hard and soft cooking method for eggs as reliable in the inactivation of SE and SH. The results of this study show that no *Salmonella* survived the hard and soft cooking methods. In fact after nine min total cooking time both cooking methods reached 63°C, the temperature which was reported by the Tharrington et al. study (2003). When eggs were scrambled, the eggs were cooked for two min, which left no visible liquid the final temperature was 81.3°C (Figure 3).

For this cooking method one egg out of the 30 that were cooked yielded viable *Salmonella* PT's 4 and 30. While PT 4 was part of the suspension used in this study, PT 30 was not, and therefore it must be assumed that PT 30 was in the egg prior to the inoculation. The APC following scramble cooking was 1.1 $\log_{10}$ cfu/g (Table 1). Baker (1990) stated that scrambled eggs should be cooked for one min to be safe, while Tharrington stated two min was needed for the egg to the 63°C safe temperature. However the results of this study show that two min did not result fully inactivate.

The over-easy method produced no eggs that were positive for SE; however, there was one egg that yielded viable SH bacteria. APC was a 1.1 $\log_{10}$ cfu/g (Table 1) and resulted in a final temperature of 82.5°C (Figure 4). Baker (1990) determined that it would take a total of five min cooking time to produce an SE free egg. The five min total time describes the cooking method because the egg is cooked for three min and then turned over and cooked for an additional two min. The over-easy cooking method used in this study required that the egg be cooked for 1.5 min and then flipped and cooked for an additional thirty sec. It could be speculated that an increase in cooking time after the egg was flipped could possibly eliminate the occurrence of *Salmonella*. When comparing the two, Baker produced a SE free product but does not follow the AEB characteristics of an over-easy egg, as was done in this study.

Sunny-side-up is much like the over-easy cooking method except that instead of flipping the egg at 1.5 min, the egg is removed from the heat completely. This method resulted in 19 positive samples out of the 30 samples that were cooked. Of those samples SH was the most viable, 16 of these were a combination of SE and SH. The three remaining samples yielded viable SE only. Of the SE PT's used; only four PT's 4, 4a, 8

and 23 were recovered and no other phage types that were not in the suspension were found. APC of the cooked product was 2.8 $\log_{10}$ cfu/g (Table 1) and the final temperature was 58.9°C (Figure 5). Baker (1990) observed that the time needed for complete kill for this cooking method was seven min. As previously stated, with the over-easy cooking method the Baker study does not follow the AEB recommendations. Tharrington et al. (2003) determined that eggs cooked in this manner without the use of the basting technique or a lid used to cover the cooking pan, produce an egg that did not reached 63°C in the recommended time. The results of this study agreed with the Tharrington et al. study in that *Salmonella* was not destroyed.

Poaching resulted in no SE or SH positive samples. The aerobic plate count numbers for the poached cooking method was < 1.0 $\log_{10}$ cfu/g (Table 1.) and a final temperature of 64.3°C (Figure 6). Using heating curves Tharrington et al. (2003) determined that between six to seven min produced the acceptable temperature of 63°C. The slight temperature differences could be from the types of pots and/or pan inserts and heating elements used. Based on the combination of studies discussed, recommendations of this cooking method are adequate.

For the free poached cooking method an egg was freely dropped into a pot of boiling water where it remained for three min. Due to the egg being dropped into the boiling water freely, the wire would not stay in the egg to measure the temperature. The ladle was then lowered and submerged into the boiling water the resulting final temperature was 48.4°C (Figure 7). When the eggs were cooked using the free poached method the temperature of the eggs did not reach 63°C. When cooked for three min the albumen was set and the yolk had begun to thicken but the cooking temperature only

reached 48.4°C. The free poached method resulted in two positive samples, a SE PT 4 and a SH. When the APC's were done, the cooked egg produced a 1.4 $\log_{10}$ cfu per gram of remaining viable cells (Table 1). Baker (1990) reported that when this boiling water method was used it took five min to achieve the complete killing of the *Salmonella* organism. The AEB recommendations for a free poached egg suggest a three to five minute cooking time. For this study the free poached method was only cooked for three min because this was the amount of time the eggs needed to obtain the characteristics of a cooked free poached egg. If the free poached egg was cooked five min *Salmonella* may have been inactivated

Based on recovery data, it would seem that SE PT 4 was still the most heat tolerant of all the SE samples that were used when it came to the frying cooking methods. It also appears that because SH was found in both boiled and fried cooking methods it could possibly be more heat tolerant than SE. This could lead to future research on SH considering that both SE and SH can be found in the reproductive tract of chickens.

Findings indicate that the AEB hard cooked, soft cooked, and poaching methods are safe. The same is not true for the over-easy and scrambled egg cooking methods, in which SE or SH were recovered from cooking product. The over-easy came back positive for SH, and scrambled came back positive for SE PT4 and PT30. The free poached egg cooking method came back positive for SH and SE PT4 in two of the 30 cooked samples. Given this low frequency of SE and SH recovery, the over-easy and scrambled cooking methods can be considered reasonably safe. The free poached method could be considered safe if cooked to the maximum of five min. The sunny-side-up cooking method had a total of 19 of 30 samples were *Salmonella* positive after

cooking, with some samples having a combination of multiple *Salmonellas*. The sunny-side-up method should be considered unsafe.

Table 1. Summary of cooking methods and recovery of viable bacteria.

Method	Initial Bacteria Population (cfu/g) APC* (raw)	Initial Bacteria Population (cfu/g) Sal** (raw)	Post-Cook Population sample (cfu/g)	Post-Cook Recovery of SE (%) Phage Type(s)	Post-Cook Recovery of SH (%)
Hard Cooked	2.0	3.5	<1.0[a]	0	0
Soft Cooked	2.0	3.5	≤1.0[a]	0	0
Scrambled	2.0	3.5	1.1[a]	1/30 (3.33%) PT 4, 30	0
Over-easy	2.0	3.5	1.1[ab]	0	1/30 (3.33%)
Sunny-side-up	2.0	3.5	2.8 [c]	8/30 (26.67%) PT 23,8,4a,4	17/30 (56.67)
Free Poached	2.0	3.5	1.4[b]	1/30 (3.33%) PT 4	2/30 (6.67%)
Poached	2.0	3.5	≤1.0[ab]	0	0

P<0.0001. Salmonella Frequency=0.1062

[a-c] Means within a row with different superscripts are significantly different at *P*<0.0001 The lowest detectable level of countable cells on the APC Petri film used is 1 log cfu/ gram.

*Naturally occurring mesophile + added sterile distilled water.

**Following addition of SE +SH inoculum. Population of inoculum suspension was 4.3 cfu/mL

Figure 1. Example of the free poached cooking method.

Figure 2. Insertion of wire thermocouple into raw egg

Figure 3. Cooked free poached egg with wire thermocouple

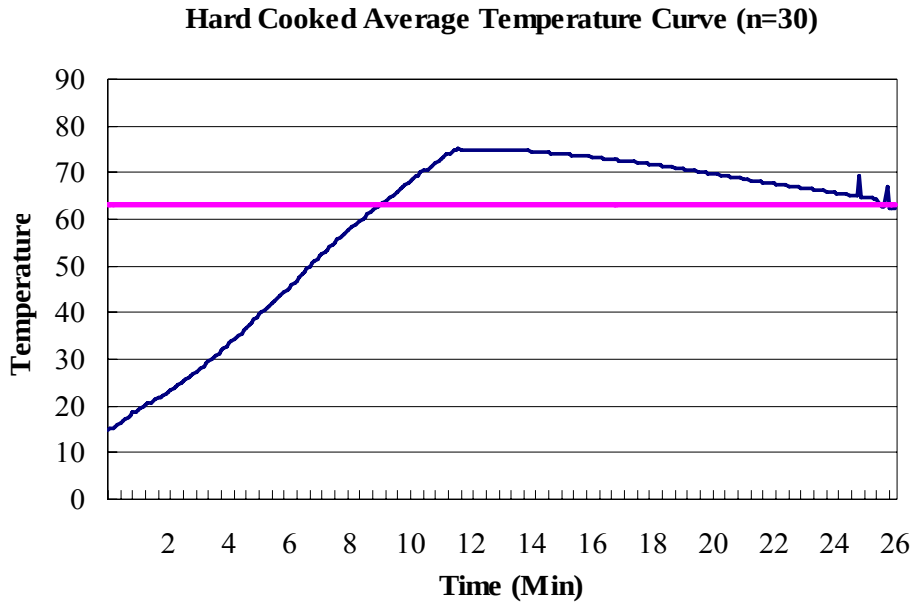

Figure 4. Internal temperature of eggs during the hard cooked. Total heating time was 26 min. The target Salmonella inactivation temperature (63.3°C) is given as the pink horizontal line.

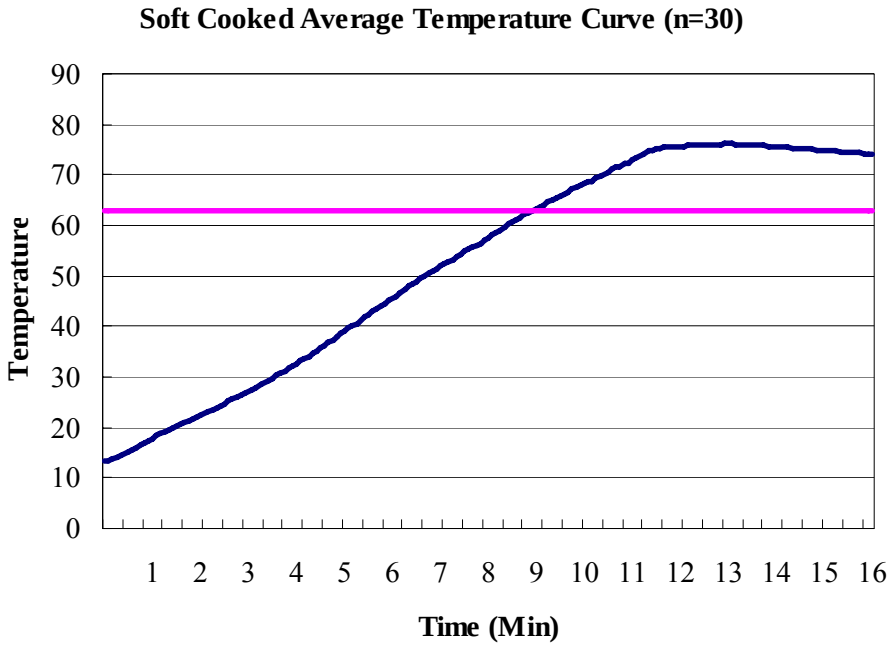

Figure 5. Internal temperature of eggs during the soft cooked. Total heating time was 16 min. The target Salmonella inactivation temperature (63.3°C) is given as the pink horizontal line.

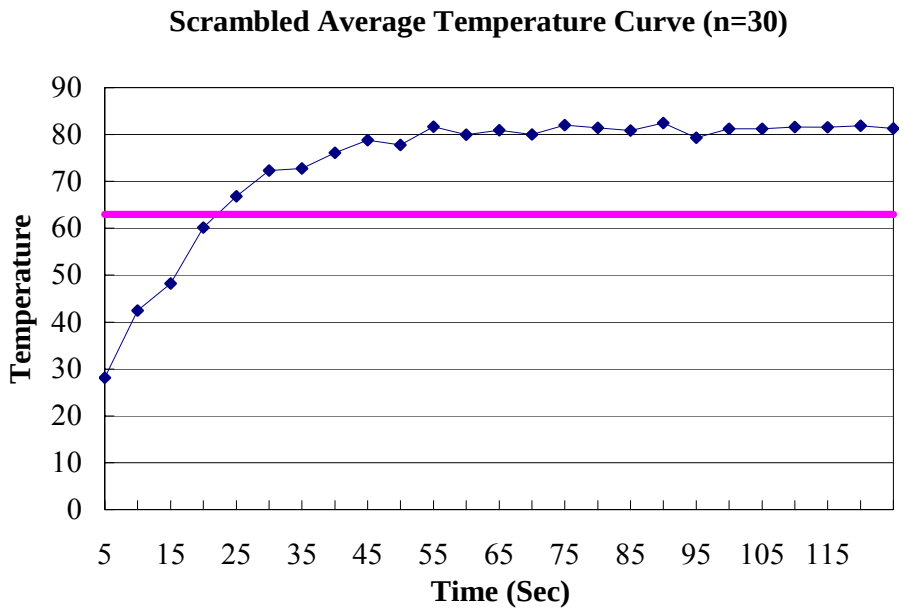

Figure 6. Internal temperature of eggs during the scrambled cooking method. Total heating time was 120 sec (2 min). The target Salmonella inactivation temperature (63.3°C) is given as the pink horizontal line.

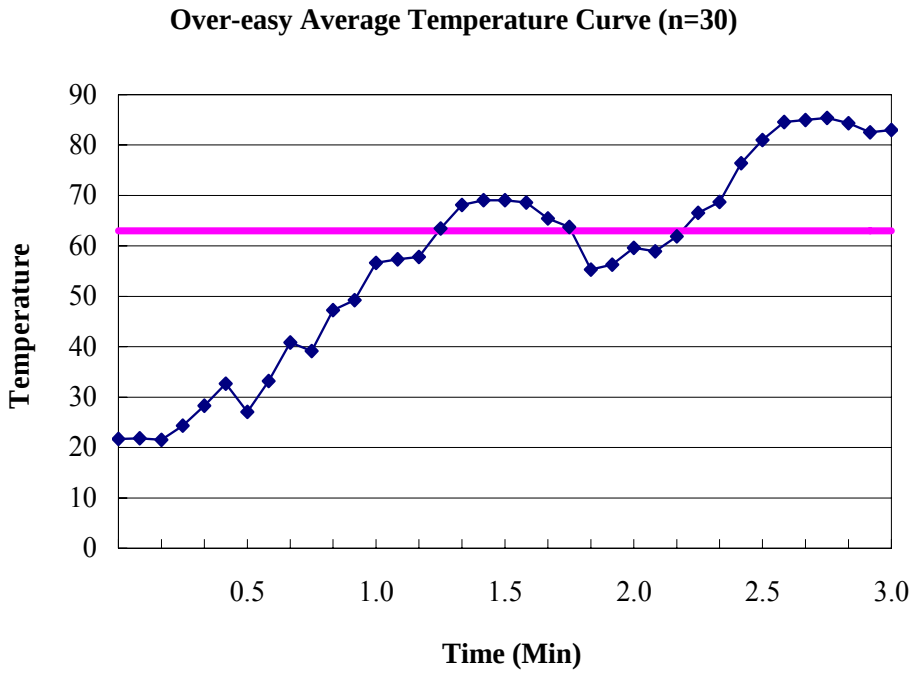

Figure 7. Internal temperature of eggs during the over-easy cooking method. Total heating time was 3 min. The target Salmonella inactivation temperature (63.3°C) is given as the pink horizontal line.

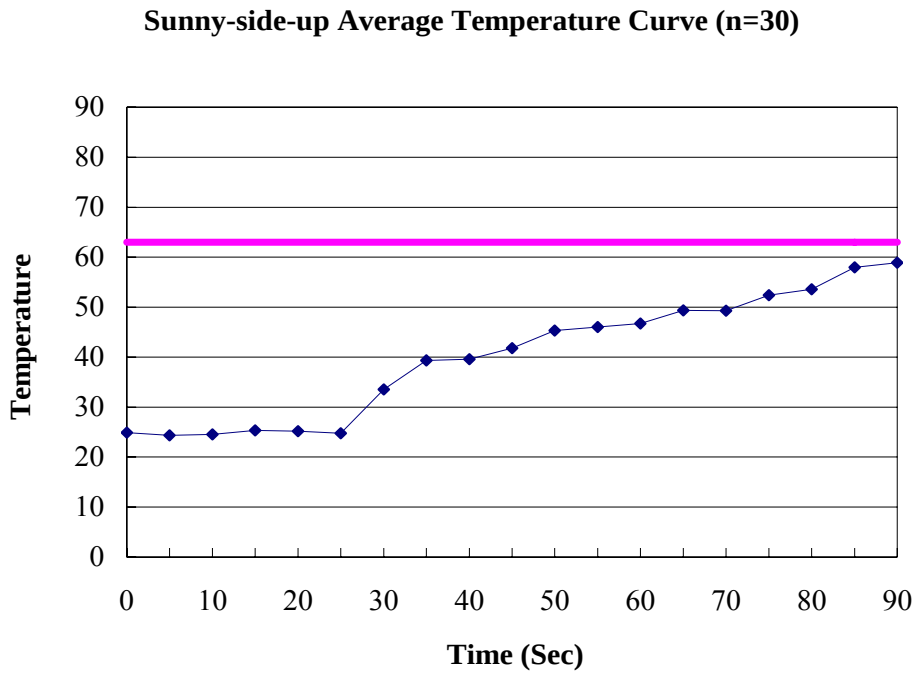

Figure 8. Internal temperature of eggs during the Sunny-side-up cooked method. Total heating time was 90 sec (1.5 min) of cooking. The target Salmonella inactivation temperature (63.3°C) is given as the pink horizontal line.

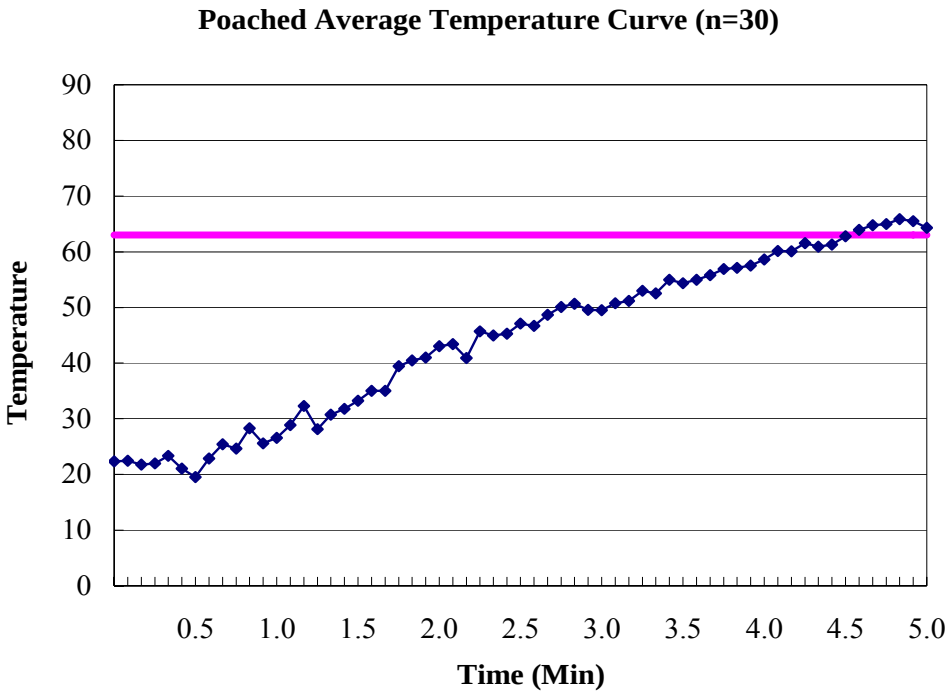

Figure 9. Internal temperature of eggs during the Poached cooked method. Total heating time was 5 min. The target Salmonella inactivation temperature (63.3°C) is given as the pink horizontal line.

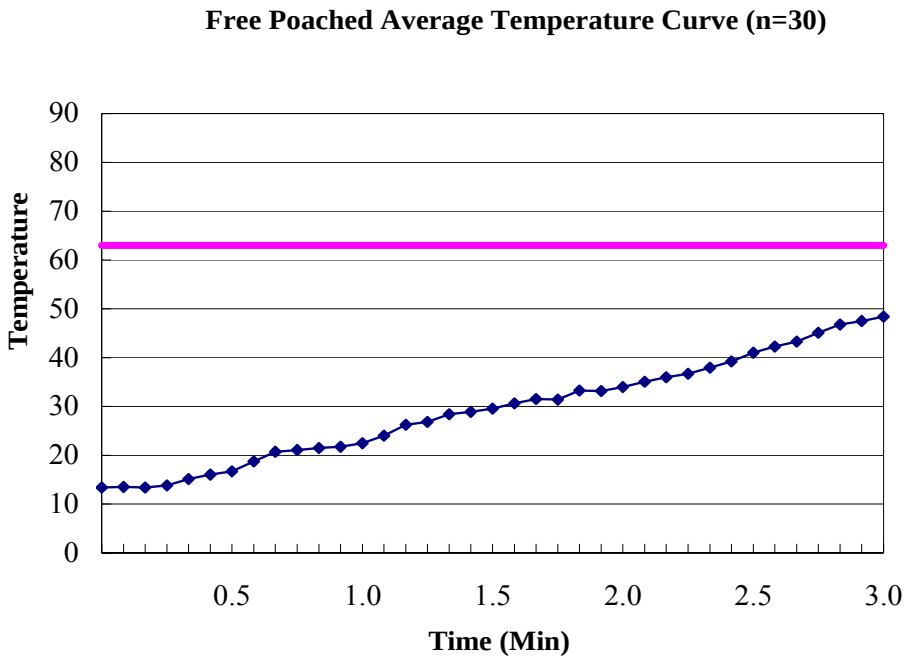

Figure 10. Internal temperature of eggs during the free poached cooking method. Total heating time was is 3 min. The target Salmonella inactivation temperature (63.3°C) is given as the pink horizontal line.

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