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Microbial Quality of Cool Water Washed Shell Eggs

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Abstract: A study was conducted to examine the effects of cool water washing on the microbial quality of shell eggs. Six dual tank wash water temperature schemes were examined for their ability to reduce naturally occurring aerobic bacteria and inoculated *Salmonella* Enteritidis (SE). The wash water schemes were: T1= 48.9°C; T2 = 48.9°C, 23.9°C; T3 = 48.9°C, 15.6°C; T4 = 23.9°C; T5 = 15.6°C; and T6 = 23.9°C, 15.6°C. All wash water tanks were maintained from 10.5-11.5 pH throughout the study. Eggs were exposed to the wash water temperature schemes in a pilot egg washer with recirculating wash water tanks. The total amount of time eggs were exposed to the wash water combinations was 60 s. Following washing, all eggs were sprayed with a 48.9°C, 200 ppm chlorine rinse solution. Eggs were stored and sampled for 9 wks. External aerobic populations were lowest for T1 (typical U.S. wash water configuration), followed by T2 and T3. Aerobic surface contamination was greatest in T5 eggs. All treatments reduced SE levels in a similar manner as detected by shell and membrane emulsion and egg contents pools after enrichment. Commercial application of cool water shell egg processing will be investigated to determine the potential of this technology to enhance the safety and quality of shell eggs.

Key words: Shell eggs, cool wash, microbial quality, wash water

Introduction

Salmonella Enteritidis (SE) is the primary food borne pathogen associated with shell eggs. In 1999, the United States Department of Agriculture (USDA) added a requirement that all shell eggs be stored in an ambient temperature of 7.2°C or lower post-processing (USDA, 1999). This was an attempt to introduce another hurdle to SE growth in eggs. Salmonellae do not multiply in eggs at temperatures below 7°C (Buchner, 2005). Gast and Holt (2000) reported difficulty in promoting SE growth in eggs stored between 10-17.5°C. Another study (Hara-Kudo *et al*., 2001) found eggs stored at 10°C were less able to support SE growth in the albumen when inoculated.

While the merit of the new refrigeration guidance was understood, scientists began to question the ability of post-processing ambient storage temperature to reduce internal egg temperatures. Anderson *et al.* (1992) documented temperature changes that occur as an egg progresses through processing. These researchers examined eggs from both inline (hen houses connected directly to the processing facility through a series of belts) and offline (hens housed off site and eggs brought to the processing facility two to three times a week) facilities. Inline eggs enter the processing line at an average temperature of 34.4°C whereas offline eggs begin processing between 15.6-20°C. Average wash water temperature recorded during this survey was 46°C. As eggs exited the washers, external temperature was 42.8°C. There was a slight drop (40.6°C) noted for shell temperature on the spools. After eggs were exposed to the blowers, shell temperature was recorded as 35°C. The internal temperature of the egg at the time of packaging was 24.4-26.7°C (offline) and 26.7-34.4°C (inline). The project continued to monitor the temperature of the center most egg of a 30 case pallet and found on average it took 142 h (5.9 d) to reach 7°C. In another study, Jones *et al.* (2002) found traditionally processed shell eggs required at least 5 d to reach an internal 7°C. Bell *et al.* (2001) and Patterson *et al.* (2001) reported that on average, shell eggs are purchased by the consumer within 19 d post-processing. With this short period of transition through processing, transport, warehousing, and distribution, eggs are not held for a great length of time at any step of the distribution chain making cooling a more challenging goal.

Wash water temperatures for all USDA shielded eggs are required to be at least 32.2°C or 11.1°C warmer than the warmest egg entering the processing line (USDA, 2005). The temperature requirements are primarily based on the work of Brant and Starr (1962) and Brant *et al.* (1966). Brant and Starr (1962) stated that egg temperature should be considerably lower than wash water temperature. In the 1966 report, Brant and colleagues suggest that wash water temperature should be 11.1°C warmer than the egg in order to prevent

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Table 1: Actual nalidixic acid resistant *Salmonella* Enteritidis inoculum concentration for each replicate

Replicate	Inoculum concentration (log cfu/ml)
1	6.11
2	5.80
3	5.85

Table 2: Temperature schemes utilized during pilot egg washing study

Scheme	Washer 1	Washer 2
1	48.9°C	48.9°C
2	48.9°C	23.9°C
3	48.9°C	15.6°C
4	23.9°C	23.9°C
5	15.6°C	15.6°C
6	23.9°C	15.6°C

spoilage organism growth within the egg during extended storage. The authors noted that at high concentrations, spoilage occurred during storage regardless of the temperature difference between wash water and eggs. Furthermore, it was recommended that wash water should contain 10^3 cfu/ml or less to prevent spoilage during storage.

Over the years, investigators have examined intervention strategies during processing to reduce the risk of SE in eggs. One of the areas of focus has been reducing egg temperature quickly. Curtis *et al.* (1995) demonstrated that cryogenic gases quickly cooled eggs before being packaged. Further work by this group found egg quality was enhanced by quick cooling and exposure to gaseous carbon dioxide (Jones *et al.*, 2002). Fajardo *et al.* (1995) reported subsequent exposure to SE after rapid cooling allowed for easier SE penetration into the egg. Conversely, Thompson *et al.* (2000) found no adverse effects of rapid cooling on SE penetration.

Lucore *et al.* (1997) reported no differences for internal microbial counts from eggs washed in 15.5°C vs 32.2°C or 48.9°C wash water. There was a statistical difference for surface counts between the treatments, but the differences were within one log. Conversely, Hutchison *et al.* (2004) found lowering wash water temperature to 25°C caused contents to become contaminated with SE and *Salmonella* Typhimurium (ST), even in the presence of 200 pm chlorine. In this study, eggs were exposed to belts covered with a manure slurry containing SE and ST. Meckes *et al.* (2003) examined spent wash water (pH 8.1 and 8.3) and found *Salmonella*, *E. coli* and total coliforms numbers decreased more rapidly at 25°C than 5 or 15°C. The current study was undertaken to determine the role of various cool water washing schemes in the pilot setting on aerobic bacterial levels and SE contamination in inoculated eggs.

Materials and Methods

Shell eggs: Nest run shell eggs were purchased from a local offline facility. All eggs utilized in the study came from the same lot of eggs from a single laying flock. It should be noted that there was an excessive amount of adhering debris/feces/egg meat on the shell surface of these eggs. One nest run egg cart was allotted to each processing day (replicate) with a total of n = 5400 eggs/cart. Two thirds of the eggs were utilized as untreated controls for aerobic population determinations and physical quality measurements (not presented in this manuscript). All eggs were stored at 7°C until processing. The remaining third were inoculated with SE.

***Salmonella* Enteritidis inoculation:** For each replicate, approximately 1800 eggs were candled and all cracked eggs were removed. Eggs were warmed in a 42°C incubator overnight. A nalidixic acid resistant SE was utilized as an inoculum. A 7L stock solution of approximately 10^6 cfu SE/ml was prepared in 7°C buffered peptone water. Actual inoculum concentrations for each replicate can be found in Table 1. Eggs were inoculated according to the procedures of Jones and Musgrove (2005). Inoculated eggs were stored overnight at 7°C before processing.

Egg processing: Eggs were processed in a fabricated pilot egg washer on three consecutive days (replicates). Inoculated eggs were processed at the end of each day. The stainless steel unit included eleven, six wide egg rollers (Sanova Engineering Corp, Elk Grove Village, IL 60007) with one row of the rollers utilized for the drive belt (n = 50 eggs/wash; 26 rpm). Spray nozzles were mounted in the top of the unit in an orientation that ensured each egg was being sprayed (average 4 psi). Unlike a commercial egg washer, the unit did not contain brushes. The plumbing of the unit allowed for dual tank washer conditions to be mimicked. Furthermore, wash water was recycled. Six wash water temperature schemes were examined in the study (Table 2). The eggs were exposed to the wash condition of each washer tank for 30 s (1 min total wash time). The pH of the wash water was maintained from 10.5-11.5 throughout the study. After washing, eggs were sprayed with a 48.9°C chlorine solution (200 ppm chlorine) according to USDA guidelines (USDA, 2005). Eggs were randomly placed into new foam cartons and allowed to air dry before the cartons were closed and placed into cardboard 15 dozen cases. Processed eggs were stored on pallets (one for each day) at 7°C for the duration of the study.

Microbial analysis: Eggs were sampled once a week for 9 weeks (day of processing = 0 wk) for microbial analysis. All eggs were candled before sampling and cracked eggs were discarded. Inoculated eggs were sampled for the presence of nalidixic acid resistant SE. Non-inoculated eggs were analyzed for total aerobic

Table 3: Total aerobic plate counts in shell rinses, shells and associated membranes, and egg contents according to wash water temperature scheme

Scheme[1]	Rinse (log cfu/ml)*	Shells and membranes (log cfu/ml)*	Egg contents (log cfu/ml)
1	4.04	2.30	0.31
2	4.48	2.45	0.31
3	4.51	2.34	0.36
4	4.69	2.43	0.33
5	4.93	2.87	0.45
6	4.83	2.74	0.49
SEM	0.03	0.12	0.08

[1] Wash water temperatures for each washer during a temperature scheme: 1 = 48.9°C, 48.9°C; 2 = 48.9°C, 23.9°C; 3 = 48.9°C, 15.6°C; 4 = 23.9°C, 23.9°C; 5 = 15.6°C, 15.6°C; 6 = 23.9°C, 15.6°C.
*Means within the column are statistically different; P < 0.01.

Table 4: Effects of wash water temperature schemes on the inoculated nalidixic acid resistant *Salmonella* Enteritidis levels on and within the shell and associated membranes after enrichment

Scheme[1]	SE positive	SE negative
1	38.20 %	61.80 %
2	44.44 %	55.56 %
3	45.56 %	54.44 %
4	46.67 %	53.33 %
5	38.89 %	61.11 %
6	45.56 %	54.44 %

[1]Wash water temperatures for each washer during a temperature scheme: 1 = 48.9°C, 48.9°C; 2 = 48.9°C, 23.9°C; 3 = 48.9°C, 15.6°C; 4 = 23.9°C, 23.9°C; 5 = 15.6°C, 15.6°C; 6 = 23.9°C, 15.6°C.

Table 5: Effects of wash water temperature schemes on the inoculated nalidixic acid resistant *Salmonella* Enteritidis levels in the egg contents after enrichment

Scheme[1]	SE positive	SE negative
1	2.22 %	97.78 %
2	1.11 %	98.89 %
3	3.33 %	96.67 %
4	3.33 %	96.67 %
5	1.11 %	98.89 %
6	1.11 %	98.89 %

[1]Wash water temperatures for each washer during a temperature scheme: 1 = 48.9°C, 48.9°C; 2 = 48.9°C, 23.9°C; 3 = 48.9°C, 15.6°C; 4 = 23.9°C, 23.9°C; 5 = 15.6°C, 15.6°C; 6 = 23.9°C, 15.6°C.

organisms. Three sampling methods were utilized for both inoculated and non-inoculated eggs: shell rinse (Jones and Musgrove, 2005), egg contents (Jones *et al.*, 2004) and shell and membrane emulsion (Musgrove *et al.*, 2005). For both egg contents and shell and membrane emulsion samples, 3 eggs are pooled with 3 pools constructed for each treatment group.

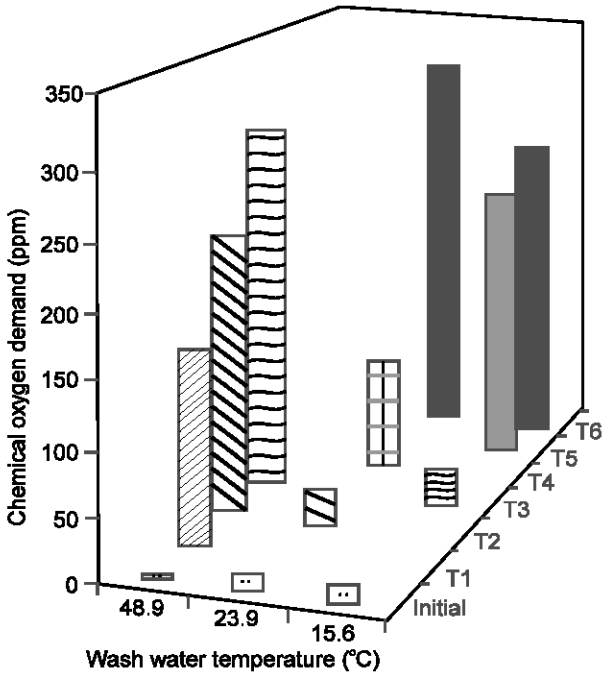

Fig. 1: Changes in chemical oxygen demand within each wash water tank throughout processing.[1]
[1]Wash water temperatures for each washer during a temperature scheme: 1 = 48.9°C, 48.9°C; 2 = 48.9°C, 23.9°C; 3 = 48.9°C, 15.6°C; 4 = 23.9°C, 23.9°C; 5 = 15.6°C, 15.6°C; 6 = 23.9°C, 15.6°C.

Total aerobic populations were enumerated on plate count agar (PCA; Difco, Becton-Dickinson, Sparks, MD 21152). Duplicate plates were spread plated with 0.1 ml of rinsate or emulsion diluent or 0.25 ml of homogenized egg contents. Plates were incubated for 48 hrs at 37°C before enumeration. Nalidixic acid resistant SE was quantified on brilliant green sulfa agar (BGS; Becton-Dickinson, Sparks, MD 21152) with 200 ppm nalidixic acid added (Sigma-Aldrich, St. Louis, MO 63178). A 0.1 ml aliquot of each sample was applied to the BGS and incubated at 37°C for 24 hrs before enumeration. The pooled egg contents and shell crush samples from the inoculated eggs were enriched in buffered peptone water and incubated at 37°C for 24 hrs then plated on BGS to detect nalidixic acid resistant SE.

Wash water analysis: A water sample was collected in a sterile specimen cup from each temperature reservoir at the end of each treatment combination. A portion of the water sample was enumerated for aerobic bacterial populations and nalidixic acid resistant SE according to the procedures outlined for shell rinses. Chemical oxygen demand (COD) and total Kjeldahl nitrogen (TKN) were determined according to the methods of Northcutt *et al.* (2005).

Statistical analysis: All data were analyzed with the SAS software system (1999). Enumerated microbial counts,

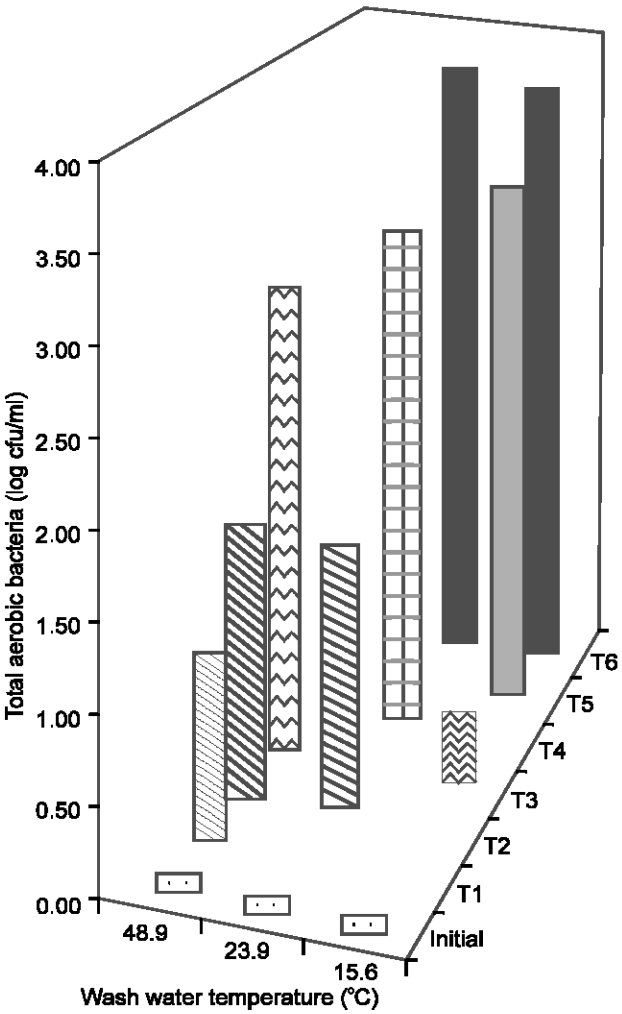

Fig. 2: Changes in total aerobic bacteria levels within each wash water tank throughout processing[1].

[1]Wash water temperatures for each washer during a temperature scheme: 1 = 48.9°C, 48.9°C; 2 = 48.9°C, 23.9°C; 3 = 48.9°C, 15.6°C; 4 = 23.9°C, 23.9°C; 5 = 15.6°C, 15.6°C; 6 = 23.9°C, 15.6°C.

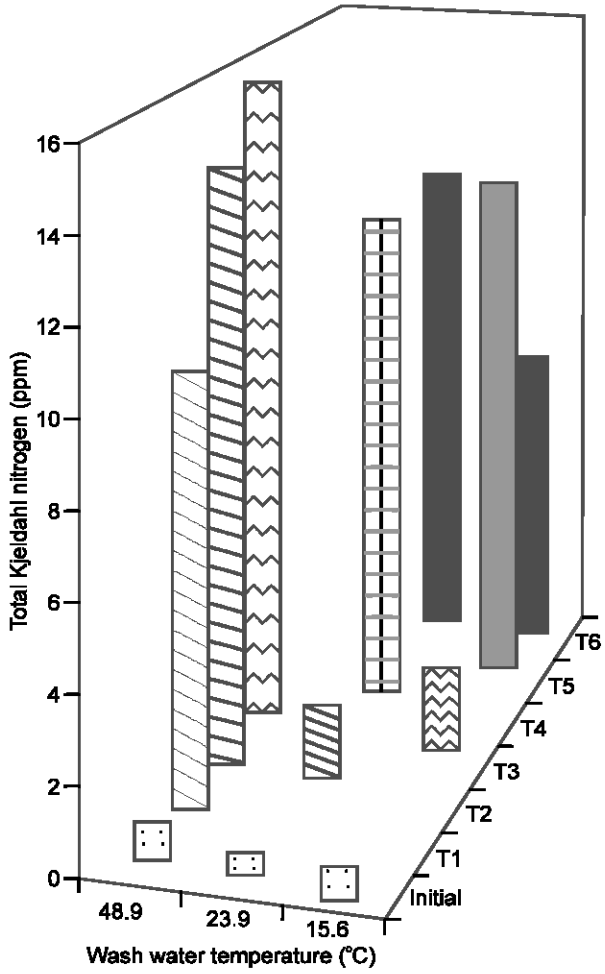

Fig. 3: Changes in total Kjeldahl nitrogen levels within each wash water tank throughout processing.[1]

[1]Wash water temperatures for each washer during a temperature scheme: 1 = 48.9°C, 48.9°C; 2 = 48.9°C, 23.9°C; 3 = 48.9°C, 15.6°C; 4 = 23.9°C, 23.9°C; 5 = 15.6°C, 15.6°C; 6 = 23.9°C, 15.6°C.

COD, and TKN were analyzed according to the general linear model with means separated by the least square method. The significance ($P < 0.05$) of positive and negative nalidixic acid resistant SE in enriched samples was determined by Chi-square analysis and the goodness of fit test.

Results and Discussion

Wash water analysis: Fig. 1 shows the changes in chemical oxygen demand (COD) within each wash water reservoir during the course of the study. COD levels increased at the greatest rate in the 48.9°C wash water reservoir, but the overall COD levels at the end of processing were not different amongst the three temperatures. Therefore, after processing a moderate number of eggs, the COD was not influenced by wash water temperature. Considering that current guidelines specify wash water to be replenished every 4 h (USDA, 2005) and the rate current washers operate in the commercial setting, COD would not be expected to be different between the wash water temperatures examined in this study.

The changes in wash water total aerobic populations are illustrated in Fig. 2. The 23.9°C and 15.6°C wash water had higher overall aerobic bacterial counts than the 48.9°C. However, the difference was within a log cfu/ml water. The eggs utilized in this study had a higher degree of contamination on the shell surface than most nest run eggs found in offline processing facilities. Therefore, the results of this study represent of a "worse case scenario", since the amount of debris (feed, feces, egg meat, etc.) introduced into the wash water during

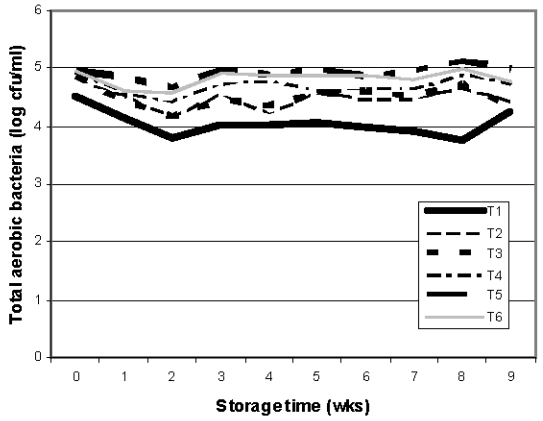

Fig. 4: Effects of wash water temperature and post-processing storage time on total aerobic bacteria isolated from shell surface rinses.[1]*

[1]Wash water temperatures for each washer during a temperature scheme: 1 = 48.9°C, 48.9°C; 2 = 48.9°C, 23.9°C; 3 = 48.9°C, 15.6°C; 4 = 23.9°C, 23.9°C; 5 = 15.6°C, 15.6°C; 6 = 23.9°C, 15.6°C.

*Significant temperature scheme by storage time interaction (P < 0.01).

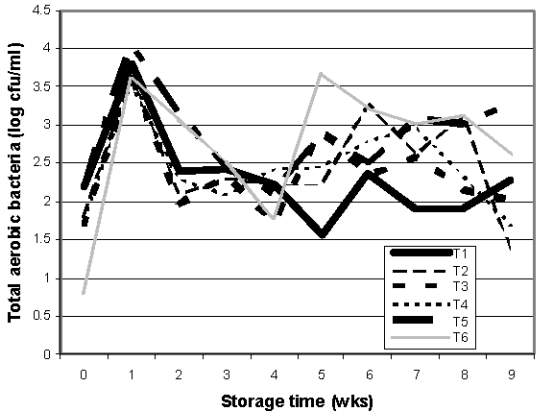

Fig. 5: Effects of wash water temperature and post-processing storage time on total aerobic bacteria isolated from shell and membrane emulsions.[1]*

[1]Wash water temperatures for each washer during a temperature scheme: 1 = 48.9°C, 48.9°C; 2 = 48.9°C, 23.9°C; 3 = 48.9°C, 15.6°C; 4 = 23.9°C, 23.9°C; 5 = 15.6°C, 15.6°C; 6 = 23.9°C, 15.6°C.

*Significant temperature scheme by storage time interaction (P < 0.01).

processing was elevated compared to "normal" commercial offline egg processing.

The total Kjeldahl nitrogen (TKN) levels present in the wash water reservoirs can be found in Fig. 3. There were no statistical differences in TKN between the wash water temperatures. The levels detected in the wash water were very low, which caused a fluctuation in results due to the normal variability of the method. During the course of washing eggs in this study, very few eggs were broken in the washer. Therefore, despite the excessive exterior dirts, there was less protein in the wash water reservoirs.

Egg microbiology: The effect of the six wash water temperature schemes on total aerobic bacterial populations can be found in Table 3. Statistical differences ($P < 0.01$) were found between the treatments for surface rinse and shell and membrane emulsion total aerobic counts. In both cases, the difference between the highest and lowest average counts was less than a log cfu/ml. For both shell rinse and shell and membrane emulsion samples, wash water scheme one (48.9°C) had the lowest aerobic counts. The highest counts were associated with wash water scheme five (15.6°C).

The interaction ($P < 0.01$) between wash water temperature scheme and storage time for shell rinse aerobic bacterial counts is illustrated in Fig. 4. As discussed previously, results were within a log for all treatment groups during each week of testing. The traditional processing temperature scheme (T1 = 48.9°C) consistently maintained the lowest aerobic bacterial level on the shell surface. Temperature schemes two and three (T2 = 48.9°C, 23.9°C and T3 = 48.9°C, 15.6°C) were the next lowest in surface aerobic bacterial levels. Temperature scheme five (T5 = 15.6°C) had the highest average aerobic population on the shell surface at each sampling time.

There was also a significant interaction ($P < 0.01$) between wash water temperature scheme and storage time for shell and membrane emulsion aerobic bacterial counts (Fig. 5). Unlike shell rinse results, no clear trend can be found amongst the temperature schemes for the shell and membrane emulsion samples. Musgrove *et al.* (2005) reported shell rinse to be a more sensitive means of detecting aerobic bacterial levels on unwashed eggs. While eggs in the current study were washed, it was in a pilot environment without brushes and the eggs utilized had a greater amount of adhering dirt than normally encountered on nest run eggs. Therefore, the results in this study indicate that shell rinse was a better method for assessing surface aerobic microbial contamination levels in this pilot processing setting.

Direct plating of shell rinses, shell and membrane emulsions, and egg contents resulted in extremely low numbers of SE being recovered. Pooled shell and membrane emulsions and egg contents were enriched to detect the presence of the inoculated nalidixic acid resistant SE. Table 4 shows the percentage of SE positive pools detected throughout storage for all of the wash water temperature schemes. Through Chi-square analysis it was determined there were no differences between the treatments for nalidixic acid resistant SE recovery. Furthermore, Table 5 presents the percentage of nalidixic acid resistant SE positive egg content pools for all wash water temperature treatments. There were no differences, according to Chi-square analysis,

between the treatments for SE recovery.

The results of this study indicate that all wash water temperature schemes investigated were equally capable of removing SE. Eggs washed in only 48.9°C wash water had the lowest aerobic bacterial contamination. Washing shell eggs initially in 48.9°C followed by a second washer temperature of 23.9°C or 15.6°C led to fewer aerobic bacteria present on the shell surface than eggs washed in a combination of 23.9°C and 15.6°C water. These data suggest there is a potential for utilizing cool water washing in the commercial setting while still producing safe eggs. Furthermore, washing eggs at a lower temperature will allow for a faster reduction of post-wash egg temperature. For these reasons, a commercial study will be conducted to compare shell eggs commercially processed at 48.9°C, 23.9°C, and a combination of the two temperatures.

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