Low Cost Forced Air Cooling of Shell Eggs

PROGRESS REPORT
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Introduction

Work during the first six months of the test centered on evaluating the effect of cooling rate on shell penetration of *Salmonella* Enteritidis (SE) and shell strength. All testing was done in the laboratory.

Materials and methods

Eggs: White shell egg samples were obtained from a flock of commercial hens which were approaching the end of their second cycle of lay in an attempt to get eggs with relatively poor shell quality. The samples were collected from the commercial processing plant after washing and oiling. Samples were picked up from the packing house and delivered same day to the laboratory on pulp fiber egg flats in a corrugated box. Eggs were candled to remove any checked eggs.
They were kept at 37 °C overnight and contaminated with SE next morning.

Salmonella: SE was obtained from Dr. Brian Walsh (UC Davis) was cultured in brain heart infusion broth (Difco) overnight at 37°C. The cultures contained $10^{8.5}$ – 109 CFU/ml.

Contamination procedure: Eggs with an internal temperature of 37°C were individually dipped in the 7°C SE culture for 3-5 seconds. They were dried at 37°C and 30% relative humidity for 3 hours before being put in cooling chambers.

Cooling: Eggs were held in a warm room (approximately 33 “C) overnight to obtain an uniform starting temperature. The eggs were then placed in a single layer in one of three container types (fast cooled, slow cooled, or very slow cooled) and moved to a cool room (approximately 1 “C). The fast cooled eggs were placed in a plastic chamber in which a fan drew air up from an opening in the bottom of the container past the eggs. The slow cooled eggs were placed in a container similiar except the container was sealed and there was no forced air flow. The very slow cooling treatment was accomplished by placing a container simliar to the slow cooling container into a larger container with one inch of foam insulation around the inner container. For each cooling rate four eggs were sacrificed to record egg temperture at the center of the egg using thermocouples and a data logger. Cooling times were very consistant between reps.

Humidity control: Calcium nitrate was placed inside all cooling chambers to maintain a relative humidity of about 50% which was the same relative humidity of the room where the control groups were kept. In the fast cooling group, eggswere cooled in stream of air with relative humidity of 75-80%. After the eggs reached the desired temperature they were kept in a box containing calcium nitrate and cultured the following day.

Egg shell culture: Shells, separated from the egg contents, were ground to a particle size of 0.25 mm or less in a sterile mortar with 10 ml lactose broth. Surface colony counts on brilliant green novobiocin agar were used to estimate high numbers of SE in the samples. Most Probable Number (MPN) with 3 replicates at each 10 fold dilution level was used to estimate low numbers.

Egg content culture: Eggs were disinfected by dipping for 5 minutes in a sanitizer (70% ethanol, or 10% Lugol’s iodine ethanol which is 1 part 10% Lugol’s iodine solution + 3 parts 70% ethanol). (In the Pennsylvania SE Pilot Project eggshells were disinfected by spraying the 10% Lugol’s iodine solution on the eggs.) Eggs were opened by the conventional method, cracking the egg with sterile metal blade and aseptically opening it into two equal parts and pouring the contents into a sterile receptacle. During preliminary testing, it became evident that the conventional method did not provide protection against contents being contaminated from the incompletely disinfected shell, so an improved method was developed. It consisted in flaming the pointed end of an egg with a small torch. Egg contents were removed with a wide mouth pipette through a hole cut at the pointed end with sterile forceps. Egg contents were put in a Whirl-pak bag, homogenated

by hand massage and incubated at 37°C overnight, then streaked on XLT4 agar and incubated overnight at 37°C.

Shell strength: Shell deformation was determined using a Marius instrument (Marius N.V., Hollantlaan 18, Utrecht, Netherland) which measures the deformation of the shell when a static load of 500 gm is applied at the equator. This method was originally developed by Schoorl and Boersma (1962). Breaking strength was determined using an Instron Universal testing machine set with a cross-head speed of 5 $mm \cdot m^{-1}$. Deformation was measured on each egg before and after cooling. Breaking strength was determined after cooling only.

Results

The superior performance of the improved method of opening eggs is illustrated in table 1. The contents of five of 29 eggs were contaminated by the conventional method and none by the improved method. The difference between the two methods is significant. (Fisher exact test, p = 0.026)

The failure of the disinfecting procedure to completely eliminate SE present in the shell is shown in Figure 1, which indicates that about 90% of SE were killed by the sanitation procedure. In other procedures we observed that SE can penetrate the shell to the shell membrane and may shielded from the disinfectant.

The pooled data from the cooling tests are presented in Table 2. Most shells in all of the cooling treatments showed SE infection. The ratio of positive shells is not significantly different for the different cooling treatments. All 125 eggs had SE negative contents, indicating that SE does not penetrate the shell membrane. These results suggest none of cooling treatments cause an increase risk of SE exposure compared with non cooled control eggs.

One additional study was done to examine possible effects of cooling on the fate of SE during storage. Experimentally contaminated and rapidly cooled eggs were stored at 20°C or 4°C at 50% relative humidity and compared to non cooled eggs stored at 20°C. Over a period of 22 days there was a gradual and similar drop in viable SE in shell + membrane (figure 2). A linear regression equation of all data is log SE = 4.04 - .1 ldays

(R2 = 0.29), which means an average drop of 90% of survivor every 10 days. The relatively high degree of scatter is largely due to differences in initial contamination among eggs (fig. 3). The contents of the eggs remained free of SE over the total period.

Previous research (Fajardo et al., 1996) indicated that the structure of egg shells may be affected by rapid cooling. In this study there were no significant differences in shell strength related to the rate of cooling (see table 3).

Conclusions

There is no indication of an adverse effect of slow or rapid cooling with respect to SE risk from eggs.

There is indication that SE is intact shell eggs decline during storage at 50% relative humidity and that the rate of decline is the same at 20°C as at 4°C; this might be mainly a result of changing water activity in the shell. This would suggest that cooling of intact eggs neither protect nor harm public health. With cracked eggs the situation is different and cooling could be expected to be protective.

The sanitation procedures used in laboratories that test eggs, does not effectively disinfect eggshells contaminated with SE and represents a risk of contamination of the contents during breaking.

Shell strength is not affected by cooling times ranging from 1.5 to 18 hours.

Refrences

Fajardo, T. A., Anatheswaran, R. C., Puri. 1996. Effect of cooling on crack development and mechanical behaviour of eggshells.Applied Engineering in Ag. 12(1):49-55.

Schoorl, P. and H.Y. Boersma, 1962. Research on the quality of the egg shell (a new method of determination). Proc. 12th World's poultry Congress p. 432.

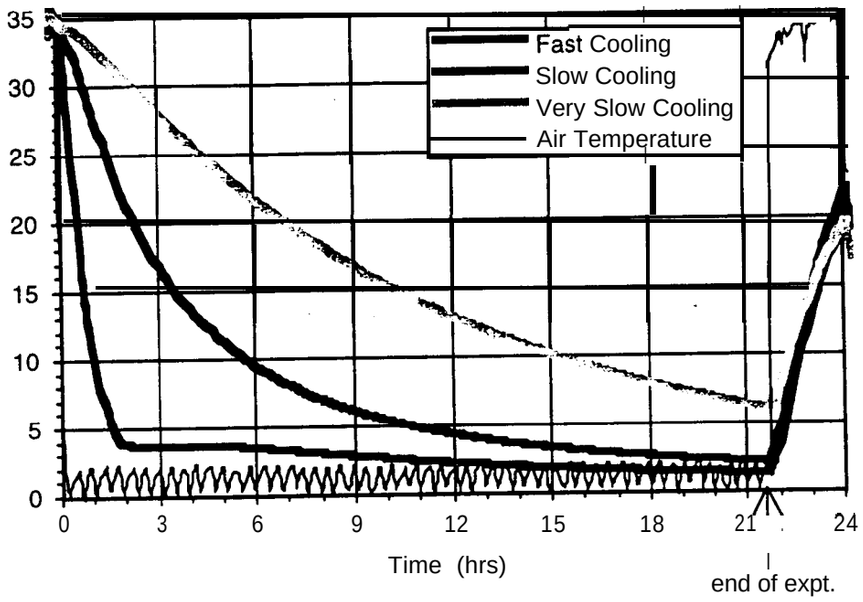

Figure 1. Example of cooling times for the three cooling treatments.

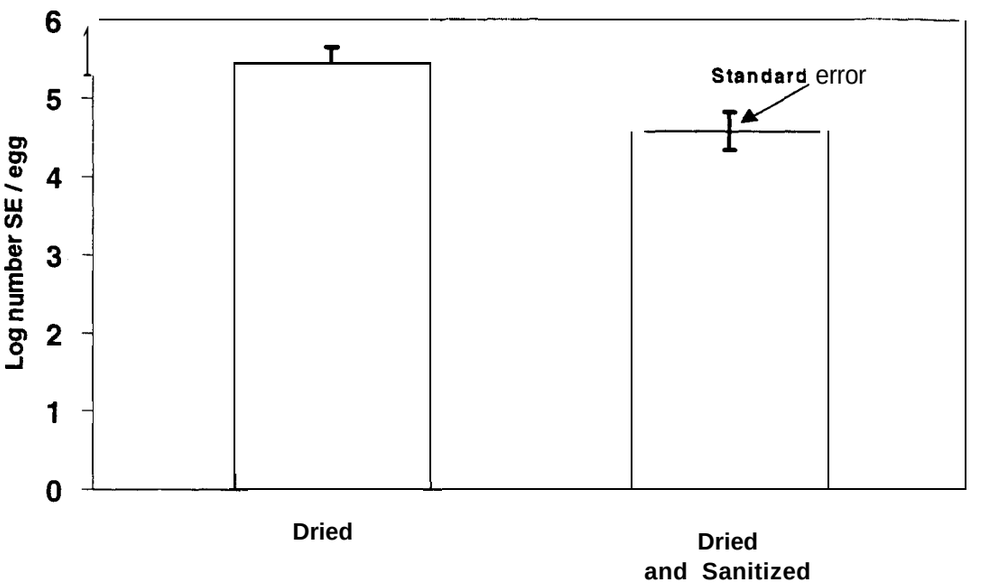

Figure 2. Mean numbers and standard errors of *Salmonella enteritidis* in /on egg shell structure (shell +membrane) after drying and after sanitation.

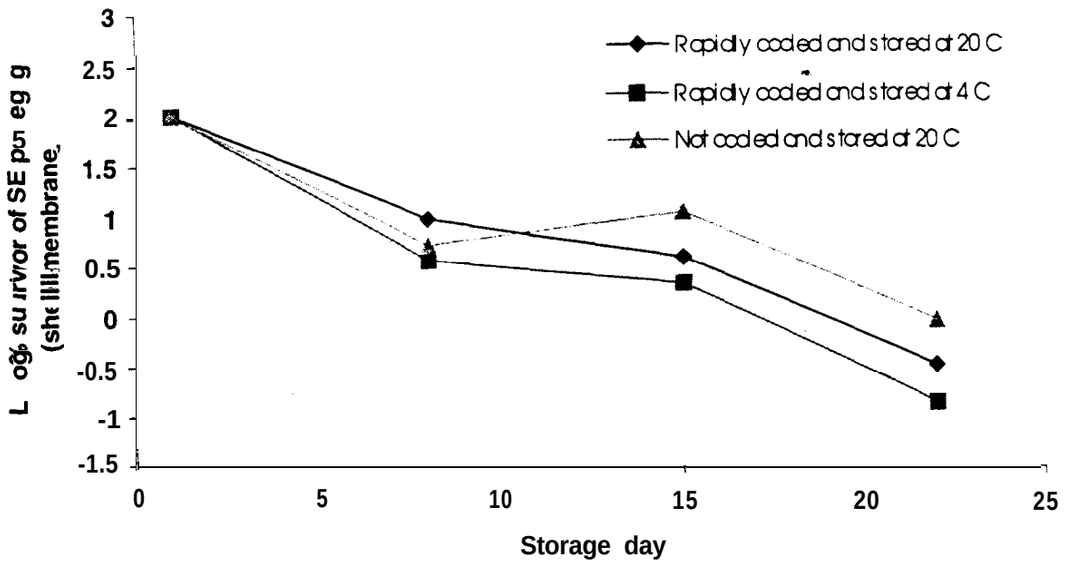

Figure 3. Long term survival of SE in the shell structure (shell + membrane) at two temperatures, cooled or not cooled eggs.

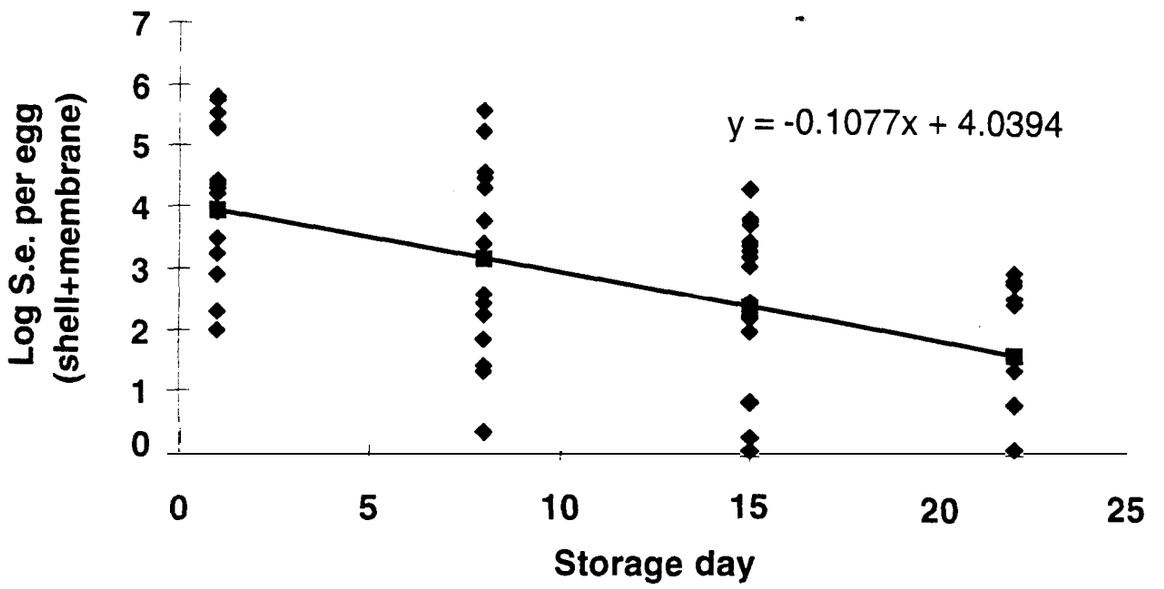

Figure 4. Regression of log survivor of SE in shell structure (shell + membrane) on storage time; eggs experimentally contaminated and stored at 50 % relative humidity at 4 ^{O}C or 20^{O}C.

Table 1. Ratios of positive egg contents with an improved and the conventional methods of content removal. Eggs experimentally contaminated [1] with *S.* Enteritidis and sanitized with Lugol's / ethanol mixture [2]

Replicate no.	Average no. of SE on shell after disinfecting [3]	Ratio of positive contents (+) / Total	
		Improved method [4]	Conventional method [5]
1	3.71	0/14	4/14
2	3.28	0/15	1/15

1) Eggs with an internal temperature of 37 C were dipped for 3-5 seconds in a 37°C culture of Se (in brain heart infusion broth) and dried at 37°C and 30 % relative humidity for 3 hours.
2) Lugol's / ethanol mixture contains 3 parts of 70% ethanol and 1 part of Lugol's solution (contains 5% iodine and 10 % potassium iodide dissolved in 75 % ethanol)
3) The eggs were dipped for five minutes in Lugol's / ethanol mixture
4) The point end of the eggs were flamed for 15 seconds with a small propane torch then egg contents were removed with a sterile pipette through a hole cut with sterile forceps.
5) The eggs were cracked by a sterile metal blade then contents were removed by breaking eggs into halves with sanitized hands.

Table 2. Ratios of *Salmonella enteritidis* positive shells and positive contents of eggs cooled to $4^{O}C$ at 3 rates of cooling Eggs were experimentally contaminated [1] and sanitized with disinfectants [2]. Pooled data from 7 experiments.

Treatments	)o el	atio po Conten
Contaminated not cooled [4]	25/30	0/30
Contaminated fast cooled [5]	33/35	0/35
Contaminated slow cooled [6]	26/35	0/35
Contaminated very slow cooled [7]	22/25	0/25

1) Eggs with internal temperature of $37^{O}C$ were dipped for 3-5 seconds in $37^{O}C$ overnight culture in BHI of *Salmonella enteritidis* culture.
2) 70 % ethanol for 15 minutes, Lugol's 1 part in 3 part ethanol for 5 minutes, 10 % Lugol's 1 part in 3 part ethanol for 5 minutes, or 0.6 % chlorhexidine diacetate + 1 % acetic acid for 5 minutes. In all experiments eggs and disinfectants were at room temperature.
3) Eggs were opened with an improved technique involving flaming the point end of an egg for 15 seconds with a small propane torch followed by removal of egg contents with a sterile pipette through a hole cut with a sterile forceps.
4) Eggs were contaminated and dried for 3 hours at 37 C and left at room temperature.
5) Eggs were contaminated and dried for 3 hours at 37 C then cooled down to around 4 –6 C in 1.5 hour.
6) Eggs were contaminated and dried for 3 hours at 37 C then cooled down to around 4 –6 C in 9-12 hour.
7) Eggs were contaminated and dried for 3 hours at 37 C then cooled down to around 4 –6 C in 19-22 hour.

Table 3. Effect of cooling rate on shell strength.

Cooling rate	Shell deformation [1], after cooling (mm x 0.001)	Deformation difference before - after cooling (mm x 0.001)	Breaking strength, after cooling (newtons)
Fast	23.6	-0.075	31.6
Slow	23.3	0.325	32.4
Very slow	23.4	0.125	32.4

[1] data are means of 5 replicate tests each with 20 eggs. No significant differences between data in the same column.

This talk was presented at the 1998 Egg Processing Workshop.