MICHAEL FOODS INC.

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November 15, 2004

04-034N
04-034N-5
Hershell Ball

Docket Clerk
U. S. Department of Agriculture
Food Safety and Inspection Service
300 12th Street, S.W., Room 102, Cotton Annex
Washington, DC 20250

Re: U.S. Department of Agriculture, Food Safety and Inspection Service, Docket No. 04-034N

Dear Sir/Madam:

The following comments and attached materials are submitted in response to publication of the quantitative risk assessments *on Salmonella Enteritidis* (SE) in shell eggs and *Salmonella spp.* in liquid egg products.

General Comments:

Comment period not sufficient and important information not provided.

The comments and submitted materials are offered for further consideration by the scientific staff responsible for preparing the risk assessment reports. Unfortunately, the public has been given a very short span of time to review the extensive draft assessments and the supporting materials presented in the Annexes. It would seem appropriate to allow additional time for review given that it is obvious that the FSIS has spent at least two years and utilized an extensive team of scientist including experts in statistics, risk assessment, microbiology, and food processing to prepare the risk assessments presented October 22, 2004. It is not reasonable to expect that individuals, impacted industry, or trade associations have had the time to conduct reviews and assemble appropriate expertise to comment in detail within the time allocated for public comments (Federal Register, Vol. 69, No. 192, October 5, 2004).

It would also be appropriate to provide information used in preparation of the drafts, but not available to the public. They include (1) several citations of "Personal Communications" used in support of develop of assumptions or analysis of data; (2) the complete presentation of the "Base Line Study" used to anchor the risk assessment for pasteurized egg products; and (3) studies conducted by Research Triangle Institute for the FSIS and used to support development of assumptions and analysis of data for the assessments.

Comments Regarding *Salmonella Enteritidis* (SE) in Shell Eggs:

Assumptions for growth of SE in pasteurized shell eggs using the estimators built from evaluation of temperature and the "Yolk Membrane Breakdown" hypothesis overestimate should be reevaluated.

In the draft risk assessment the there is a brief discussion (Chapter 3 Exposure Assessment, pages 38-39) about the "yolk membrane breakdown" hypothesis that acknowledges the event(s) or process(s) is not well understood or defined. The hypothesis relates some change in the yolk membrane that that allows rapid growth of SE in an egg that also appears to be related to the storage temperature history of an egg. It is well accepted that both albumen quality and yolk membrane integrity are affected by temperature history. For shell eggs that have not been treated to stabilize the albumen for example by refrigeration, oiling the shell, thermostablization,

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or pasteurization it is expected that there will be deterioration of the yolk membrane as indicated by reduction in yolk index or weakness and fragility of the membrane.

Shell eggs that have been treated to stabilize interior quality of the egg as measured by albumen quality (Haugh Units) will also have associated maintenance of the yolk membrane quality. Oiling the shells, theromostabilization, and pasteurization of shell eggs stabilizes albumen quality and yolk membrane quality. Stadelman's (1986) chapter, The preservation of quality in shell eggs. In Egg Science and Technology, 3rd edition. The Haworth Press Inc., Binghamton, NY. gives a good overview of the relationships of albumen and yolk qyality and methods to preserve quality. Schuman et al. (1997) in Jouranl of Applied Microbiology Vol. 83, 438-444, presented results showing that pasteurization of shell eggs did not harm the albumen quality as measured by Haugh Units or yolk membrane quality as measured by yolk index.

Earlier unpublished research conducted at the University of Missouri-Columbia on pasteurization of shell eggs reported Haugh Units after four weeks of storage at 22.2°C for pasteurized eggs that would indicate albumen quality equal to that of USDA Grade A or AA eggs (report entitled Thermal Destruction of *Salmonella Enteritidis* in Shell Eggs, prepared by H. R. Ball is attached).

Quality Attributes of Thermally Treated Shell Eggs with and without Oiling after Four Weeks Storage at 22.2 °C (72°F) and 7.2 °C (45°F).

Treatments	Albumen pH	Albumen pH	Haugh Units	Haugh Units
No Oil	**22.2 °C (72°F)**	**7.2 °C (45°F**	**22.2 °C (72°F)**	**7.2 °C (45°F)**
No Heat	9.3	9.2	20	60
56.75°C, 36 min.	9.2	8.9	78	82
57.5 C° 23 min.	9.2	9.1	74	82
Oiled				
No Heat	8.0	8.1	58	70
56.75°C, 36 min.	7.9	8.2	80	80
57.5 C° 23 min.	8.0	8.1	81	82

Schuman et al. (1997) reported Haugh Unit values after treatment in a 58°C water bath of 80.7 Haugh Units. That observation determined within hours of heat treatment is similar to the Hugh Unit data above. As noted in Schuman et al. (1997), the thermal treatments improve Haugh Units but had no effect on yolk index.

The Missouri data and Schuman et al. (1997) show a positive effect on the indicator of albumen quality with no effect on yolk index. The data also shows that the effect is maintained through at least 4 weeks of storage at 72°F. Since the bulk of the prior literature shows positive relationships between maintenance of albumen quality and yolk membrane quality for eggs in general, it could reasonably be assumed that the positive albumen quality result that occurs with shell egg pasteurization also maintains yolk membrane quality.

Maintenance of egg quality indicators even at temperatures above 45°F argue against acceptance of assumptions for growth of SE in pasteurized shell eggs using the estimators built from evaluation of temperature/time and the "Yolk Membrane Breakdown" hypothesis for non-pasteurized shell eggs.

Assumptions for SE surviving shell egg pasteurization should be reviewed and lowered.

The United States Department of Agriculture, Agriculture marketing Service and the U.S. Food and Drug Administration have jointly established a requirement that shells eggs designated as pasteurized must be subjected to a treatment that yields a minimum 5-log reduction of viable salmonellae (Federal Register 62(185):49955-49957. Docket PY-97-008). Because of this

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existing regulation it is not clear why time an effort was devoted to evaluation of 3-log reduction processes. Those process would not be by regulation pasteurization processes or be expected to have creditability for food safety. In contrast to the 3-log reduction approach, the portions of the risk assessment for pasteurized egg products seemed to refer to USDA Egg Products Inspection regulation minimums in those discussions.

Some of the discussions in the draft report and Annex discuss the lack of information about the lethality of SE located in other portions of the egg other than the center of the yolk. Schuman et al. (1997), Hou et al. (1996) (Food Microbiology, 13, 93-101.), and Brackett et al. (2001) (Journal of Food Protection 64, 934-938) report destruction of SE in the center of the yolk, assumed to the worst case situation because of potential high numbers and slowest portion of egg to heat. Although the heating medias were different in Schuman et al. and Bracket et al., the time-temperature curves were essentially the same with essentially equal lethality reported.

Because of conduction heating, heat is transferred from the media through the shell, shell membranes, albumen, yolk membrane and final to center of the yolk. Although time-temperature profiles have not been fully developed for the different portions of the eggs during pasteurization processes evaluated, it is logical to assume that those portions nearest the heating media reach temperatures of the media from one to five minutes or less than time required to reach temperature in the center of the yolk.

The University of Missouri studies described above and attached, showed up to 6-log reductions of SE inoculated on the surface of the yolk membrane in less than 27 minutes in 57.5 °C water bath, less than 32 minutes in 56.7 °C water bath, and less than 45 minutes in 56 °C water bath.

Schuman et al. (1997) reported center yolk temperatures of 55.3 to 56.2 °C and log reductions of 4.3 and 4.83 respectively for eggs held 35 minutes in a 57 °C water bath.

Bracket et al. (2001) reported center yolk temperatures of 56.12 and 56.18 °C and log reductions of 6.13 and 6.21 for eggs held in humid heated air for 30 minutes at 57.2 °C.

The residence time of 27 minutes in a 57.5 °C water bath used in the Missouri study is approximately the come-up time required to achieve pasteurization temperatures if the pasteurization process is define by time at temperature in the center of the yolk. The risk assessment draft did not adequately define shell egg pasteurization as used in the context of the report. Definition including time and temperature as well as minimum required log reduction at a specific location in the egg should be included.

When considering the conductive nature of heat transfer in water immersion or humid air heating, if minimum 5-log reduction processes defined for center yolk are used it is unlikely that there would be any survivors in any portion of the egg outside of the yolk.

Based on reported D-value of approximately 2 minutes at 56.7 °C for pH 8.8 egg white (UEA/AEB, 2002, International Egg Pasteurization Manual), an optimum egg white pH for best visual qualities of pasteurized shell eggs) and assuming that the temperature of the egg white from shell to the yolk membrane was at 56 °C in 30 minutes (Bracket et al. 2001) or 35 minutes (Schuman et al. 1997) the log reductions would be 15 and 17.5 respectively for SE in the albumen.

If 5-log reduction process for center yolk, i.e., time and temperature at center of the yolk, are used there will always be 2-3-log reduction occurring in the yolk as the center yolk temperatures are approaching the process control temperature. Seven to 8-log reductions would be expected for the total process with no survivors in the albumen.

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Given the above and assuming that minimum legal pasteurization process must deliver a 5-log reduction minimum in the center of the yolk, it seems reasonable that the assumptions for survival of SE after pasteurization should be lowered.

At this time there are only two producers of pasteurized shell eggs. The process used by Michael Foods, Inc. is defined for a 5-log minimum reduction in the center of the yolk.

Comments Regarding *Salmonella spp.* In Liquid Egg Products:

50,000 illnesses attributable to *Salmonella spp.* In liquid egg products seems unreasonable.

Given that there have been no documented illnesses attributable to *Salmonella spp.* from pasteurized liquid egg products it seems reasonable to question the assumptions used to develop the estimate of 50,000 illnesses per year. The estimates seemed to be anchored based on the incidence of *Salmonella spp.* positive egg white samples found in the base-line study. There could also be some fundamental issues with assumptions used to estimate numbers surviving, growth post-pasteurization, and portions of egg consumed in prepared foods.

The draft report and Annex discuss the broad assumptions that equal portions of each type of egg would be consumed and that the analysis did not deal with food formulation or preparation practices that in themselves would not allow illnesses to develop.

Although the base line study reported finding positive samples, we do not know if the producing plants would have also determined that the product was contaminated and held for rework or disposal. The study did not consider the possibility that a significant portion of positive product could be detected at the plant level and not allowed to move to distribution. If the risk assessments can use assumptions of survival of *Salmonella spp.* and subsequent growth, it would seem reasonable to also use assumptions that quality and food safety programs would prevent a portion of positive product from moving to market.

A correction for intervention of quality programs should be included in the determination of risk.

The base line study as reported lacks critical information.

At this time the details of the base line study have not been fully presented. For example one must assume at this time that all samples were sent as liquid samples with sufficient refrigeration and insulation to keep them at temperatures less than 40°F. That detail has not been discussed.

For samples having the higher estimates of *Salmonella spp.* we do not have confirmation that temperature of the samples were known at time of reception.

The discussions in the Annex and draft report indicate that there could be uncertainty in the uniformity of sampling which could impact on the results.

We do not know the pasteurization process associated with the samples, minimally the temperature and hold time. This is especially critical for understanding the data relative to survival of *Salmonella spp.* reported in egg white samples. Did the processes include use of pH adjustment as permitted as a process aid? Were they with or without hydrogen peroxide? Were they after hot room treatment for dried whites?

Other critical information would be total aerobic plate count for the raw egg samples prior to pasteurization and relation to estimated content of *Salmonella spp.*

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New data describing the pH effect on lethality of *Salmonella spp.* in white based egg substitute.

During the public meeting where the risk assessments were presented. There was an invitation for additional information on several topics that relate to effectiveness of pasteurization processes. The pH of egg white has been recognized as being important to lethality of egg white pasteurization processes. The UEA/AED (2002) study reported lethality at pH values 7.8, 8.2, 8.8, and 9.3. D-vales for egg white at 9.3 were significantly lower than those for the lower pH values. The lower pH values are more consistent with fresher egg generally used for processing. The UEA/AEB (2002) report indicated a pH effect with lethality generally higher as pH increased.

Included with these comments are an internal report and raw data evaluating the effect that pH of an egg substitute (98% egg white) has on lethality of *Salmonella spp.* As pH increased from 8.2 to 9.0, D_{135oF} decreased from 1.02 to 0.69 minutes. The results provide additional information that generally supports the understanding that lethality of egg white based liquid egg products is enhanced at higher useful pH values. This provides an approach that has long been recognized as an effective aid to pasteurization of whites.

As noted above, knowing the details of the pasteurization processes applied to the egg white samples that were positive for *Salmonella spp.* would be useful. It would also be useful to understand the general use of pH control for assisting egg white pasteurization.

Concluding Comments:

The USDA, FSIS staff and others contributing to the risk assessments have devoted may hours to data collection and data analysis. The drafts provide an excellent base for discussion with the intent to enhance public safety by reducing the risk of illnesses due to *Salmonella spp.* It is our conclusions that time to study the drafts should be extended and that the additional disclosure of some of the critical data be provided.

We also believe that the estimated illnesses attributed to pasteurized shell eggs and pasteurized liquid egg products are over stated. Specific points of concern and suggested reasons for reconsidering some of the assumptions used are presented above.

It is difficult for me to adequately review the "science" and "statistical" theories used in developing the various equations to assign risk. However there seems to be some opportunity to further enhance the understanding of the characteristics of pasteurized shell eggs and egg products that may allow building of assumptions that are more closely related to on going experience and science/technology of the products under study.

Respectfully,

Hershell Ball, Jr.

Hershell Ball, Jr., Ph.D.
Vice President
Michael Foods, Inc.

Enclosures

HRB Research & Development
3245 Grand Oak Lane
New Hill, NC 27562
919-387-8199

June 11, 1993

Connie M. Armentrout
Coordinator
University Patents & Licensing
University of Missouri System
509 Lewis hall
Columbia, MO 65211

Dear Ms. Armentrout:

Enclosed is a revised draft of a manuscript and analysis of data provided to me by Dr. Vandepopuliere [redacted]

Please have copies of the draft sent to Joe, Jesse, and Owen for their comments.

Sincerely,

Hershell Ball, Jr.

Hershell Ball, Jr.

cc: J. M. Shapiro

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Draft 6-93
NRB

THERMAL DESTRUCTION OF SALMONELLA ENTERITIDIS IN SHELL EGGS

Introduction

It is well know that Salmonella organisms have been associated with egg products for many years. More recently Salmonella enteritidis (SE) has been detected within the shell egg. It is currently a major concern. SE is heat labile and can be destroyed by a relatively low temperature. The objectives of this study were to destroy SE within the shell egg and evaluate the processing treatment on interior egg quality.

Thermal treatments of shell eggs to prevent embryonic growth in fertile eggs, to reduce the incidence of spoilage during long term storage, and maintain internal quality received considerable research attention during the decade of 1943 to 1953. Stadelman (1986) presents a concise review of that research. The heating of shell eggs in hot water or oil at various temperatures and times has been named "thermostabilization." The egg industry at that time was very different in that most of the eggs were produced by small flocks and the majority of eggs used by the food industry were collected as seasonal surpluses in the Spring. As a result of the production practices in those days, the eggs were more likely to lose interior quality, or become unfit for human consumption because of undesirable bacterial growth or embryonic development. As assessed in Stadelman's review, there were several articles published concerning "thermostabilization" methods to help overcome those problems. During that period,

Dr. E. M. Funk was granted a U.S. Patent for thermostabilizing shell eggs (Funk, 1947). While there were discussions of reducing bacterial rots in shell eggs by thermostabilization, it is interesting to note that the researchers involved or others to date have not suggested that those treatments would be capable of resulting in sufficient destruction of Salmonella sp. to consider those treatments as pasteurization.

As egg washing technology improved, positive benefits from high wash water temperatures were noted. Bierer and Barnett (1961) reported that as the temperature of wash water was increased, the number of recoveries from Salmonella contaminated eggs decreased. Washing at 150° F for either 1 or 3 minutes resulted in complete kill of S. pullorum and S. gallinarum, and 99.5 - 100% kill of S. typhimurium.

The increased occurrence of outbreaks of gastroenteritis due to S. enteritidis related to the consumption of grade A shell eggs (Lin et al., 1988) has resulted in several studies concerning the control of that organism. Phelps (1992) stated that when layers were inoculated intravenously with 10^5 and 10^8 SE, heavy infection of the ovaries occurred and some infection persisted for several weeks. Nearly all of the ovarian infections were confined to the interstitial tissue and not to the yolk contained in the large follicles. Infections of the ovary did not result from contamination from infected air sacs

and none of the eggs contained SE. Hens infected orally gave similar results to those following intravenous injection although the number of isolations found in the ceca and cloaca were higher. SE was isolated from less than 0.3% of the eggs into which SE bacteria were injected, whereas 10 times as many were cultured in eggs that were contaminated on the shells only.

Gast and Beard (1991) inoculated laying hens orally with a phage type 13a strain of SE. Only 3% of the freshly laid eggs and 4% of the eggs held for 7 days at refrigerator temperature sere identified as having SE contaminated contents, whereas SE was isolated from the contents of 16% of the eggs held for 7 days at room temperature. Most contaminated eggs contained generally less than 10 cells/ml and rarely exceeding 100/ml.

Materials and Methods

Two experiments were conducted to determine the thermal resistance of SE (phage type 8) in artificially infected shell eggs and the resulting changes in interior quality due to elevated processing temperatures. During the first experiment fresh shell eggs weighing approximately 62 grams each were obtained form the University research unit. The eggs were dipped in an iodoform solution, excess solution was removed with a catlin gauze and permitted to air dry on sterile plastic egg flats. Each egg was inoculated with 10^6 viable cells from a 24 hour Trypticase soy broth culture of SE (phage type 8). The

shell was perforated with a sterile blunt 18 gauge needle. A sterile blunt glass needle on a 10u liter pipet was used to inject the culture near the yolk surface and the hole in the shell was then sealed with a small piece of aluminum foil and Super Glue. Groups of 36 eggs each were subjected to temperatures of 22.2 (unheated control), 56, 56.75 and 57.5°C. Eggs within a temperature-group were subjected to a range of heating time periods ranging from 15 to 45 minutes. The study was replicated in time. Heating was carried out in a shaking water bath equipped with polyethylene egg flats perforated with numerous 1 cm holes to increase water circulation around the eggs.

Immediately following heat treatment, each egg was broken separately and the albumen plus yolk was mixed for 30 seconds in a sterile Stomacher bag containing 200 ml of lactose broth using a Stomacher Lab - Blender 400[1]. The mixed egg content was incubated in a sterile glass container for 24 yours at 39°C. A representative culture was then transferred to selenite-cystein broth and incubated for 24 hours at 39°C. The incubated culture was streaked on brilliant green agar plates and incubated for 24 hours at 39 C. The suspect colonies were transferred to TSI slants.

The second experiment was conducted to evaluate the effect of heating, oiling and storage on interior egg quality. Four storage treatments of zero, one, two and four weeks were used,

each with oiled and non-oiled eggs. The eggs were heated in a water bath at 56.75°C for 36 minutes and 57.5°C for 23 minutes. Eggs were oiled following heat treatment. Thirty eggs from the control and each treatment were stored at room temperature (22.2°C) and 7.2°C.

A group of 14 eggs from each variable was used to determine pH, foam volume, whipping time, foam depth, foam stability, grade and a second group of 14 eggs was used to evaluate Haugh units.

Results and Discussion

Microbiology

Table 1 presents the results of the thermal treatments on the survival of S. enteritidis inoculated into shell eggs. As temperature increased, the time required to obtain salmonella negative eggs decreased. At 56°C, exposure time required to obtain no positive eggs was greater than 41 minutes. At 56.75 and 57.5°C, exposure times greater than 28 and 23 minutes, respectively, were required to obtain eggs negative for salmonella. Times at temperatures where none of the twelve inoculated eggs were positive, were used in a regression equation to determine the thermal death time curve (TDTC) presented in Figure 1. The equation for the line is:

$$\log t = -0.1216*T + 8.4274$$

where time is minutes and T is temperature °C. The R^2 ~~for t is~~ = 0.86.

Since the line is based on a relatively small number of samples over a limited temperature range, it is probably best to consider the line as a workable approximation or an apparent F_o line for S. enteritidis in shell eggs. The temperature range and times used to obtain the data were selected with the intent of determining ~~that~~ if commercially reasonable thermal treatments would have sufficient lethality for Salmonella sp. It is expected that the increasing the number of samples and extending the temperature range would result is some changes in the slope of the line, especially at lower temperatures (Cotterill et al., 1973). Based on concerns for the interior quality and their use in cooking, the practical upper temperature range would probably be less than 60°C (Funk 1947). At temperatures in the range of 55 to 65°C, Cotterill et al. (1973) generally found linear TDTC for destruction of S. oranienburg. It is anticipated that the F_o line for Salmonella sp. in shell egg is also linear over that temperature range.

It is established that different strains of Salmonella sp., the type of egg product, and other environmental conditions will effect the thermal inactivation of Salmonella sp. Shah et al. (1991) presented D values for 17 strains of S. enteritidis in whole egg ranging from 13.7 to 31.3 seconds at 60°C. The average D was 19.2±5.4 sec. and was reported to be similar to previous data. Cotterill et al. (1973) and USDA (1969) provide data showing the influence of egg product type, pH, salt, and sugar on

the thermal resistance of Salmonella sp. When evaluating the thermal resistance of Salmonella sp. in intact shell eggs, the location of the bacteria within the egg becomes important. The thermal resistance of Salmonella sp. in different egg products is as follows: plain yolk> whole egg or pH 7 egg white> pH 9 egg white (USDA, 1969).

In this study, the culture was placed in the egg white near the surface of the yolk. The consensus of those actively studying S. enteritidis infection of shell eggs is that the bacteria is found in the egg white of naturally infected eggs produced by infected hens (Gast and Beard, 1992; Beard, 1993). The apparent F_o line was plotted in Figure 2, a redrawing of Figure 6 from the Egg Pasteurization Manual (USDA, 1969). This allows a visual evaluation of the thermal processes applied to intact shell eggs relative to accepted minimal pasteurization processes for liquid egg products. Minimal pasteurization processes are expected to produce a 9 log reduction in the numbers of Salmonella sp.

When comparing the apparent F_o line and actual processes to the lines for pH 9 egg white and whole egg or pH 7 egg white, the shell egg processes seem to be more than adequate to achieve reductions of S. enteritidis sufficient for an accepted pasteurization process for protection of public health. The pH of the eggs in this study ranged from 8.4 to 8.6 which is typical for shell eggs the age of those used in this study.

If the cells were in the yolk, the processes would still be considered to be substantial. Although natural infections of the yolk are not expected at the time of ovulation, it is clear that under adverse handling conditions, S. enteritidis can be introduced into the egg and grow to very high numbers in the yolk (Hammack et al., 1993). At 56.5°C (134°F), if the cells were in the yolk, the minimum holding time would be 36.42 minutes for an adequate pasteurization process. Since the apparent F_o line crosses the USDA yolk pasteurization line at about 134°F, it may be prudent to select thermal treatments for shell eggs at temperatures above 134°F.

Quality and Function

Quality and functional attributes of shell eggs heated at 56.75 and 57.5°C with and without oiling are summarized in Table 2. The expected ability of oiling egg shells to maintain fresh egg pH and interior quality is evident. The egg white pH of the oiled eggs is clearly lower than for the unoiled eggs regardless of storage temperature. The thermal treatments did not seem to have an effect on egg white pH, but did seem to have an impact on interior quality as indicated by the Haugh unit values. For the non-thermally treated eggs, oiling held egg white pH and resulted in higher Haugh values at both storage temperatures. Oiling the thermally treated eggs appeared to help maintain interior quality if they were stored at room temperature (22.2°C). The thermal treatments alone, provided good protection of interior quality.

All thermally treated eggs regardless of oiling or storage temperature would be considered high A or AA quality grades. There seemed to be less correlation of egg white pH with interior quality than might have been expected. This is particularly so when comparing the egg white pH and Haugh units of oiled and unoiled eggs. That result suggests ~~that~~ the thermal treatments are stabilizing interior quality independently of deterioration mechanisms related to change in egg white pH. Funk (1947) claimed that heating shell eggs for 5 to 40 minutes at temperatures of 60 to 43.4°C, respectively, would maintain interior quality without impairing the whipping qualities. However, he did not define quality or whipping qualities.

In this study, the whipping qualities as indicated by whip volume and whip time were adversely effected by the thermal treatments. This indicates that the thermal treatments were substantial and parallel damage that is expected when liquid egg white is pasteurized. Oiling or storage temperature did not seem to have an effect on function of the egg white.

Thermally treated eggs, when broken out onto a plate, appear quite similar to unheated eggs with the exception of some slight opaqueness on the edges of the albumen. The normal shape of the thick egg white is maintained and there appears to be the normal amount of outer thin albumen. The yolk membrane may exhibit some weakness. Although yolk indices were not determined, trained observers note some flattening of the yolk relative to unheated

controls. The yolk membranes of heated shell eggs did not exhibit any additional fragility over the four weak storage and seemed to withstand handling for Haugh unit determinations as expected for eggs of the same interior quality.

Conclusions

The microbiological results show that the thermal processes applied were sufficient to impart substantial destruction of Salmonella enteritidis that had been introduced into the egg. The apparent F_0 line, when compared to pasteurization process that achieve 9 log reduction of Salmonella sp. in various egg products, indicates that the thermal treatments should be effective even if the bacteria were present in large numbers in the yolk. However the uncertainty about extrapolating the F_0 line below the treatments evaluated make it prudent to choose treatments more severe than 134°F for 36.5 minutes. It is also concluded that others have not anticipated that thermal processes, such as those described here, would result in a in-shell pasteurization process for eggs.

The maintenance of interior quality was expected from previous research. The loss of functional power was not expected, but is probably related to the more severe thermal treatments applied relative to those taught by Funk (1947).

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1.Tekmar company, P. O. Box 37202, Cincinnati, OH 45222.

Figure 1

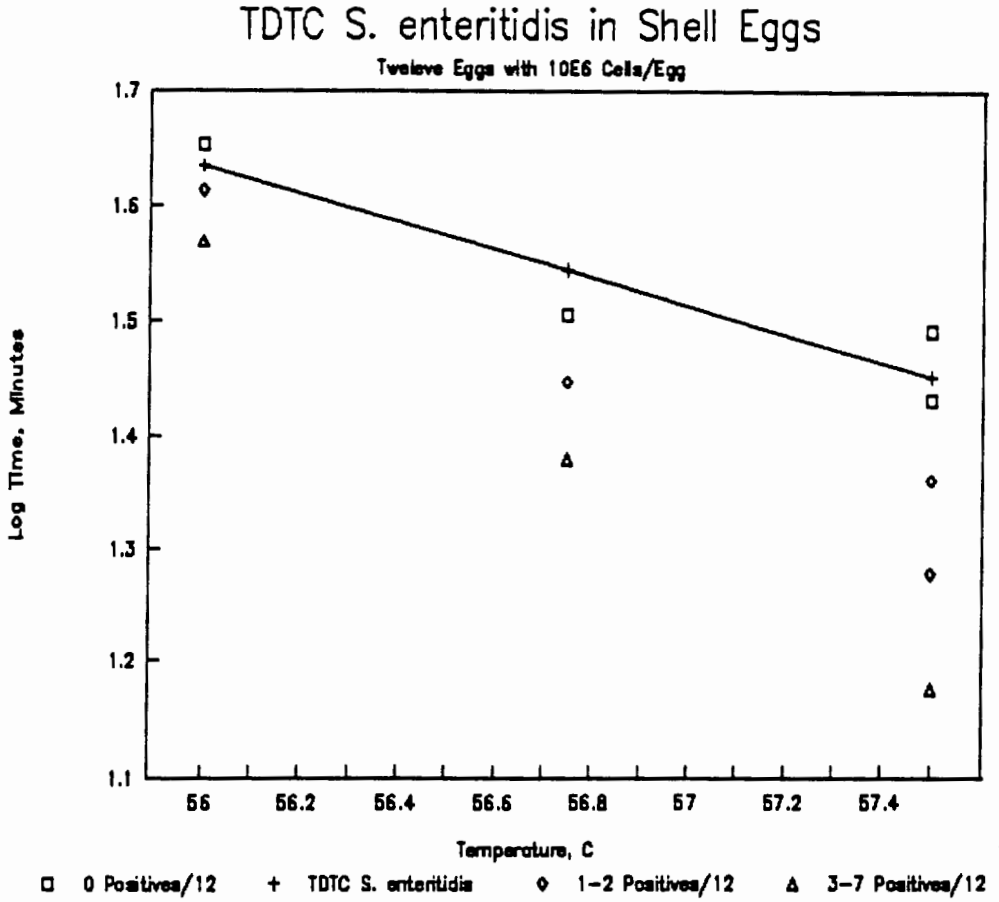

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Figure 2

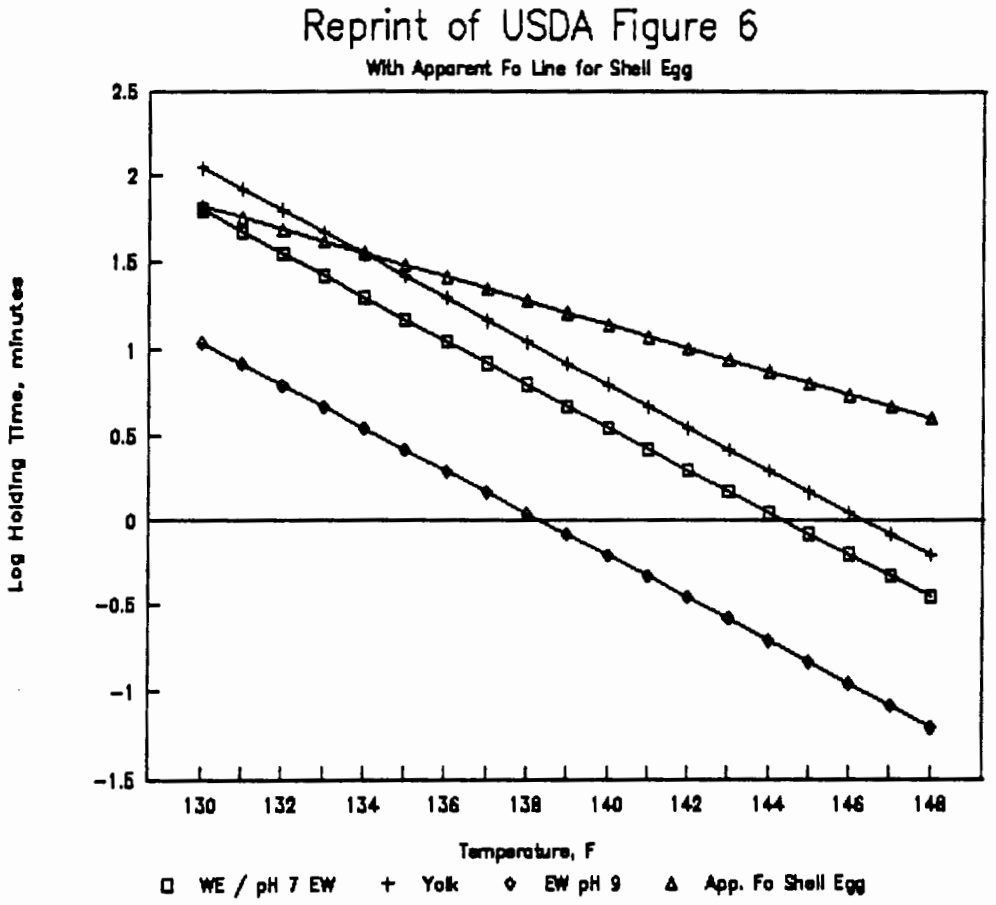

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Table 1. Number of samples positive after heating at 56, 56.75, and 57.5°C.

Time in Waterbath	Temperature of Water					
	56C		56.75C		57.5C	
min.	*No.-	No.+	No.-	No.+	No.-	No.+
15					12 -	4
16			12 -	11		
19					12 -	2
20			12 -	8		
23					12 -	2
24			12 -	7		
27					12 -	0
28			12 -	2		
29	12 -	3				
31					12 -	0
32			12 -	0		
33	12 -	6				
37	12 -	4				
41	12 -	1				
45	12 -	0				

* No. - No. + . Number of samples heated - number positive.

Table 2. Quality and functional attributes of thermally treated shell eggs with and without oiling after four weeks storage at 22.2 or 7.2°C.

	Egg White pH		Haugh Unit		Whip Volume[a]		Whip Time[b]	
No Oil	22.2C	7.2C	22.2C	7.2C	22.2C	7.2C	22.2C	7.2C
No Heat	9.3	9.2	20	60	1,000	900	40	45
56.75°C, 36 min.	9.2	8.9	78	82	550	650	220	110
57.5°C, 23 min.	9.2	9.1	74	82	750	600	280	130
Oiled								
No Heat	8.0	8.1	58	70	950	800	45	45
56.75°C, 36 min.	7.9	8.2	80	80	550	650	190	200
57.5°C, 23 min.	8.0	8.1	81	82	600	700	200	210

[a] Whip Volume in ml.
[b] Whip time in min.

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Immersion heat treatments for inactivation of *Salmonella enteritidis* with intact eggs

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J.D. SCHUMAN, B.W. SHELDON, J.M. VANDEPOPULIERE AND H.R. BALL JR. 1997. The effects of water-bath immersion heat treatments on the inactivation of *Salmonella enteritidis* within intact shell eggs were evaluated. Six pooled strains of *Salm. enteritidis* (*ca* 3×10^8 cfu, inoculated near the centre of the yolk) were completely inactivated within 50–57·5 min at a bath temperature of 58°C and within 65–75 min at 57°C (an 8·4 to 8·5-*D* process per egg). Following the initial 24 to 35-min come-up period, semilogarithmic survivor curves obtained at 58 and 57°C yielded apparent decimal reduction times (*D*-values) of 4·5 and 6·0 min, respectively. Haugh unit values increased during heating, while yolk index and albumen pH values were unaffected. Albumen clarity and functionality were affected by the thermal treatments; therefore, extended whip times would be required for meringue preparation using immersion-heated egg whites. Immersion-heated shell eggs could provide *Salmonella*-free ingredients for the preparation of a variety of minimally-cooked foods of interest to consumers and foodservice operators.

INTRODUCTION

Because of the public health significance and economic importance of foodborne salmonellas, a high research priority has been directed at the reduction of salmonellas in the food supply. Foods of animal origin including meat, poultry, eggs and dairy products are widely recognized as the primary vehicles for salmonellosis outbreaks in the US (Roberts 1990). For example, between 1968 and 1977, Grade A shell eggs and foods containing raw eggs were implicated in 20·3% of the documented salmonellosis outbreaks in the USA (Bryan 1981). *Salmonella enteritidis* (SE), an egg-associated serotype, was responsible for 380 salmonellosis outbreaks in the USA between 1985 and 1991, involving 13 056 illnesses and 50 deaths (Mishu *et al.* 1994). Grade A shell eggs were implicated in 82% of these outbreaks. The Centers for Disease Control and Prevention (1996) recently reported that the percentage of clinical *Salmonella* isolates identified as SE (and therefore having a probable egg association) increased from 5% in 1976 to 26% in 1994.

Shell eggs may become contaminated with SE by two possible routes: via contamination and passage through the shell exterior or by a transovarian transmission route (Board 1996). Recent SE outbreaks have generally involved Grade A eggs that have met both state and local requirements for shell quality and that have undergone shell washing with disinfectants (St. Louis *et al.* 1988). Although many different *Salmonella* serovars have been isolated from the surface of egg shells, only SE has been isolated from the contents of intact eggs using currently-accepted aseptic sampling methods (Humphrey 1994). These factors imply that the transovarian route of SE transmission is the more plausible route of shell egg infection.

Based on epidemiological evidence, Gast and Beard (1992) suggested that egg-associated human SE outbreaks are generally the result of a series of three independent events. Firstly, SE-contaminated eggs must be produced by infected hens. Secondly, contaminated eggs must be subjected to improper food handing practices that permit multiplication of SE to infectious levels. Thirdly, SE-contaminated eggs must be undercooked or consumed raw. Many products, such as Caesar salad dressing, hollandaise sauce, eggnog and home-made ice cream, have been implicated in SE outbreaks because they typically receive little or no heat treatment prior to consumption (St. Louis *et al.* 1988). Moreover, certain

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traditional egg cooking practices (i.e. 'sunny-side' frying, soft poaching, marginal hard-cooking) may be inadequate to kill *Salmonella* in eggs (St. Louis *et al.* 1988).

Long-term control of SE infections will require an examination of the ecology of this organism in poultry flocks and may depend ultimately on either its elimination from flocks, or on the pasteurization of shell eggs or liquid egg. During the 1940s and 1950s, the thermal treatment of shell eggs to prevent embryonic growth in fertile eggs, to reduce the incidence of spoilage during long-term storage and to maintain internal quality received considerable research attention. Stadelman (1995) presents a concise review of that research with particular attention directed toward the practice of 'thermostabilization', a patented process (Funk 1947) whereby shell eggs are placed in heated water or oil to extend storage life and prevent spoilage and deterioration of egg shell quality. At the time of the present research, no studies evaluating the potential for such treatments to inactivate bacterial pathogens within internally-infected or inoculated shell eggs were available in the published literature. Hou *et al.* (1996) recently documented the feasibility of combining water-bath and hot air oven processing to reduce viable SE populations within inoculated intact shell eggs. The objectives of the present research were to assess the SE-inactivation potential of 57° and 58°C water-bath immersion heat treatments for fresh, intact shell eggs, and to evaluate the effects of selected immersion heat treatments on albumen functionality and egg interior quality attributes.

MATERIALS AND METHODS

Shell eggs

Nest-run brown shell eggs (62 ± 2 g per egg) were obtained within 1 d of laying from a single flock of Arbor Acre hens at the North Carolina State University Poultry Research Unit. The eggs were individually washed in warm soapy tap water, rinsed twice in sterile deionized water and air-dried at room temperature for 2 h.

Bacteria and culture conditions

The six SE isolates used in this study and their sources were as follows: Benson-1 (human clinical) obtained from Dr F. T. Jones (North Carolina State University, Raleigh, NC, USA); ATCC 4931 (human clinical, type strain) obtained from the American Type Culture Collection (Rockville, MD, USA); and strains ME-14 (poultry manure), ME-15 (shell egg transfer belt), ME-16 (shell egg transfer belt) and ME-18 (live poultry), all obtained from Dr H. M. Opitz (University of Maine, Orono, ME, USA). The isolates were maintained on tryptic soy agar (TSA; Difco Laboratories, Detroit, MI, USA) slants at 4°C.

Working stock cultures were prepared by transferring a loopful of parent culture to 10 ml of tryptic soy broth (TSB; Difco) (37°C, 24 h). By transferring 60 μl from each working stock culture to a centrifuge tube containing 30 ml of TSB (37°C, 24 h), stationary-phase cultures containing 9·1–9·6 log cfu ml^{-1} were obtained. The 30 ml cultures were centrifuged for 15 min at $9000 \times g$ (4°C), and the cell pellets were each resuspended in 3 ml of sterile 0·1% w/v peptone water (PW) and pooled into a single sterile test tube to yield the final six-strain inoculum suspension.

Inoculation protocol

A droplet of Duro™ Super Glue® (Loctite Corp., Cleveland, OH, USA) was placed on the approximate geometric centre along the equatorial axis of each shell egg. A 9·5 mm diameter rubber septum (Microsep® F-174; Supelco, Bellefonte, PA, USA) was then affixed to each egg at the glue droplet site and permitted to dry at room temperature for 2 h. The eggs were held overnight at 4°C and allowed to warm to room temperature before inoculation. Eggs were individually inoculated near the geometric centre of the yolk with *ca* 8·5 log cfu of concentrated, pooled SE cells in 50 μl of sterile PW. This was accomplished by perforating the septum and the egg using a 2·54 cm/23 gauge sterile needle (Becton Dickinson, Franklin Lakes, NJ, USA) coupled to a calibrated 50 μl repeating syringe (Hamilton Co., Reno, NV, USA). Inoculated eggs were held at room temperature for $\leqslant 1$ h prior to immersion heating. Preliminary trials involving inoculation of 50 μl of aqueous tracer dye into the yolk followed by a standard hard-cooking procedure demonstrated that this inoculation procedure provided consistent placement of the dye near the centre of the yolk with no detectable inoculum drift (data not shown).

Immersion heating apparatus

Each set of 20 shell eggs was placed in a perforated plastic egg flat affixed to a 29×27 cm Plexiglas™ plate (0·5 cm thick) (McMaster-Carr Supply Co., Atlanta, GA, USA). Four Plexiglass posts ($22 \times 4{\cdot}5$ cm) placed at each corner were used to position and anchor an upper Plexiglas plate (29×27 cm) at a fixed height of 9·5 cm above the base plate. Three holes were drilled through the upper plate to permit placement of type T hypodermic thermocouple probes (3·8 cm/21 gauge; Omega, Stamford, CT, USA) perpendicular to the septum side of two uninoculated control eggs or within the water-bath. By perforating the septum and shell with the thermocouples, the internal temperature at the geometric centre of control eggs was monitored and recorded at 5 s intervals via a personal computer (LabTech Notebook™ software; Laboratory Technologies, Wilmington, MA, USA).

All heating trials were conducted by submerging the Plexi-

glas egg tray apparatus into a preheated circulating water-bath (Model W19; Haake, Paramus, NJ, USA) containing 16·8 l of deionized water. The water-bath was equipped with a calibrated temperature control module (Model DC1; Haake) accurate to ±0·05°C. Periodic calibration checks of the system were made using a standardized mercury-in-glass thermometer. During immersion heating, the upper surface of each shell egg was approximately 2·5 cm below the surface of the water in the bath.

Salmonella inactivation trials

For the SE inactivation trials, eggs were submerged in a preheated 58°C water-bath for up to 65 min or in a 57°C water-bath for up to 85 min. These thermal processes were selected based on the results of initial SE-inactivation experiments (unpublished data). At selected intervals, sets of three eggs were removed from random positions within the egg tray, rapidly transferred to a sterile plastic beaker containing 1 l of ambient (22°C) sterile deionized water, stirred and permitted to cool for 5 min. Each egg was gently wiped dry using sterile tissues and the contents were aseptically broken out into a tared Stomacher® 400 lab blender bag (Seward Medical, London, UK), diluted 1 : 10 (w/w) with chilled (7°C) sterile lactose broth, blended for 60 s using a Stomacher lab blender (Model 400; Tekmar, Cincinnati, OH, USA) and held at 4°C for up to 50 min. Serial dilutions of this homogenate were prepared in PW and portions (0·1–0·33 ml) were surface-plated onto pre-poured TSA plates. Plates were held at room temperature for 3 h (resuscitation period) and overlaid with tempered (45°C) xylose lysine deoxycholate (XLD; Difco) agar as described by Strantz and Zottola (1989). The overlay was permitted to solidify at room temperature and the plates were incubated at 37°C for 48 h. Black or black-centred colonies were enumerated as SE, and suspect isolates were confirmed by re-isolation onto XLD agar plates (37°C, 24 h) and biochemical confirmation in triple sugar iron (TSI; Difco) agar slants (37°C, 24 h). At the time of plating onto TSA, the remaining blended egg/lactose broth (*ca* 520 ml) was retained as an enrichment and incubated at 37°C for 24 h. After mixing, 1 ml of enrichment broth was transferred to 10 ml of selenite cystine broth (SC; Difco) (37°C, 24 h). A loopful of each SC enrichment was then streaked for isolation onto a prepoured XLD plate, and presumptive *Salmonella* isolates were selected and confirmed on TSI slants as previously described.

Egg interior quality evaluation

Based on the *Salmonella* inactivation data, shell eggs subjected to selected immersion heat treatments were evaluated to assess the effects of such treatments on egg interior quality and albumen functionality. Sets of 20 eggs were immersion heated as described previously and, at selected time intervals, groups of six eggs were removed and cooled in 2 l of ambient deionized water (1 h, 22°C), dried gently using tissues and stored at 4°C for 18–24 h. All interior quality tests were performed on eggs permitted to warm to approximately 22°C by incubation at room temperature for 3·5 h. Three eggs per bath temperature/dwell time were weighed and individually broken out onto clean 20 cm^2 glass plates and Haugh unit measurements were determined using an Ames micrometer (Model S-64128; B.C. Ames, Waltham, MA, USA) as previously described (Haugh 1937). The shape of the yolk (unseparated from the albumen) was also characterized by determining the yolk index as detailed by Funk (1948).

The albumen from the remaining three eggs per time/temperature combination was collected using a stainless steel egg separator, pooled in a glass beaker and transferred to a blender (Waring® model 31-BL-91; Dynamics Corp. of America, New Hartford, CT, USA) jar coupled to a variable power transformer. Pooled albumen samples were blended at 30% transformer power until a standard viscosity was achieved (i.e. a flow time of 10 s through a no. 3 Zahn viscometer cup). Two 8 ml portions of the blended albumen were transferred to 16 × 100 mm glass test tubes and centrifuged for 4 min at speed 3 in a Clay Adams Safety-head® centrifuge (Becton Dickinson, Parsippany, NJ, USA). Duplicate 3 ml aliquots of albumen supernatant fluid were transferred to individual disposable cuvettes for determination of albumen clarity, defined as per cent transmission (relative to deionized water) using a spectrophotometer at a wavelength of 550 nm. The pH of two 4 ml samples of non-centrifuged albumen was determined using an Accumet® model 10 pH meter (Fisher Scientific, Pittsburgh, PA, USA) calibrated using buffers of pH 7·00 and 10·00.

The functionality of blended, non-centrifuged albumen was assessed by determining whip times and whip volumes. Whip time was defined as the time (min) required for 60 g of blended albumen to achieve a soft peak condition (as judged by a trained analyst) when blended at speed 10 in a KitchenAid® blender (Model K45; Hobart, Troy, OH, USA) bowl coupled to a timer/power supply. Immediately after completing the above procedure, the foam was transferred to a 12·2 cm diameter glass cylinder resting on a glass plate. The upper surface of the foam was gently levelled, the foam height was measured and the whip volume (ml) calculated as described by Ball and Winn (1982).

Experimental replication and statistical analysis

Duplicate *Salmonella* inactivation trials were conducted for each target water-bath temperature tested. To assess the impact of immersion heating on albumen functionality and interior egg quality, triplicate trials were carried out at each water-bath temperature. A fresh set of 20 eggs was used for

each of the above experimental replicates (statistical blocks). For each water-bath temperature, values for interior egg quality factors and functionality were analysed as a function of dwell time using a one-way analysis-of-variance procedure (alpha = 0·05; SAS 1989). Mean separation was accomplished by comparison of each pair of treatment means using Student's *t*-test (LSD, alpha = 0·05; SAS 1989).

RESULTS

Inactivation of *Salmonella enteritidis*

Water-bath immersion heat treatments which completely inactivated the SE inoculum were identified (Tables 1 and 2). The eggs had an initial internal temperature of approximately 21°C and a blended pH of 7·2. Preliminary trials demonstrated that an immersion heating period of 24–35 min was necessary for the centre of the eggs to reach a temperature within 2°C of the water-bath temperature (Tables 1 and 2). At 58°C, viable SE populations (initially at 6·7 log cfu g^{-1} of internal contents) were reduced by 3·9–5·0 log cycles during the initial 35 min come-up period (Table 1). Within 50 min, viable SE populations had been reduced to $<1·0$ log cfu g^{-1} using the direct plating procedure, although the pathogen was detected in one of six eggs via enrichment testing (detection limit = 1 cfu per egg). Immersion heating at 58°C for $\geqslant 57·5$ min was effective in eliminating all detectable SE by both the plating and enrichment procedures. Based on the mean mass of egg contents (52 g per egg), the lethality of the 58°C immersion treatment was equivalent to an 8·4-*D* process per egg (Table 2).

Immersion heating at 57°C reduced viable SE populations (initially at 6·8 log cfu g^{-1}) by 2·0–2·5 log cycles during the initial 35 min come-up period (Table 2). Within a dwell period of 65 min, pathogen levels had been reduced by $>6·8$ log cycles g^{-1}, although one of six eggs tested positive for *Salmonella* via the enrichment protocol. Immersion heating at 57°C for $\geqslant 75$ min inactivated all detectable salmonellas (i.e. an 8·5-*D* process per egg; Table 2). In summary, immersion heat treatments of 50–57·5 min (at a water-bath temperature of 58°C) and 65–75 min (at 57°C) were determined to be salmonella-cidal at the inoculum level tested (i.e. an 8·4–8·5-*D* inactivation of SE per egg).

Interior egg quality and functionality

The impact of selected immersion heat treatments on egg interior quality and functionality is summarized in Table 3. Dwell time variables included unheated controls and eggs heated for 35 min (the approximate end of the come-up period) and for two additional time/temperature processes previously shown to yield an 8·4 to 8·5-*D* reduction in viable SE (Tables 1 and 2). Immersion heating for $\geqslant 35$ min at 58° or 57°C increased the Haugh unit values and reduced the clarity of the albumen relative to the unheated controls ($P \leqslant 0·05$; Table 3). These results reflect the impact of the thermal processes on the albumen proteins and on the height of the broken-out egg white. In contrast, yolk index values

Table 1 Thermal inactivation of *Salmonella enteritidis** in shell eggs subjected to low-temperature, long-time immersion heating at 58°C

Dwell time in 58°C water-bath (min)	Mean egg centre temperature (°C)		Survivors (log cfu g)†		Samples *Salmonella*-positive by enrichment testing‡	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
0	21·2	20·6	6·67	6·73	3/3	3/3
24	55·9	55·9	4·88	5·35	3/3	3/3
35	57·0	57·6	2·81	1·77	3/3	3/3
42·5	57·2	57·0	1·10	0·87	3/3	1/3
50	57·1	57·5	$<1·0$	$<1·0$	1/3	0/3
57·5	57·1	57·5	$<1·0$	$<1·0$	0/3	0/3
65	57·6	57·6	$<1·0$	$<1·0$	0/3	0/3

* Six-strain pooled inoculum.

† Determined by surface plating onto tryptic soy agar overlaid with xylose lysine deoxycholate agar (37°C, 48 h). The initial inoculum consisted of 50 μl of concentrated cells injected into the geometric centre of each intact shell egg, with a hold period of <1 h at room temperature prior to heating. The mean mass of total egg contents was 52·0 g per egg ($n = 42$). Therefore, a negative *Salmonella* enrichment test is equivalent to an 8·4-*D* process per egg ($n = 3$ eggs per dwell time).

‡ No. of samples *Salmonella*-positive/no. of samples tested (minimum detection limit = 1 cfu per egg).

Table 2 Thermal inactivation of *Salmonella enteritidis** in shell eggs subjected to low-temperature, long-time immersion heating at 57°C

Dwell time in 57°C water-bath (min)	Mean egg centre temperature (°C)		Survivors (log cfu g)†		Samples *Salmonella*-positive by enrichment testing‡	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
0	21·1	19·6	6·77	6·80	3/3	3/3
35	55·3	56·2	4·3	4·83	3/3	3/3
45	55·5	56·3	2·83	3·62	3/3	3/3
55	56·8	56·6	1·00	1·41	2/3	3/3
65	56·2	56·4	<1·0	<1·0	1/3	0/3
75	56·2	56·9	<1·0	<1·0	0/3	0/3
85	56·2	57·0	<1·0	<1·0	0/3	0/3

* Six-strain pooled inoculum.
† Determined by surface plating onto tryptic soy agar overlaid with xylose lysine deoxycholate agar (37°C, 48 h). The initial inoculum consisted of 50 μl of concentrated cells injected into the geometric centre of each intact shell egg, with a hold period of <1 h at room temperature prior to heating. The mean mass of total egg contents was 52·0 g per egg (n = 42). Therefore, a negative *Salmonella* enrichment test is equivalent to an 8·5-D process per egg (n = 3 eggs per dwell time).
‡ No. of samples *Salmonella*-positive/no. of samples tested (minimum detection limit = 1 cfu per egg).

Table 3 Interior quality and functional attributes* of shell eggs subjected to low-temperature, long-time immersion heating

Bath temperature (°C)	Dwell time (min)	Haugh units†	Yolk index	Albumen clarity (% Transmission at 550 nm)	Albumen pH	Whip time (min)	Whip volume (ml)
58	0	$66·8^{b} \pm 8·0$	$0·39 \pm 0·04$	$45·4^{a} \pm 11·6$	$9·1 \pm 0·0$	$1·5 \pm 0·0$	$388^{a} \pm 30$
	35	$80·7^{a} \pm 2·8$	$0·39 \pm 0·03$	$16·0^{b} \pm 7·2$	$9·0 \pm 0·2$	$16·7 \pm 10·8$	$302^{ab} \pm 6$
	57·5	$79·1^{a} \pm 6·7$	$0·40 \pm 0·02$	$8·0^{b} \pm 5·4$	$9·0 \pm 0·0$	$32·9 \pm 37·2$	$223^{b} \pm 75$
	65	$81·0^{a} \pm 4·0$	$0·38 \pm 0·02$	$5·4^{b} \pm 4·0$	$9·0 \pm 0·1$	$15·2 \pm 6·7$	$312^{ab} \pm 62$
57	0	$59·7^{b} \pm 4·0$	$0·39 \pm 0·03$	$60·7^{a} \pm 8·0$	$9·1 \pm 0·1$	$1·5^{b} \pm 0·7$	449 ± 114
	35	$78·3^{a} \pm 5·5$	$0·42 \pm 0·02$	$37·8^{b} \pm 9·6$	$9·1 \pm 0·1$	$16·1^{a} \pm 6·3$	295 ± 29
	65	$77·8^{a} \pm 7·8$	$0·38 \pm 0·02$	$20·4^{c} \pm 2·5$	$9·1 \pm 0·1$	$12·2^{a} \pm 5·5$	287 ± 37
	75	$75·2^{a} \pm 7·6$	$0·36 \pm 0·07$	$21·7^{c} \pm 5·2$	$9·1 \pm 0·1$	$10·4^{a} \pm 0·6$	334 ± 66

* Data are means ± S.D. (n = 9 eggs per treatment/time combination from three separate trials).
† The mean mass of eggs processed at 57°C was 62·8 g/egg (n = 72); the mean mass of eggs processed at 58°C was 63·1 g/egg (n = 72).
[a–c] For each bath temperature, values in a column with a different superscript are significantly different ($P \leq 0·05$).

were unaffected by immersion heating ($P > 0·05$), indicating that in-shell pasteurization processes do not adversely affect the spherical shape of the yolk. Similarly, the immersion heat processes evaluated did not yield differences ($P > 0·05$) in albumen pH values. Immersion heating at 57°C had a less adverse effect on whip volume and yielded more consistent whip times (i.e. smaller S.D.) relative to heating at 58°C (Table 3). Subsequent scale-up work on the in-shell pasteurization process has allowed identification of process and egg quality control factors which help to minimize such adverse effects on albumen quality.

DISCUSSION

The inactivation of SE within intact shell eggs via immersion heating is a conduction thermal process. Based on the *Salmonella* enumeration data obtained after heating shell eggs for up to 42·5 min at 58°C or 55 min at 57°C (Tables 1 and 2), semilogarithmic survivor curves relating the viable SE population (log cfu g^{-1}) to the heating time were plotted for each bath temperature and experimental trial (data not shown). With the omission of the initial unheated control point, linear survivor curves ($r^2 \geq 0·95$) were obtained, indi-

cating that the inactivation of yolk-inoculated SE followed first-order kinetics over the 18·5–20 min heating interval evaluated. The survivor curves obtained at 58° and 57°C traversed 3·3–5·5 log cycles and yielded apparent decimal reduction times (*D*-values) of 4·5 and 6·0 min, respectively. These *D*-values, although based on a limited number of sampling intervals, are comparable to the kinetic data reported by Humphrey *et al.* (1990). In that study, cells of *Salm. enteritidis* PT4 were suspended in blended egg yolk and dispensed into screw-capped 46 mm glass vials prior to immersion heating in a water-bath at 55° and 60°C. Approximate *D*-values of *Salm. enteritidis* PT4 at 58° and 57°C (extrapolated) were 3·7 and 6·6 min, respectively (Humphrey *et al.* 1990).

At the time the present research was designed and conducted, no studies evaluating treatments specifically designed to eliminate SE or other bacterial pathogens from internally-infected or inoculated shell eggs were available in the literature. Recently, Hou *et al.* (1996) reported that a combination of water-bath heating (25 min at 57°C) followed by hot-air heating (60 min at 55°C) reduced the viable SE population (single-strain inoculum) within inoculated shell eggs by 7 log cycles.

In the present study, several elements were incorporated into the final experimental design to provide a large safety factor with regard to *Salmonella* inactivation. Firstly, a pooled, six-strain inoculum was used to compensate for strain-to-strain variations in thermal resistance. Secondly, stationary-phase cells of SE were used in the inoculum suspension because cells in this phase of growth are generally several-fold more heat-resistant than cells harvested in the log phase (Hansen and Riemann 1963; Garibaldi *et al.* 1969b). Thirdly, the inoculum was injected at or near the geometric centre of the egg, predicted to be the slowest-heating point during immersion heating; therefore, micro-organisms located in the albumen would be subjected to an even more lethal thermal process. Lastly, inoculation into the yolk permitted a conservative estimation of process lethality to SE. The near neutral pH of the yolk and its high solids and lipid content relative to the albumen impart thermal protection to salmonellas localized in the yolk. Previous studies have demonstrated that the relative thermal resistance of *Salmonella* spp. in various egg components is as follows: resistance in plain yolk > whole egg or pH 7 egg white (supplemented with $Al_2(SO_4)_3$) > pH 9 egg white (Garibaldi *et al.* 1969b; Humphrey *et al.* 1990). For example, Garibaldi and co-workers reported that the heat resistance of *Salm. typhimurium* was 1·5 times greater in plain yolk than in blended liquid whole egg; at 54°C, this strain was 9·5 times more heat resistant in yolk than in pH 9 egg white. Recently published research indicates that when naturally- or artificially-infected hens produce eggs internally contaminated with SE, the pathogen is typically detected only in the albumen or on the outside of the vitelline membrane (Gast and Beard 1990; Humphrey 1994).

The question of what constitutes an adequate level of *Salmonella* inactivation (via immersion heating or any other pasteurization process) is closely linked to the pathogen levels expected to occur in contaminated eggs. In surveys of 46 egg processing plants, Garibaldi *et al.* (1969a) reported that, of 287 samples of commercially-broken raw liquid egg, 95 per cent contained less than one *Salmonella* cell g^{-1} and none contained >110 cells g^{-1}. Gast and Beard (1992) reported that SE populations in the contents of shell eggs laid by experimentally-infected hens averaged 220 cfu per egg on the day of lay, and 184 cfu per egg after 7 d at 7·2°C. In the light of these studies, in-shell egg 'pasteurization' treatments which effect an 8·4 to 8·5-*D* inactivation of SE would provide a wide margin of consumer safety.

Of all the egg components, the egg white is the most sensitive to heat (Elliott and Hobbs 1980). As conduction heating processes, in-shell pasteurization treatments subject the shell membranes and outer thin albumen to the most severe heating, with the minimum thermal damage predicted to occur at the geometric centre of the egg. Slosberg *et al.* (1948) reported that even momentary heating of egg white above 57·2°C resulted in a loss of foaming power. US Department of Agriculture minimum pasteurization requirements for unsupplemented liquid egg whites (3·5 min at 56·7°C; Anon 1995) have been shown to markedly increase whip times for meringue preparation (Elliott and Hobbs 1980). Hou *et al.* (1996) reported that the thermal processing of intact shell eggs resulted in altered egg white viscosity, turbidity, Hunter hue values and lysozyme activity relative to unheated shell eggs.

Due to *Salmonella* safety concerns, Grade A shell eggs are currently regarded as a 'potentially hazardous food' (FDA 1990). The present studies were designed to simulate 'worst-case' *Salmonella* contamination conditions, and thus should be useful in defining what constitutes an acceptable level of SE inactivation in immersion-heated shell eggs. Potential end-users of in-shell pasteurized eggs would include consumer households and foodservice operations interested in the preparation of items such as soft-boiled, soft-poached and 'sunny-side' fried eggs, French toast, custards, sauces (e.g. hollandaise, bernaise), Caesar salad dressing and 'home-made' ice cream and eggnog. Broken-out whole egg and yolk from in-shell pasteurized eggs could be used as ingredients in the above products while minimizing the likelihood of introducing salmonellas to finished foods or to food preparation areas via egg ingredients. Although immersion-heated albumen requires extra beating time to form meringues, independent test kitchen trials have demonstrated that the whites may be used in a variety of recipes (e.g. sponge cakes, soufflé omelettes, meringues, chiffon desserts) with flavour, texture and appearance comparable to items made using conventional Grade A shell eggs (Thoms, personal communication).

Defining the appropriate refrigerated shelf-life for in-shell pasteurized eggs is a final important product development issue. Research in our laboratories has demonstrated that the interior quality (albumen pH, Haugh units, albumen foaming power) of immersion-heated eggs (with or without oiling) did not deteriorate further during 4 weeks of storage at 7° or 22°C (unpublished data). Physicochemical, sensory and microbiological tests should also be used to define the refrigerated shelf-life of in-shell pasteurized eggs (a 'keep refrigerated' statement and consumer 'use-by' dates would also need to be provided on the external packaging). With the incorporation of sensory and shelf-life studies of this type, the production of *Salmonella*-free shell eggs for the foodservice and consumer egg markets appears to be an achievable goal.

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Thermal Inactivation Kinetics of *Salmonella* spp. within Intact Eggs Heated Using Humidity-Controlled Air

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ABSTRACT

The heat resistance of six strains of *Salmonella* (including Enteritidis, Heidelberg, and Typhimurium) in liquid whole egg and shell eggs was determined. Decimal reduction times (*D*-values) of each of the six strains were determined in liquid whole egg heated at 56.7°C within glass capillary tubes immersed in a water bath. *D*-values ranged from 3.05 to 4.09 min, and significant differences were observed between the strains tested ($\alpha = 0.05$). In addition, approximately 7 $\log_{10}$ CFU/g of a six-strain cocktail was inoculated into the geometric center of raw shell eggs and the eggs heated at 57.2°C using convection currents of humidity-controlled air. *D*-values of the pooled salmonellae ranged from 5.49 to 6.12 min within the center of intact shell eggs. A heating period of 70 min or more resulted in no surviving salmonellae being detected (i.e., an 8.7-log reduction per egg).

Despite decades of research and attempted control programs, salmonellosis continues to be one of the most prevalent forms of bacterial foodborne illness. It is estimated that 1,412,498 cases of salmonellosis occur each years in the United States *(11)*. Although a variety of salmonellae serotypes can be associated with eggs, not all are equally responsible for salmonellosis associated with these foods. In recent years, it has become apparent that *Salmonella* serotype Enteritidis is the serotype most often associated with salmonellosis, particularly with egg products *(10)*. From 1985 to 1998, 796 outbreaks of *Salmonella* Enteritidis, involving 28,689 illnesses, 2,839 hospitalizations, and 79 deaths, were reported to the Centers for Disease and Prevention *(3)*. Indeed, the percentage of clinical *Salmonella* isolates associated with foodborne disease outbreaks and identified as *Salmonella* Enteritidis has increased from approximately 5% in 1976 to 26% in 1994 *(2)*, and this serotype accounted for 55% of all foodborne salmonellosis cases in 1997 *(4)*. Foods of animal origin are responsible for many of the cases, but an estimated 82% of the *Salmonella* Enteritidis outbreaks are specifically associated with grade A shell eggs or foods containing raw or undercooked eggs *(3)*.

Traditional recommendations for reducing salmonellae in eggs have focused on eliminating contamination of external surfaces of eggshells. However, *Salmonella* Enteritidis has been isolated from internal portions of hen eggs *(8)*, and evidence suggests transovarian infection of eggs by this bacterium *(8, 9)*. It is estimated that the rate of transovarian contamination by *Salmonella* Enteritidis is about 1 in every 20,000 eggs produced in the United States. Although this rate is quite low, there are definite regional and producer-to-producer variations in risk. In addition, considering that an estimated 47 billion shell eggs are sold each year in the United States *(13)*, as many as 2.3 million *Salmonella* Enteritidis–contaminated shell eggs could reach consumers.

The fact that raw or undercooked eggs are responsible for a disproportionate number of *Salmonella* Enteritidis illnesses has prompted strong governmental action to reduce and ultimately eliminate egg-borne salmonellosis. In 1999, The President's Council on Food Safety announced an action plan to eliminate *Salmonella* Enteritidis illnesses due to eggs *(13)*. The plan will use two interrelated strategies to accomplish this goal. The first is to improve *Salmonella* Enteritidis testing on the farm to minimize the spread of the organism in layer flocks. The second strategy is to identify lethal treatments that can be applied to eggs and egg products that would kill any *Salmonella* Enteritidis that might remain in the eggs. Standards exist *(17)* for the pasteurization of liquid egg products, which are primarily used in food service and food processing. However, about 70% of the eggs produced in the United States are sold as shell eggs. Hence, it would be prudent to develop techniques that could also eliminate *Salmonella* Enteritidis in these products.

A variety of potential processing techniques for the reduction or elimination of salmonellae from shell eggs have been described. The pasteurization of intact shell eggs by immersion in hot water and air ovens has received the most attention. Stadelman et al. *(15)* subjected eggs inoculated with *Salmonella* Enteritidis to heated air or heated water and found that the time required for heated air or water to eliminate *Salmonella* Enteritidis was too long to be of practical use to the egg industry. In a separate publication *(7)*,

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however, this same research group found that exposing eggs to hot (57°C) water for 25 min followed by exposure to 55°C air for 60 min was able to effect a 7-log reduction of *Salmonella* Enteritidis in shell eggs. More recently, Schuman et al. *(14)* described the ability of a water immersion heat treatment of 58°C for 65 to 75 min to provide an 8.4-decimal process for shell eggs. These investigators demonstrated that by adjusting the hold time under optimized conditions, various degrees of process lethality may be delivered to the center of intact shell eggs, while preserving the essential quality attributes of a fresh shell egg. Regulations in many countries outside the United States currently prohibit the spray washing or immersion of shell eggs intended for the commercial table egg market. Hence, additional research to identify egg processing approaches that effectively pasteurize shell eggs without the use of water immersion was of interest. The overall objective of the experiments described herein was to determine the efficacy of heated air processing as a means to effectively eliminate salmonellae within intact shell eggs. Preheating and holding of the intact eggs were accomplished using a convection oven capable of maintaining relatively high humidity conditions.

MATERIALS AND METHODS

Experimental design. The overall research project was divided into two separate but interrelated phases. In phase 1, we determined the decimal reduction times (*D*-values) of six strains of *Salmonella* in liquid whole egg (LWE) in glass capillary tubes at temperatures to be tested in phase 2 of the project. In phase 2, the thermal inactivation kinetics of a six-strain cocktail of salmonellae in shell eggs was determined using a humidity-controlled convection heating system.

Heating menstrum: LWE. Ultrapasteurized LWE from a recent production lot was supplied by Michael Foods, Inc. (Gaylord, Minn.). On the day of testing, duplicate samples of uninoculated LWE were analyzed for pH (Accumet model 15 with Accumet flat surface polymer combination body electrode, catalog no. 13–620–289, Fisher Scientific, Pittsburgh, Pa.), total solids (Association of Official Analytical Chemists official method 925.30), standard plate counts (plate count agar, Becton Dickinson Microbiology Systems, Becton Dickinson and Co., Sparks, Md.), and total coliform counts (VRBA agar, Difco Laboratories, Detroit, Mich.).

Heating menstrum: shell eggs. Nest-run shell eggs were obtained from a local single flock within 24 h of being laid. Eggs were sorted to obtain eggs weighing 62 ± 2 g each. Eggs were washed in warm, soapy tap water until the shell surfaces were free of visible debris, rinsed by immersion in a beaker of ambient temperature sterile water, and allowed to air dry on plastic egg trays (flats) for 2 h. Eggs were then inspected to remove any eggs with visible cracks or checks. The eggs were stored overnight at room temperature (22°C) so that the age of eggs at the time of heating was approximately 48 h after lay. As an indicator of egg freshness, the separated whites (albumen) of three uninoculated eggs were collected and gently blended and the pH determined on the day of egg inoculation or heating.

Cultures: *Salmonella* strains. Six *Salmonella* strains were used in this study: *Salmonella* Enteritidis Benson-1 (phage type 8, human clinical), *Salmonella* Enteritidis ME-14 (phage type 14B, poultry manure), *Salmonella* Enteritidis ME-18 (phage type 14B, live poultry), *Salmonella* Heidelberg UN-L (unknown source), *Salmonella* Enteritidis H3353 (phage type 4, egg), and *Salmonella* Typhimurium (DT104, human clinical). All cultures were maintained on tryptic soy agar (TSA; Difco) slants at 4°C. Cultures were activated in tryptic soy broth (TSB; Difco) at 37°C at least twice for 24-h periods before being used. Strains H3353, DT104, and UN-L were provided by Dr. Larry Beuchat at the University of Georgia Center for Food Safety and Quality Enhancement and strains Benson-1, ME-14, and ME-18 were provided by Dr. James Schuman of Michael Foods.

Culture preparation: LWE. Twenty-four-hour cultures of each strain were sedimented by centrifugation (approximately 1,800 × *g*, 10 min) (IEC Clinical Centrifuge, International Equipment Co., Needham Heights, Mass.), and pellets were washed once in 0.1% peptone water (Difco) and recentrifuged. Each cell pellet was resuspended and mixed well in 10 ml of LWE to yield an initial inoculum level of approximately 6.8×10^8 CFU/ml of LWE. During preliminary work, each culture was streaked for isolation onto xylose lysine desoxycholate (XLD; Difco) agar to verify colony morphology typical of *Salmonella*.

Culture preparation: shell eggs. An inoculum cocktail consisting of the six strains described in phase 1 was used to inoculate the eggs. Working stock cultures were prepared by transferring a loopful of each culture to 10 ml of TSB and incubating at 37°C for 24 h. Sixty microliters of each 24-h culture was added to a centrifuge tube containing 30 ml of TSB and incubated at 37°C for 24 h. The 30-ml cultures were centrifuged for 15 min at 9,000 × *g* (4°C), and the cell pellets were each resuspended in 3 ml of sterile 0.1% (wt/vol) peptone water and pooled into a single sterile test tube to yield the final six-strain inoculum suspension (9.5 to 10.8 log CFU ml^{-1}).

Thermal inactivation in capillary tubes. Fifty microliters of each strain was dispensed into sterile capillary tubes (Kimax-51, 0.8 to 1.10 by 90 mm, Kimble Products, Vineland, N.J.) using a 1-ml syringe (Becton Dickinson) with a 22-gauge, 4-in. hypodermic needle with deflection point (Popper & Sons, Inc., New Hyde Park, N.Y.). The capillary tubes were then heat sealed and stored in an ice slurry for up to 2 h before use. Ten minutes before heating, each set of capillary tubes was brought to room temperature by immersion in a tray of ambient temperature water (approximately 22°C). Sets of tubes were then fully immersed in a preheated circulating water bath (Thermomix 1480, B. Braun, Melsungen, West Germany) at 56.7°C (134°F). For each trial, a thermocouple was placed in a capillary tube of uninoculated LWE to document come-up times and actual egg temperatures. At 5-min intervals, duplicate tubes were removed from the bath and rapidly cooled in an ice water slurry and held for up to 1 h before plating. Tubes were removed at 5, 10, 15, 20, and 25 min after reaching the time-zero target temperature. Two experimental trials were conducted for each of the six *Salmonella* strains.

Thermal inactivation in shell eggs. Sixty eggs were placed into two 30-egg-capacity perforated plastic egg flats with the blunt end of the egg facing up. The eggs in one flat were each inoculated by placing 50 μl of concentrated, pooled *Salmonella* suspension into the geometric center of the egg, yielding a final inoculum of approximately 5×10^8 CFU per egg. This was accomplished by placing a piece of high-adhesive, moisture- and heat-resistant tape (Anchor Continental Inc., Columbia, S.C.) over the blunt end and perforating the eggshell through the tape using a 2.54-cm, 23-gauge sterile needle (Becton Dickinson, Franklin Lakes, N.J.) coupled to a calibrated 50-μl repeating syringe (Hamilton Co., Reno,

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Nev.). Inoculated eggs were held at room temperature for less than 1 h before heating. Preliminary trials using a dye placement technique as described by Chantaraponanont et al. *(5)* were done to confirm that cultures were inoculated into the geometric center of eggs. Briefly, this technique involves injecting 50 μl of dye into the yolk followed by a standard hard-cooking procedure to demonstrate consistent placement of the dye near the center of the yolk with no detectable inoculum drift.

The second flat of eggs was used to record the temperature of the eggs during heating. Tape was affixed to six eggs. An empty egg flat was placed over the eggs, and type T hypodermic rigid thermocouple probes (38 by 1.6-mm diameter, Omega, Stamford, Conn.) were placed into the six eggs and secured to the top flat to measure the temperature at the geometric center of control eggs. The temperature data were collected at 1-min intervals via a HOTMUX Temperature Data Logger (DCC Corporation, Pennsauken, N.J.) connected to a personal computer. The flat of eggs with the thermocouples was placed at the right back corner of a Combitherm Model 7.14G oven (Alto-Shaam Inc., Menomonee Falls, Wis.). The flat of inoculated eggs was placed in the left front corner of the oven. The oven was set on "Biosteam" mode at a temperature of 57.2°C (135°F) and started. This oven setting optimizes the relative humidity level in the chamber to efficiently heat foods at temperatures below 100°C. Sets of three eggs were randomly removed from the oven for analysis after each of the following treatment times: at 0 min (unheated controls) and after 30, 40, 50, 60, 70, and 80 min of heating. At each of these times, three inoculated eggs were removed from the oven and immersed in a beaker containing 1 liter of ambient sterile water, stirred, and permitted to cool for 5 min. Two independent trials were performed.

Micobiological analysis of LWE. Tubes were briefly immersed in 70% ethanol and allowed to dry before transferring to individual sterile test tubes containing 9.9 ml of 0.1% peptone water (pH 7.0) and crushed using a sterile glass rod. The test tubes containing the crushed capillary tubes were vortexed after which 1:10 serial dilutions were prepared in peptone water. One milliliter of each serial dilution was placed onto duplicate TSA plates and uniformly distributed using sterile bent glass rods. TSA plates were incubated for 48 h at 35°C, and colonies were counted to determine surviving CFU/ml of liquid egg. For the latter two dwell times yielding survivors, one representative colony from each countable (30 to 300 colonies) plate was streaked for isolation onto XLD agar and incubated for 48 h at 35°C to verify its identity as *Salmonella*.

Micobiological analysis of shell eggs. After 5-min cooling in ambient temperature (approximately 22°C) water, the eggs were wiped dry with clean tissues and aseptically cracked open (sterile knife edge) and the contents broken into a sterile stomacher bag. The egg contents were then diluted 1:10 (wt/wt) with chilled (4°C) sterile lactose broth (Difco) and blended for 30 s using a stomacher 400 (Seward, London, UK). Samples were serially diluted in peptone broth, and 1.0 ml of each dilution was placed onto TSA and uniformly distributed using sterile bent glass rods. Plates were then incubated at 35°C for 48 h. After incubation, plates with between 30 and 300 colonies were replica plated onto XLD agar as described by Michalski et al. *(12)*. Plates were incubated at 35°C for 24 h at which time colony morphology was confirmed as typical for *Salmonella*.

All remaining egg and lactose broth mixture not used in plating was incubated for 24 h at 35°C to determine presence of viable salmonellae *(1)*. Selective enrichment was carried out by transferring 1 ml of enrichment to 10 ml of selenite cystine (Difco) broth (35°C). A loopful of each selenite cystine enrichment was streaked for isolation onto XLD agar (35°C, 24 h) to determine whether viable salmonellae were present. All suspect colonies were further confirmed on triple sugar iron (Difco) agar slants.

TABLE 1. *Salmonella spp.* D-*values at 56.7°C*

Salmonella strain	*D*-value
Salmonella Enteritidis Benson-1	3.45 AB[a]
Salmonella Enteritidis ME-14	4.09 A
Salmonella Enteritidis ME-18	3.82 AB
Salmonella Heidlberg UN-L	3.05 B
Salmonella Enteritidis H3353	3.16 B
Salmonella Typhimurium DT104	3.13 B

[a] Values followed by the same letter are not significantly different ($P > 0.05$).

Statistical analysis. Populations were converted to $\log_{10}$ values and plotted versus time (min or s). A commercial spreadsheet program (Microsoft Excel version 7.0) was used to perform analyses of variance and linear regression; *D*-values were calculated as the negative reciprocal of the slope of the thermal inactivation curves. The correlation coefficient (r^2) was 0.97 or greater for all trials, indicating that the inactivation curves (log number versus time) were linear and *D*-values could be calculated directly from them.

RESULTS AND DISCUSSION

Egg products used in these experiments varied slightly in composition but in all cases were within normal ranges for fresh eggs. The pH of LWE used with strains ME-14, ME-18, UN-L, and H3353 was 7.40 and total solids were 23.95%. The pH was 7.07 and the total solids were 23.63% for eggs inoculated with *Salmonella* Enteritidis Benson-1 and *Salmonella* Typhimurium DT104. Plating the LWE onto plate count agar and VRBA demonstrated that the background microflora in the ultrapasteurized liquid egg product was minimal (<10 CFU g^{-1}).

The thermal resistance of individual strains in LWE also varied slightly. The *D*-values for salmonellae heated at 56.7°C (134°F) in capillary tubes ranged from 3.05 to 4.09 min, depending on the strain (Table 1). The determination of *D*-values in LWE provided a simple and easily reproducible means of comparing the relative heat resistance of the strains in the pooled inoculum used in phase 2. Very few data exist in the published literature on the heat resistance of salmonellae in LWE heated at temperatures as low as 56.7°C. Data published by the U.S. Department of Agriculture suggest a *D*-value of 2.25 min in LWE at this temperature *(17)*. The method by which *D*-values are determined can have profound effects on the values collected. The use of large volumes of samples or slow heating can result in longer come-up and come-down times. Donnelly et al. *(6)* observed that the use of capillary tubes as sample vessels enabled essentially instantaneous heating and cooling, resulting in very precise control over the amount of heat to which cells were exposed. More recently, Michalski et al. *(12)* used capillary tubes to more precisely determine the *D*-values of salmonellae in LWE and found *D*-values of about 1.5 min at 58°C. Results of experiments described

TABLE 2. *Thermal inactivation of* Salmonella *spp. in shell eggs subjected to low-temperature, long-time heating at 57.2°C in a humidity-controlled convection heating system*

Total process time (min)	Internal temperature[a] (°C)		Survivors (log CFU g^{-1})		Viable *Salmonella* present[b]	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
0	23.25	22.94	6.98	7.01	3/3	3/3
30	56.12	56.18	6.13	6.21	3/3	3/3
40	56.65	56.62	5.11	5.05	3/3	3/3
50	57.05	56.82	2.82	3.43	3/3	3/3
60	56.85	56.76	0.82	1.30	1/3	3/3
70	56.98	56.98	<1.0	<1.0	0/3	0/3
80	56.98	56.88	<1.0	<1.0	0/3	0/3

[a] The internal temperature for trial 2 is the average of five eggs. Egg no. 6 was omitted because the thermocouple was pulled out at the beginning of the heating cycle.

[b] Samples positive by enrichment/samples tested. The mean mass of internal egg contents was 52 g per egg. Therefore, a negative enrichment test represents an 8.7-decimal process per egg.

in this article, which used a similar procedure as Michalski et al. *(12)*, indicated a mean D_{57}-value of about 3.4. It is also important to be sure that both injured and fully viable cells are enumerated. Both Michalski et al. *(12)* and the experiments described herein used a nonselective medium for recovery to be sure that the exclusion of injured cells was minimized.

Table 2 presents the results of shell egg pasteurization experiments conducted using a humidity-controlled convection heating system. During the first 40 min of processing, a 2-log reduction of viable salmonellae was observed. Heating eggs at 57.2°C for total process times of 60 and 70 min in a humidity-controlled convection heating system enabled the destruction of 6 and 7 log CFU g^{-1} salmonellae, respectively. Moreover, we were unable to recover any viable salmonellae from eggs heated for 70 min (ie, an 8.7-D log pasteurization process per egg; Table 2).

Microbial populations of salmonellae recovered from shell eggs heated in a humidity-controlled convection heating system were converted to $\log_{10}$ values for each sampling time (Table 2). The *D*-values for the inoculum pool were calculated as the negative reciprocal of the slope of the survivor curve obtained by linear regression analysis (slope only includes those populations determined after the eggs had reached the target heating temperature). The mean *D*-values for trials 1 and 2 were 5.49 (r^2 = 0.98) and 6.12 (r^2 = 0.98), respectively. These results were consistent with the *D*-value data reported by Schuman et al. *(14)* for intact shell eggs pasteurized using a water immersion process. These prior investigators reported a mean *D*-value of 6.0 min for *Salmonella* Enteritidis strains inoculated into the center of shell eggs heated by immersion in a 57.0°C (134.6°F) circulating water bath. Although Stadelman et al. *(15)* and Hou et al. *(7)* did not specifically calculate *D*-values, these research groups implied or presented *D*-values for *Salmonella* Enteritidis of approximately 4.5 min when heated at 57°C within intact shell eggs. However, precise *D*-values can be difficult to determine in this type of research, since the inoculated eggs are typically subjected to a long (24- to 35-min) come-up time, and the variability introduced by using cocktail of various salmonellae precludes precise determinations of *D*-values.

During the last decade, there has been a growing interest on the part of the egg industry, regulatory agencies, and consumers to identify means to more effectively address *Salmonella* food safety concerns linked to uncooked or undercooked shell eggs. This has spawned development work on a variety of egg processing technologies intended to produce safer eggs. The U.S. Food and Drug Administration, in conjunction with the U.S. Department of Agriculture, Agricultural Marketing Service, has established a requirement that to be designated as "pasteurized," intact shell eggs must be subjected to a process that yields a minimum 5-log reduction of viable salmonellae *(16)*. The heating system described in the present research provides a suitable system to accomplish a 5-log or greater pasteurization process for shell eggs, while providing an alternative to processes that rely on water immersion as a means to heat and maintain the temperature of the eggs.

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ORIGINAL ARTICLE

Pasteurization of intact shell eggs

H. Hou, R. K. Singh, P. M. Muriana* and W. J. Stadelman

The feasibility of pasteurization for destruction of Salmonella enteritidis *in artificially-inoculated intact shell eggs was investigated. Water-bath and hot air ovens were used for pasteurization of intact shell eggs under different conditions using these two heating methods, either individually or in combination. Eggs pasteurized in a 57°C circulating water-bath for 25 min gave reductions in* S. enteritidis *ATCC 13076 of about 3 log cycles whereas heating at 55°C in a hot air oven for 180 min gave a 5 log reduction of* S. enteritidis. *A combination of the two methods (water-bath heating at 57°C for 25 min followed by hot-air heating at 55°C for 60 min) produced 7 log reductions in* S. enteritidis *ATCC 13076 in shell eggs. Examination of lysozyme activity and other physical properties of egg white upon heating indicated that the overall functionality of pasteurized shell eggs are acceptable under the heating conditions defined in this study. This process may be used to produce safe, pasteurized shell eggs.*

Introduction

Salmonella enteritidis is recognized as an egg-associated pathogen that has been implicated in foodborne disease outbreaks in the United States and other countries (Humphrey et al. 1991a). *S. enteritidis* infections have shown a dramatic increase because of the consumption of raw egg or undercooked egg-containing foods. Considering that more than 65 billion fresh shell eggs are sold annually in the United States, shell eggs may pose a serious health hazard to humans if they are contaminated with *Salmonella* (Mishu et al. 1991). *S. enteritidis* may originate from transovarian transmission *in utero* to the developing preovulatory follicles or by trans-shell transmission, in which *Salmonella* gains access to the egg by penetration into the shell through the cuticle (Barnhart et al. 1991, Board and Fuller 1994, Fajardo et al. 1995, Saeed and Koons 1993). The USDA has regulations on the requirement of pasteurization and inspection of liquid eggs and egg products (7CFR59, 21CFR160) and processes for batch, HTST, ultrapasteurization, and the use of chemical preservatives have been defined (Brant et al. 1968, Sourky et al. 1971, Swartzel et al. 1989). Although there are several methods of preservation and sanitation of shell eggs such as washing, rapid chilling, irradiation and ultrasonic treatment, they do not destroy *Salmonella* which is harbored inside shell eggs (Catalano and Knabel 1994). Ionizing radiation (6 kGy) has recently been used to produce *Salmonella*-free eggs but this process also lowered the quality of the irradiated eggs (Vanlith et al. 1995).

Pasteurization is an effective method to destroy *S. enteritidis* in liquid eggs but it has not been applied to shell eggs. In intact shell eggs, heat could cause quality changes in the egg components. The functional properties of

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eggs (coagulation, foaming, and emulsification) are mainly a result of its complex protein content (Herald and Smith 1988, Shimada and Matsushita 1980). Some of these proteins can be denatured at a temperature as low as 60°C. Therefore, the preservation of shell eggs by heat is a difficult task because destruction of the microbial load implies loss of functional properties. The purpose of this project was to develop a process for gentle pasteurization of shell eggs to kill *S. enteritidis* using water-bath and hot-air-oven heating systems, and to investigate quality and functional properties of the egg components in pasteurized shell eggs.

Materials and Methods

Preparation of Salmonella enteritidis

Salmonella enteritidis ATCC 13076 was obtained from the American Type Culture Collection (Rockville, MD, USA). In order to obtain a streptomycin-resistant (str^r) isolate for use in our shell egg pasteurization studies, 0·1 ml of an overnight culture of *S. enteritidis* was plated onto tryptic soy (TS; Difco Laboratory, Detroit, MI, USA) agar plates containing 50 μg ml^{-1} streptomycin sulfate (Fisher Biotech, Fairlawn, NJ, USA) and incubated at 37°C. A str^r colony (50 μg ml^{-1}) was streaked onto TS agar containing 100 μg ml^{-1} streptomycin. A resulting str^r colony was cultured in TS broth and maintained as a frozen stock (–20°C) by freezing in double-strength TS broth containing 10% glycerol. A working culture was obtained by inoculating 0·1 ml glycerol stock culture into 10 ml TS broth and incubating at 37°C for 24 h.

Inoculation of shell eggs

Rather than using cells suspended in a buffer/aqueous medium for inoculation, *S. enteritidis* ATCC 13076 (str^r) cells were resuspended in egg yolk (*c.* 10^6–10^7 cfu ml^{-1}) for injection into yolks of test eggs. Shell eggs and a 100 μl gas-tight glass syringe were sanitized with 70% ethanol. A small puncture hole was made on the blunt end of the shell eggs and 50 μl of the *S. enteritidis* ATCC 13076-egg yolk mixture was injected into the yolk of each egg; the hole was then sealed with fast drying DUCO cement (Devcon Corporation, Wood Dale, IL, USA) and the eggs were held on ice. When the glue dried, the eggs were pasteurized at specified conditions and then plated to determine residual *S. enteritidis* ATCC 13076 cell counts after pasteurization. Eggs were sanitized and aseptically broken into sterilized beakers containing sterile magnetic stir bars; the liquid whole eggs were then mixed for 5 min on a magnetic stirrer (Lab-line Inc., Melrose Park, IL, USA). Finally, serial dilutions (0·1% Bacto-peptone, Difco) were made and surface-plated in duplicate onto TS agar (+100 μg ml^{-1} streptomycin). The plates were incubated at 37°C for 2–3 days and colony forming units (cfu) were enumerated.

Each pasteurization data point represents the mean of a triplicate series of inoculated shell eggs that were sampled and plated in duplicate. In addition to the inoculated eggs used for graphical data points, several negative and positive controls were also examined. The temperatures of two uninoculated eggs (temperature control) were recorded before, and during, each pasteurization period in order to confirm the temperature profile of the experimental eggs. Temperatures were recorded with a digital thermometer (Model HH 81 & HH 82, Omega Engineering, Inc., Stamford, CT, USA) with a fine-wire probe. Each pasteurization experiment also included: (1) three inoculated eggs plated without heating to demonstrate the initial levels of *S. enteritidis* ATCC 13076 (recovery control), and (2) three uninoculated (unheated) eggs plated to demonstrate the absence of str^r cells in our test eggs.

Pasteurization equipment and conditions

Two types of equipment were used: a water-bath set at 57°C (Blue Electric Company, Blue Island, IL, USA) with a water circulating pump (Little Giant Pump Company, Oklahoma City, OK, USA); and a hot air oven set at 55°C with a forced-air circulating fan (Thelco, Model 26, Precision Scientific, Co.,

Springfield, MO, USA). Preliminary experimentation indicated that 57°C for the water bath and 55°C for the hot air oven were maximum temperatures that could be used without denaturing the egg components. Water-bath heating times of 10, 15, 20 or 25 min were used. The hot air oven heating times were 1, 2, or 3 h. Eggs were immediately cooled on ice after pasteurization.

Heating procedures and pasteurization evaluation

Three heating methods were used: hot air heating, water-bath heating, and a combination of the two methods in which water-bath heating was used first followed immediately by transfer and continued heating in a hot air oven. One dozen inoculated shell eggs were pasteurized by each method for each holding time. Temperatures were recorded from the center of the egg yolk (coldest point) with a digital thermometer after making a small hole for insertion into the blunt end of the controlled egg. Heated eggs were collected at specific time intervals, broken aseptically and plated to enumerate surviving *S. enteritidis* ATCC 13076. The data were used to plot cfu ml^{-1} and temperature vs time curves to provide information on the effectiveness of the time–temperature regimen on *S. enteritidis* ATCC 13076-inoculated shell eggs.

Functionality tests on pasteurized intact shell eggs

The Haugh unit represents the height of the egg white after breaking the shell and pouring it on to a glass plate. This was measured with a commercial micrometer attached to a tripod (Mattox & Moore, Indianapolis, IN, USA). The Haugh unit indicates the relationship of the height of the egg white to the weight of the egg (Stadelman 1990) and is a measurement of albumen quality; as albumen quality decreases, the Haugh unit decreases.

The pH of the egg white was measured by a pH meter (Chemcadet, Model 5984-50, Cole Palmer Inc., Chicago, IL, USA) after eggs were pasteurized and held at 4°C overnight.

The viscosity of the egg white is indicative of protein changes that may occur during heating. Viscosity was measured with a Brookfield digital viscometer (Brookfield Engineering Laboratories, Inc., Stoughton, MA, USA). The viscometer was set at 30 r min^{-1}, spindle number 31 and the measurement was recorded after 1 min at a constant temperature of 20°C. Before measuring, the egg white was mixed with a wooden tongue depressor in order to homogenize the thick and thin sections of egg white.

The turbidity of the egg white was used to determine the thermocoagulation of egg albumen. Turbidimetric measurements were done on a spectrophotometer (Milton Roy Co., Rochester, NY, USA) at 600 nm using water as a standard (Shimada and Matushita 1980). An increase in the turbidity of egg white correlated with an increased absorbance and a decrease in the opalescence of the albumen.

Yolk index is defined as the height of the yolk divided by the width of the yolk (Stadelman 1990). Similar to the Haugh unit, the yolk index decreases with a decrease in egg quality. The yolk index reflects the spherical shape of egg yolk.

The exterior L, a, b color values of egg white and egg yok, before and after heating, were measured with a Hunterlab colorimeter (Model D25-PC2, Hunter Associates Laboratory, Reston, VA, USA). Four measurements were done on each 30 ml sample. The values of hue, chroma and delta E (ΔE) were calculated as follows (Clydesdale and Francis 1975):

$$\text{Hue}=\tan^{-1}(b/a)$$
$$\text{Chroma}=(a^2+b^2)^{1/2}$$

and

$$(\Delta E=(\Delta L^2+\Delta a^2+\Delta b^2)^{1/2})$$

where, ΔL, Δa, Δb are the differences of the measured L, a, b values from the Hunterlab standard yellow color plate (#C2-30638) and the L, a, and b values for egg white and egg yolk, respectively.

The effect of heat treatment on foaming ability and foam stability was also examined by a modified method of McKellar and

Stadelman (1955). Three eggs were broken, and the egg white was separated from the egg yolk with an egg-yolk separator. One hundred milliliters of egg white from control (unheated) or from heated eggs were gathered into a 600 ml plastic beaker. The egg white was mixed with an electrical egg mixer (Black & Decker Inc., Denver, CO, USA) set at the lowest position for 3 min. A ruler was then used to measure the height of the foam as an indicator of the foaming ability. The unfoamed liquid was immediately transferred to a 100 ml graduated cylinder by pouring through a funnel. The volume of the liquid was recorded as drainage volume (ml). Periodically (2–3 min) additional liquid was poured from the plastic beaker where the egg white was mixed into the measuring cylinder and the total volume was continuously recorded. The foaming stability is represented by the volume of egg white remaining in the foam phase relative to control eggs.

Lysozyme activity of egg white

Micrococcus lysodeikticus ATCC 4698 was used to study lysozyme activity (Worthington 1990). The OD at 450 nm of a cell suspension of *M. lysodeikticus* cells was observed after adding lysozyme. The rate of change in OD of the cell suspension was used to determine the units of lysozyme activity in the test samples relative to standard lysozyme solutions.

A 48 h *M. lysodeikticus* ATCC 4798 TS broth culture was harvested by centrifugation (15 000 *g*, 10 min, 10°C) and resuspended with 0·1 M sterilized potassium phosphate buffer (pH 7·0) and diluted to a concentration that gave an OD of about 0·8 at 450 nm. Standard lysozyme solutions were prepared from crystallized lysozyme (Sigma Co., St. Louis, MO, USA) with sterile glass-distilled water. The assay temperature was kept at 25°C. The lysozyme solutions were kept on ice until assayed. Egg albumen was obtained by separating white from yolk and removing the chalazae with a clean tweezer and the thick and thin portions were homogenized with a wooden tongue depressor. Egg white (1 ml) was diluted with sterile glass-distilled water. A 2·9 ml sample of cell suspension was pipetted into a cuvette and incubated for 4–5 min in order to achieve temperature equilibration. Then, 0·1 ml of an appropriately diluted enzyme or egg white sample was added to the cuvette and the OD at 450 nm was recorded. The slope of the initial linear portion of the curve was calculated by regression analysis. The lysozyme activity in 0·1 ml egg sample was calculated by Eqn (1):

$$\text{Lysozyme activity (Enzyme Units)} = \frac{\text{Rate in egg solution } (\Delta OD_{450}/\text{min}) \times \text{lysozyme std. units}}{\text{Rate in lysozyme std. solution } (\Delta OD_{450}/\text{min})}$$

Storage stability of pasteurized intact shell eggs

Two experimental approaches were examined to test the storage stability of intact shell eggs. In one approach, a triplicate series of shell eggs inoculated with *S. enteritidis* ATCC 13076 were pasteurized by the water-bath–hot-air-oven combination method, cooled on ice, and stored at 4°C for up to 30 days; during this period, each series of eggs were sampled weekly and plated on TS agar (plus streptomycin). In another approach, uninoculated shell eggs were subjected to microbial surface contamination by immersion in a slurry of micro-organisms in floor dust and debris collected from the floors of hen houses (10 g dust+90 g water) and then pasteurized by the combination method. The shell eggs were immersed in the slurry for 5 min, blotted dry, pasteurized by the combination method, stored for up to 30 days as above, and sampled weekly on TS agar.

Statistical analysis

All data in this study was obtained from a triplicate series of experimental runs. Statistical Analysis Software (SAS 1988) was used for analysis of variance at 95% confidence limits.

Destruction of S. enteritidis

Figs 1, 2, and 3 show the destruction of *Salmonella* and related temperature profiles using three different pasteurization methods. The maximum destruction of *S. enteritidis* ATCC 13076 in shell eggs by water-bath

heating was approximately 3 log cycles (Fig. 1) whereas a 5 log cycle reduction in *S. enteritidis* ATCC 13076 was obtained by hot air heating (Fig. 2). A combination of water-bath and hot air heating achieved a 7 log reduction of *S. enteritidis* ATCC 13076 without 'cooking' the eggs (Fig. 3).

In a 57°C water-bath (Fig. 1), the temperature of the yolk increased rapidly from 4·0°C to 53°C in the first 15 min and then increased slowly to 56°C by 20 min. The microbial counts of the inoculated egg samples shows low destruction rates for the first 15 min and then a high level of kill between 15–25 min (53–56°C). *Salmonella* destruction can be divided into two phases: first is the lag phase in cell destruction during the temperature 'come-up' phase (from 0–15 min) and the second is the logarithmic decay phase (from 15–25 min) once lethal temperatures are attained. During the temperature increase phase, cell destruction is low (<1 log cycle reduction), whereas during the second phase the temperature increased to 56°C and further holding for 10 min gave a 2–2·5 log reduction of *S. enteritidis* ATCC 13076. The use of a 57°C water-bath gave a maximum temperature increase without protein denat-

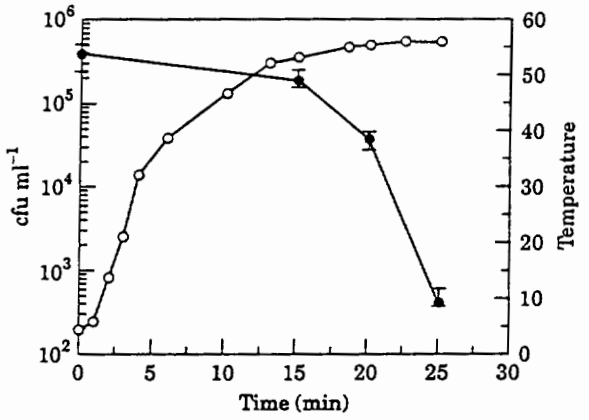

Figure 1. Microbial count reduction of *Salmonella enteritidis* ((-●-) cfu ml^{-1}) and temperature ((-O-) °C), increase in shell eggs during heating with 57°C hot water for up to 25 min. Each data point represents the mean of triplicate determinations. High and low values are represented by error bars.

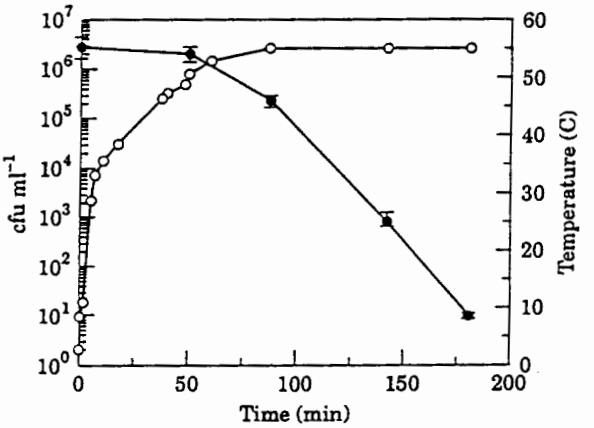

Figure 2. Microbial count reduction of *Salmonella enteritidis* ((-●-) cfu ml^{-1}) and temperature ((-O-) °C) increase in shell eggs during heating with 55°C hot air for up to 3 h. Each data point represents the mean of triplicate determinations. High and low values are represented by error bars.

uration for up to 30 min. Prior exploratory work indicated that extended incubation times for more than 30 min often resulted in denatured egg white proteins as a result of the egg white reaching a critical temperature of 57°C (data not shown). Therefore, the maximum allowable destruction of *S. enteritidis* ATCC 13076 in shell eggs by water-bath heating without egg white denaturation was approximately 3 logs (Fig. 1).

A hot air oven was also examined as an alternative heating medium. In a 55°C hot air oven (Fig. 2), the temperature of the yolk increased from 4·0–50°C in 50 min and then it increased slowly to 55°C by 180 min. The microbial counts of egg samples shows low destruction rates up to 50 min, but significant microbial reduction between 50–180 min. Cell destruction can again be divided into two phases: the lag phase from 0–50 min followed by the logarithmic decay phase from 50–180 min after lethal temperatures were reached and maintained. The maximum destruction of *S. enteriditis* ATCC 13076 in shell eggs attained by hot air heating was a 5 log reduction (Fig. 2). Differences in the heating properties of the water-bath and hot air oven are because of the type of heat transfer. In the water-bath, heat is transferred from hot water to the egg shell via convection and then by conductive heat transfer from outside to inside the egg. The hot air oven heats by convection and radiation heat transfer to the surface of the egg and then via conduction within the egg.

Although the hot air oven was more effective in reduction of *S. enteritidis* ATCC 13076, the extensive heating time required was undesirable (Fig. 2). This prompted the use of a combination of the two methods which would provide the quick heating obtained by water-bath heating followed by the extended incubation provided by hot air heating, both of which could be conveniently implemented into a commercial process. A combination of the two methods (water-bath at 57°C for 25 min following hot air oven at 55°C for 1 h) showed that the temperature rose to 55°C during the water-bath period (3 log cycle reduction) followed by a further reduction (4 logs) obtained by hot air heating, for a total destruction of *S. enteritidis* ATCC 13076 of 7 logs (Fig. 3). The change in temperature from water to hot air was not smooth because the eggs were cooled during the period of switching equipment and this is reflected in both the temperature and *S. enteritidis* ATCC 13076 profiles (Fig. 3). Because this was the critical temperature at which the rate of *S. enteritidis* ATCC 13076 reduction was greatest, a rapid transition from water-bath to hot air heating may provide a similar

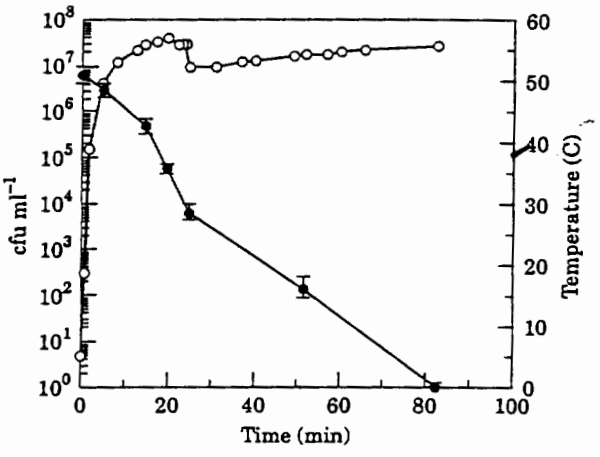

Figure 3. Microbial count reduction of *Salmonella enteritidis* ((-●-) cfu ml^{-1}) and temperature ((-O-) °C) increase in shell eggs during heating with 57°C hot water for up to 25 min followed with 55°C hot air for up to 57 min. Each data point represents the mean of triplicate determinations. High and low values are represented by error bars.

reduction of *Salmonella* in less time. It would also be expected that a commercial process, which could provide greater movement of shell egg contents during the heating and holding process may also provide better conductive heat transfer in order to reach critical bactericidal temperatures sooner.

Functionality of intact shell eggs upon pasteurization

The physical properties of shell egg contents such as color, foaming ability, foam stability and lysozyme activity of egg white or yolk of fresh eggs were compared with eggs subjected to water-bath heating at 57°C (Fig. 4) or the combination pasteurization method (Table 1). Among them, the Haugh unit, pH, yolk index, and color showed no significant differences ($P>0{\cdot}05$) between fresh and pasteurized eggs suggesting that the heating regimen did not grossly affect these egg quality characteristics (Table 1). However, egg white viscosity, turbidity, and hue value showed significant differences ($P<0{\cdot}05$) (Table 1). The decrease in the viscosity and increase in turbidity of egg white indicated partial protein denaturation (Fig. 4) and is consistent with that observed by Seideman et al. (1963). A higher hue value indicated that the heated egg white has more 'blue' hue than that of fresh egg. Foaming ability was enhanced as indicated by both the height of the foam formed upon uniform beating and by its stability during subsequent drainage of foam returning to the liquid fraction (data not shown). The enhancement in foaming ability and stability may be explained as an unfolding of protein and an increase in surface hydrophobicity of egg white. Mine et al. (1990) demonstrated by circular dichroism spectra that heat-induced changes in egg white protein already occurred at 60°C and likely occurs to a lesser degree at slightly lower temperatures comparable with what we have used in this study. Lysozyme activity was reduced nearly by half after pasteurization by the combination method (48% reduction; Fig. 4). However, it was evident that most of the reduction in activity (41% reduction) occurred during heating in water, half of which occurred in the last 5 min of the water-bath heating phase (from 25–30 min; Fig. 4). This could be explained by the temperature of the egg white slowly approaching the temperature of the water-bath; transfer to 55°C hot air heating for an additional 60 min resulted in a slight heat loss (Fig. 3) which minimized any further reduction in lysozyme activity (Fig. 4). Because lysozyme

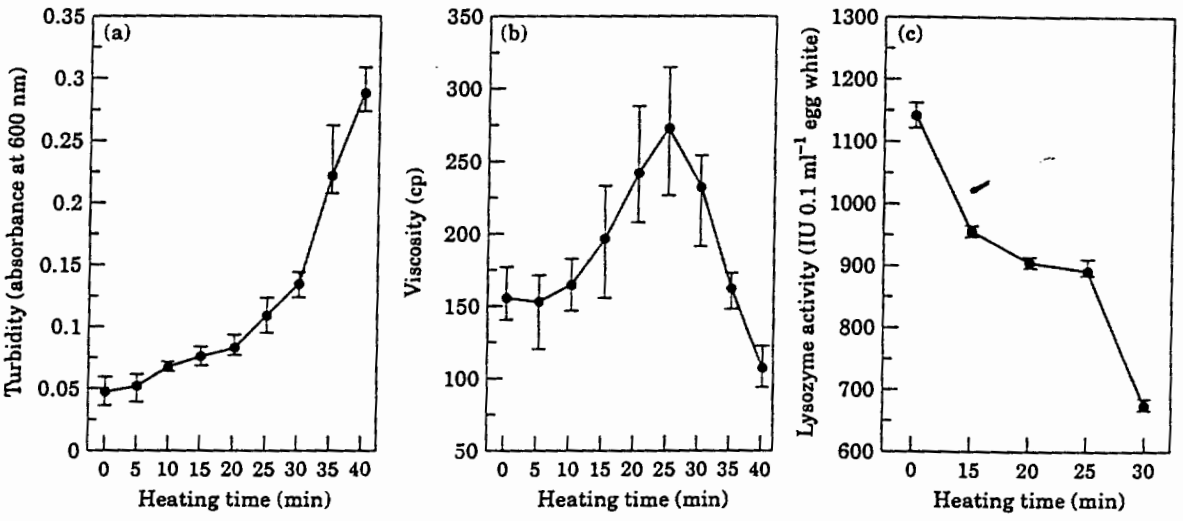

Figure 4. (a) Analysis of turbidity, (b) viscosity, and (c) lysozyme activity of egg white before and after heating of intact shell eggs in a 57°C water-bath. Each point is the mean of triplicate determinations. High and low values are represented by error bars.

Table 1. Analysis of physical properties of shell egg components from raw and pasteurized (combination method) intact shell eggs

Test or assay	Values obtained for:	
	Raw shell eggs	Pasteurized shell eggs
Egg Yolk		
Yolk index	0·45 (0·13)	0·36 (0·09)
Color values		
Hue	171·9	162·7
Chroma	39·1	41·7
Delta E	37·5	41·3
Egg white		
Haugh unit	70·3 (3·0)	75·3 (5·9)
pH	9·23 (0·22)	9·32 (0·16)
Viscosity (cp)	150 (9·5)	100 (6·8)
Turbidity (AU † 600 nm)	0·102 (0·05)	0·228 (0·13)
Foaming ability (cm)	3 (0·44)	4 (0·50)
Color values		
Hue	186·3	69·9
Chroma	1·8	2·44
Delta E	57	53·6

Values are the means of a triplicate series of independent analyses; standard deviations are listed in parentheses.

is considered an antimicrobial component of egg white, a minimal loss of lysozyme activity would further enhance the safety aspects of pasteurized intact shell eggs. Therefore, the combination method incorporated only a 25 min water-bath heating step to eliminate the portion of the water-bath heating which resulted in the most dramatic loss of egg white lysozyme activity, increase in turbidity, and decrease in viscosity (Fig. 4).

Although we examined the effect of the combination pasteurization process on lysozyme, the major antimicrobial component of egg white, we did not further examine the effect of the process on other inhibitory features of egg white such as conalbumen, avidin, or ovoinhibitor. The combination pasteurization process did not affect the storage stability of the pasteurized shell eggs held at 4°C for 30 days when challenged (yolk inoculation with *S. enteritidis* ATCC 13076 or surface contamination with hen house dust slurry) before pasteurization. Viable cells were not obtained from either of the two stability tests. The inability to recover str^r *S. enteritidis* from the inoculated eggs is indicative of the level of pasteurization obtained (7 log reduction) whereas the lack of contamination from surface-exposed eggs indicates that physical or chemical defenses are still functional in the pasteurized intact shell eggs. Although shell eggs contaminated with *S. enteritidis* either by transovarian infection or surface penetration may possess *S. enteritidis* in egg components other than in the yolk, inoculation of yolk as the position furthest from the source of heating likely provides a conservative estimate of the effectiveness of the current process.

In a recent study, Vanlith et al. (1995) recovered *S. enteritidis* from yolk-inoculated shell eggs heated in water at 57°C for 30 min and concluded that this pasteurization method was ineffective. The data presented herein demonstrated the use of a combination of water-bath and hot-air heating to implement a heating and holding time process that results in a 7 log reduction of *S. enteritidis* and the pasteurization of intact shell eggs. Because *S. enteritidis* occurring in naturally infected eggs have mainly been found in low numbers (i.e. <10 cfu egg^{-1}; Humphrey et al. 1989, 1991b), the current process provides more than a 6 log cycle safety margin for shell eggs. This process was tailored to minimize aesthetic or quality changes in the egg components and demonstrates the feasibility of extended-time non-

destructive heating to provide pasteurized shell eggs for safety-conscious consumers who have a preference for undercooked, shell-egg products. Such a process can be conveniently incorporated into existing egg processing lines that already include a heated sanitary wash; the hot air heating could be introduced subsequent to this step. Because of their reduced microbial burden, pasteurized intact shell eggs may also be used to extend shelf life if used for production of liquid pasteurized egg.

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The following is information that considers the impact of pH on lethality of pasteurization on *Salmonella spp.* in egg white based egg substitute. The data is also compared to results of studies reported by the American Egg Board/United Egg Association in the "International Egg Pasteurization Manual" (UEA/AEB, 2002).

They are offered for consideration by FSIS scientist charged with the finalization of the risk assessment for *Salmonella spp.* in pasteurized egg products. The information is abstracted from a repot of a study commissioned as part of process validation work conducted for the benefit of Michael Foods, Inc.

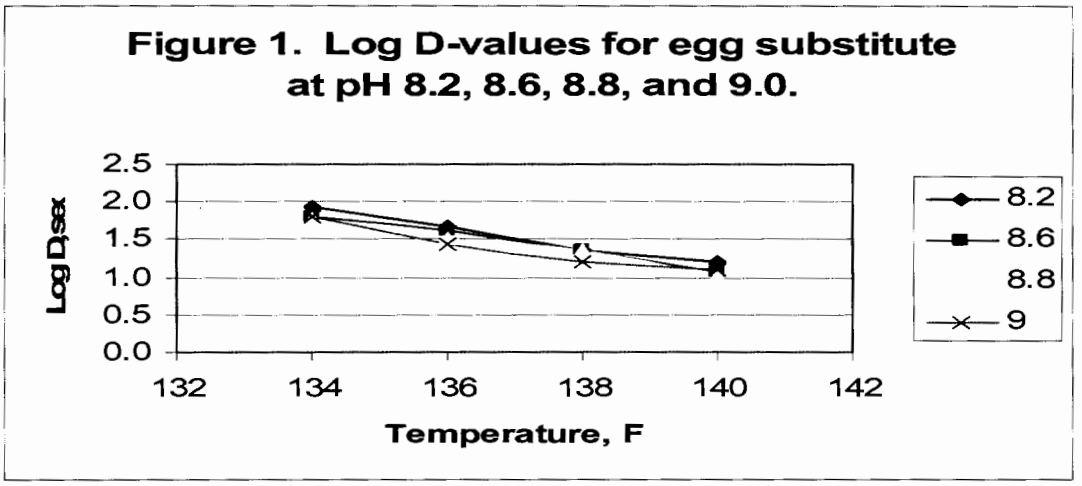
Background and Analysis:

Background information relating to pasteurization of egg products is found in the USDA Egg Pasteurization Manual (USDA, 1969), Egg Science and Technology (Stadelman and Cotterill, 1995), and the recently published International Egg Pasteurization Manual (UEA/AEB, 2002). The International Egg Pasteurization Manual (IEPM) is the most recent literature review and discussion of the current state of knowledge presented in the published literature. It also presents new data from thermal death time studies for a wide range of egg products including one egg substitute formula.

Due to the limited data available regarding the destruction of *Salmonella sp.* in egg substitutes and the documented effects of pH on inactivation of Salmonella sp. in egg white, further evaluations of pH on the inactivation of *Salmonella sp.* in our basic egg substitute formulation was commissioned.

The study was conducted by Silliker, Inc., Research Center and reported to us in a final report dated December 31, 2003. The report is attached as Appendix A. The following discussion summarizes data from the report used as the foundation for a for an egg substitute.

Figure 1 presents comparison of the D-values based on data presented in Tables 1, 2, 3, and 4 of the Silliker report.



Results from the present study show that as pH of the egg substitute product increased, the effectiveness of the thermal treatment in inactivating *Salmonella sp.* increased. The figure also indicates that the pH effect on inactivation of *Salmonella sp.* was uniform over the range of temperatures used in the study. The Z-values were 7.9, 8.7, 8.0, and 8.4 for product at pH 8.2, 8.6, 8.8, and 9.0, respectively. They are typical of Z-values for a wide range of *Salmonella sp.* (USDA, 1969).

The above data is in general agreement with the results reported in the IEPM for inactivation of *Salmonella sp.* in egg white at pH values 7.8 to 9.3.

D-values from the IEPM for the egg substitute and D-values from the Silliker report were essentially the same at temperatures at or above 134 °F (Table 1). The egg substitute formula used in the IEPM study had a solids content of 15.7% where as the egg substitute formula used in the Silliker study contained 12% solids (see attachment B for formulation). Regardless of the solids content, both studies report D-values for egg substitutes that are lower than comparable D-values for pure egg white at the same pH as shown in Table 1.

Table 1. D and Z-values for egg white and egg substitutes at pH 8.8.

	Egg White*	Egg Substitute IEPM**	Egg Substitute Silliker***
$D_{54.4C\ (130F)}$	6.99 min	2.09 min.	3.21 min
$D_{56.7C(134F)}$	1.99 min	0.92 min	1.02 min
$D_{57.7C(136F)}$	1.11 min	0.44 min	0.57 min
Z, °C(°F)	4.11(7.4)	4.99(8.9)	4.44(8.0)

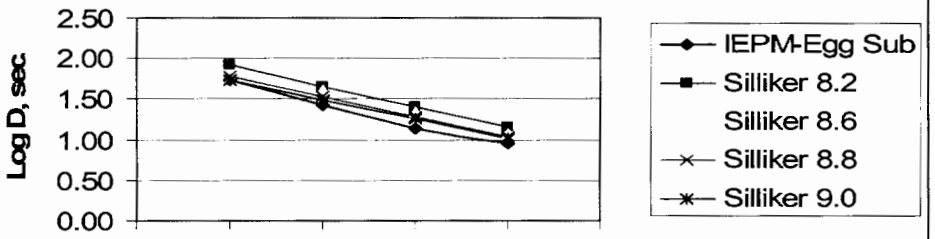
***Table 10, page 16 International Manual**

** Table 26, page 19 International Manual

*** From Silliker report

Figure 2 includes the log D for the egg substitute reported in the IEPM compared to the data reported in the Silliker study. Over the temperature ranges studied the pH effect seems to be uniform. Results from the IEPM study and from our Silliker study are in good agreement and provide the basis for determining adequate pasteurization processes for egg substitutes with solids content ranging from about 12% to about 16% with pH values at time of pasteurization ranging from 8.2 to 9.9.

Figure 2. Log D-values, sec. for egg substitutes data from IEPM and Silliker Report



Conclusion and recommendation:

Since data from two separate studies report comparable results for inactivation of *Salmonella sp.* in egg substitute formulations, minimum pasteurization conditions can be recommended for egg substitutes containing up to 16% solids with pH values ranging from 8.2 to 9.0.

Table 2 presents a comparison of expected log reductions from a minimum pasteurization process defined by a hold time of 7 minutes at 135°F.

Table 2. Log reductions of Salmonella sp. in egg substitutes achieved by minimum pasteurization treatment of 135°F for 7 minutes.

pH	$D_{(135° F)}$, min.	Log reductions in 7 min.
8.2	1.02	6.9
8.6	0.78	9.0
8.8	0.77	9.1
9.0	0.69	10.2

A pasteurization process minimum of 135°F with a 7 minute hold time should provide sufficient safety factor for egg substitute formulas containing up to 16% solids and with pH values from 8.2 to 9.0.

References:

Stadelman, W. J. and Cotterill, O. J., 1995. Egg Science and Technology, 4th edition, Food Products Press, The Haworth Press, Inc., 10 Alice Street, Binghamton, NY.

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Appendices:

Appendix A

Silliker Study Report

SILLIKER
Assuring Quality Worldwide

[redacted]

Research Report (RPN 8574)

Thermal Inactivation of *Salmonella* in Michael Foods' Egg Substitute Formula

December 31, 2003

prepared for

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USDA/FSIS Docket #04-034N
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Objective

The objective of this study was to determine the thermal inactivation rate (D and z values) for *Salmonella* in an Egg Substitute product at pH's of 8.2, 8.6, 8.8, and 9.0.

Conclusions

When the pH value of the product was 8.2, the mean D-values were 84.2 seconds at 134°F, 46.7 seconds at 136°F, 22.5 seconds at 138°F, and 15.5 seconds at 140°F, respectively. When the pH value of the product was 8.6, the mean D-values were 61.2 seconds at 134°F, 40.0 seconds at 136°F, 22.0 seconds at 138°F, and 12.0 seconds at 140°F, respectively. When the pH value of the product was 8.8, the mean D-values were 60.5 seconds at 134°F, 33.1 seconds at 136°F, 22.4 seconds at 138°F, and 10.1 seconds at 140°F, respectively. When the pH value of the product was 9.0, the mean D-values were 63.9 seconds at 134°F, 26.4 seconds at 136°F, 15.8 seconds at 138°F, and 12.2 seconds at 140°F, respectively. The z values were 7.9°F for pH8.2, 8.7°F for pH8.6, 8.0°F for pH8.8, and 8.4°F for pH9.0, respectively.

Materials and methods

Food Product

Michael Foods Egg Products Co. (MFEPC) provided thirty-six cartons of an egg substitute product as a pasteurized packaged refrigerated liquid with low initial aerobic plate counts on 9/26/2003. The cartons were labeled "Papetti Foods, [redacted] 29750, Fat Free, Cholesterol Free Pasteurized, Homogenized, P-1610-3260A, Exp. Dec. 24. 03-501991." The product was stored at 40°F for the duration of the study. Experiments were performed from September through December.

Test Organisms

A culture composite of *Salmonella* containing 6 strains each was used for the study. Strains were obtained from the Silliker Laboratories Research (SLR) culture collection. Several strains were selected to increase the genetic variation. Strain labels and descriptions are as follows:

<u>*Salmonella*</u>

S. heidelberg (SLR 539) – Silliker Laboratories Research culture collection (originally isolated from liquid whole egg).
S. enteritidis (SLR 1840) - ME-14 (poultry manure)
S. enteritidis (SLR 1841) – ME-15 (shell egg transfer belt)
S. enteritidis (SLR 1843) - ME-18 (live poultry)
S. enteritidis (SLR 1844) – Benson-1 (human clinical isolate)
S. typhimurium (SLR 1846) –Tm-1 (type strain used extensively in liquid egg pasteurization studies conducted by USDA-ARS, Western Regional Research Laboratory, Albany, CA).

Each strain was individually cultivated in Trypticase Soy Broth (TSB) at 35°C for 24 hours prior to initiation of each portion of testing. Equal volumes of each strain were

[redacted]

combined to form a composite culture of *Salmonella*. The composite was centrifuged, washed once with 40 mL of Butterfield's phosphate buffer, centrifuged a second time and suspended in 80 mL of egg product to obtain a uniform suspension.

Experimental Procedure

The product pH was adjusted to pH values of 8.2, 8.6, 8.8, or 9.0 using 1 N HCl or 1 N NaOH. The HCl or NaOH was added slowly to the product to prevent gelation and/or precipitation and was mixed gently to prevent foaming. A verification of pH after inoculation and before filling capillary tubes was performed.

Michael Foods established 134°F, 136°F, 138°F, and 140°F as the four temperatures to be used for the study. Three trials at each temperature were performed. Testing was performed using capillary tubes to minimize long come up times. For each trial, the inoculated egg suspension was distributed in 0.05 mL aliquots to 24 sterile capillary tubes using a micopipettor. The capillary tubes were heat-sealed at each end with a flame. Heat treatments were performed immediately to avoid pH shifts.

Twenty-two of the capillary tubes were individually tied to a wire and placed in heated water baths. Capillary tubes were pulled at 8 exposure times. Pull times began when the temperature control reached the target temperature. The target was to pull a sample per one log reduction. Two capillary tubes were pulled at the first 5 time intervals and 4 tubes at the last 3 time intervals. The tubes were immediately cooled in an ice bath for at least 5 minutes. The capillary tubes were sanitized using 70% ethanol. The capillary tubes were then air-dried and opened with a sterile wire cutter for analysis. Two unheated capillary tubes served as inoculated controls to obtain initial populations of *Salmonella*. Two non-inoculated controls were analyzed for aerobic bacteria.

Temperature data were collected using a Yokogawa DR240 portable hybrid recorder (Shenandoah, GA) with T-Type thermocouples to monitor the water bath and product temperature in 3-second intervals.

Microbiological Analysis

Inoculated samples were aseptically drained into test tubes. Serial dilutions were analyzed using the pour plate technique with Tryptic Soy Agar (TSA). For the first 5 time intervals dilutions were performed starting at a 1:100. For the last 3 time intervals 2 tubes were analyzed starting at a 1:100 dilution. The remaining 2 tubes were used to analyze a 1:10 dilution.

Plates were incubated at 35°C for 48 hours, colonies counted, and two colonies from a countable plate per time interval were streaked on selective agar (Xylose Lysine Desoxycholate) plates to verify that the colonies were *Salmonella*. XLD Plates were incubated at 35°C for 24 hours.

Uninoculated product was analyzed for aerobic plate count using Tryptone Glucose Yeast agar (TGY) incubated at 35°C for 48 hours and for absence of *Salmonella* per 25

[redacted]

gram analytical unit using the USDA procedures (www.fsis.usda.gov/OPHS/microlab/mlgbook.htm. Isolation and identification of *Salmonella* from meat, poultry and egg products. Chapter 4.02).

An inoculation study was conducted to verify that inoculation of *Salmonella* did not change the pH of the products for the duration of the study. The pH of the inoculated product was analyzed over 180 minutes.

Data Analysis

The base ten logarithms of the plate counts were plotted against time for each temperature and the best fit line was statistically determined by least squares linear regression. The D value is the time required, in seconds, for the population to decrease by 90% or 1-log when held at a certain temperature. Mathematically, it is the negative inverse of the slope of the regression line. The Z values for the product were also determined. The z-value represents the number of °F required for the D value to proceed through one log cycle. Mathematically, it is the negative inverse slope of the regression line of the logarithm of D values and temperature.

Results and Discussion

For all the uninoculated samples tested, the aerobic plate counts in the samples varied from 0 to 7 colony forming units per gram. The uninoculated product was found to be negative of *Salmonella* per 25-mL analytical unit.

The addition of the composite culture of *Salmonella* into the egg product did not change its pH value when the pH value of the egg product was at pH8.2 or pH8.6. When the pH value of the egg product was at pH8.8 or 9.0, addition of the composite culture slightly decreased the pH values. A small amount of NaOH was added to the product to bring the pH values back to target levels, pH8.8 and pH9.0. After inoculation and adjustment, the pH of the product remained stable at the target levels for the duration of the study.

Experimentally determined D-values and correlation coefficients (r^2) for *Salmonella* in the Better'n Eggs product for pH 8.2, 8.6, 8.8, and 9.0 are presented in Tables 1, 2, 3, and 4. Graphs of the data used to calculate these values are presented in Figures 1-16. When the pH value of the product was 8.2, the mean D-values were 84.2 seconds at 134°F, 46.7 seconds at 136°F, 22.5 seconds at 138°F, and 15.5 seconds at 140°F (Table 1). When the pH value of the product was 8.6, the mean D-values were 61.2 seconds at 134°F, 40.0 seconds at 136°F, 22.0 seconds at 138°F, and 12.0 seconds at 140°F (Table 2). When the pH value of the product was 8.8, the mean D-values were 60.5 seconds at 134°F, 33.1 seconds at 136°F, 22.4 seconds at 138°F, and 10.1 seconds at 140°F (Table 3). When the pH value of the product was 9.0, the mean D-values were 63.9 seconds at 134°F, 26.4 seconds at 136°F, 15.8 seconds at 138°F, and 12.2 seconds at 140°F (Table 4). Variations in D-values are commonly observed. Common sources of variation are the strains used, daily culture preparation, and/or laboratory testing. The variations observed appear typical based upon our experiences.

[redacted]

The calculated z values and correlation coefficients (r^2) for *Salmonella* in the Better'n Eggs product for pH 8.2, 8.6, 8.8, and 9.0 are shown in Table 5. Graphs of the data used to calculate these values are presented in Figures 17-20. The calculated z values were 7.9°F for pH8.2, 8.7°F for pH8.6, 8.0°F for pH8.8, and 8.4°F for pH9.0.

The effect of pH on the D values of *Salmonella* in [redacted] product is shown in Table 6. At 134°F, the D values decreased from 84.2 seconds to 61.2 seconds when the pH values increased from 8.2 to 8.6. Further increasing the pH values did not have any effect on the D values. At 136°F, the D values decreased as the pH values increased. At 138°F, the D values remained at about 22 seconds when the pH values increased from 8.2 to 8.8, and then decreased to 15.8 seconds when the pH values further increased to 9.0. At 140°F, the D values decreased from 15.5 seconds to 12.0 seconds when the pH values increased from 8.2 to 8.6. No apparent trend in the D values was observed when the pH values were increased from 8.6 to 9.0.

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[redacted]

Table 1. Experimental D Values (seconds) and r^2 Values of *Salmonella* in [redacted] at pH 8.2

Temperature	134°F		136°F		138°F		140°F	
	D value	r^2	D value	R^2	D value	r^2	D value	r^2
Trial 1	77.9	0.92	45.1	0.94	22.4	0.92	15.3	0.93
Trial 2	96.1	0.80	46.4	0.85	21.9	0.91	13.4	0.85
Trial 3	78.6	0.87	48.7	0.80	23.2	0.94	17.9	0.91
Average	84.2	NA	46.7	NA	22.5	NA	15.5	NA
Standard Deviation	10.3	NA	1.8	NA	0.7	NA	2.3	NA

NA = not applicable

Table 2. Experimental D Values (seconds) and r^2 Values of *Salmonella* in [redacted] at pH 8.6

Temperature	134°F		136°F		138°F		140°F	
	D value	r^2	D value	R^2	D value	r^2	D value	r^2
Trial 1	62.8	0.90	44.4	0.92	24.9	0.86	14.2	0.87
Trial 2	53.0	0.94	35.6	0.93	19.5	0.91	11.4	0.93
Trial 3	59.5	0.87	34.0	0.91	21.7	0.9	10.5	0.96
Average	61.2	NA	40.0	NA	22.0	NA	12.0	NA
Standard Deviation	2.3	NA	6.2	NA	2.7	NA	1.9	NA

NA = not applicable

Table 3. Experimental D Values (seconds) and r^2 Values of *Salmonella* in [redacted] at pH 8.8

Temperature	134°F		136°F		138°F		140°F	
	D value	r^2	D value	R^2	D value	r^2	D value	r^2
Trial 1	69.1	0.75	32.1	0.97	23.8	0.93	9.4	0.96
Trial 2	55.4	0.98	32.9	0.96	18.8	0.95	9.4	0.95
Trial 3	56.9	0.92	34.2	0.98	24.6	0.81	11.5	0.94
Average	60.5	NA	33.1	NA	22.4	NA	10.1	NA
Standard Deviation	7.5	NA	1.1	NA	3.1	NA	1.2	NA

NA = not applicable

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Liquid Egg Substitute Formula
Michael Foods, Inc. Egg Division/Papetti's
Revision #: 001
Revision Date: July 18, 2003

Ingredient Listing:

Egg Whites (98%), Water, Natural Flavor, Sodium Hexametaphosphate, Guar Gum, Xanthan Gum, Color (includes beta carotene and annatto).

Physical Parameters
1. Total Solids on Air Oven: 11.8% - 12.2%
2. Total egg white content – 98.07%