Membrane Damage and Viability Loss of *Escherichia coli* K-12 and *Salmonella* Enteritidis in Liquid Egg by Thermal Death Time Disk Treatment

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**ABSTRACT**

Bacterial injury, including leakage of intracellular substance and viability loss, of *Escherichia coli* K-12 (ATCC 23716) and *Salmonella* Enteritidis (ATCC 13076) inoculated in liquid egg white and liquid whole egg was determined by thermal death time disk. *E. coli* K-12 and *Salmonella* Enteritidis were inoculated in liquid egg white and liquid whole egg to a final count of 7.8 log CFU/ml and were thermodenatured with thermal death time disks at room temperature (23°C), 54, 56, 58, and 60°C from 0 to 240 s. Sublethal injury, leakage of intracellular substances, and viability loss of *E. coli* K-12 and *Salmonella* Enteritidis was investigated by plating 0.1 ml on selective trypticase soy agar containing 3% NaCl, 5% NaCl, sorbitol MacConkey agar, and xylose lysine sodium tetradecylsulfate and nonselective trypticase soy agar. No significant differences on percent injury or viability loss for *E. coli* K-12 and *Salmonella* populations were determined in all samples treated at 23°C. Sublethal injury occurred in *E. coli* and *Salmonella* populations at 54°C or above for 120 s. Viability losses for both bacteria averaged 5 log at 54°C or above for 180 s, and the surviving populations were below detection (<10 CFU/ml). Thermal treatment at 40°C and above led to membrane damage, leakage, and accumulation of intracellular ATP from 2 to 2.5 log fg/ml and UV-absorbing substances of 0.1 to 0.39 in the treated samples. These results indicate similar thermal injury/damage on both *E. coli* and *Salmonella* membranes as determined by the amount of inactivation, viability loss, and leakage of intracellular substances of bacteria.

Microbial contamination of eggs as well as its economic implications to the poultry industry has been reported (6, 38). *Salmonella* is the most frequently reported cause of foodborne outbreaks of gastroenteritis in the United States (8, 9). Diseases caused by *Salmonella* (salmonellosis) have been steadily increasing as a public health problem in the United States since reporting began in 1943 (30). In recent years, *Salmonella* in eggs has emerged as a major concern for consumers and health agencies, and the risk of salmonellosis is greater when the egg is used as an ingredient in a food (32). Liquid eggs, homogenized as whole egg or separated into white and yolk, are used as ingredients in a wide variety of processed products, including bakery and confectionary products, drinks, infant products, dressings, and noodles (15).

A 1-year survey of 20 egg-breaking plants in the United States found 6 to 20% of unpasteurized liquid egg samples to contain *Salmonella* Enteritidis (19). The presence of *Salmonella* Enteritidis in liquid egg was implicated as the source of salmonellosis infection (5, 32). Increase in the incidence of human food poisoning caused by *Salmonella* Enteritidis was reported in Europe and the United States (3, 8, 9, 26). However, pasteurized egg products have not been involved in any egg-associated outbreaks of gastroenteritis (8, 9, 26). The reason can be attributed to the U.S. Department of Agriculture (35) requirement that liquid egg be heated at 60°C and held for no less than 3.5 min to achieve a reduction of more than 3.0 log CFU/ml *Salmonella* (15).

Physical and chemical treatments are used in food processing to eliminate or at least reduce the presence of pathogenic and spoilage microorganisms in foods (1, 4, 13). The use of thermal processing to inactivate foodborne pathogens in food systems has been reported (28, 29, 37). Thermal processing of juice and liquid egg to inactivate foodborne pathogens might impair the characteristic flavor of the juice (1) and lead to coagulation of liquid egg (11, 13, 31).

Quality attributes of liquid egg product are heat sensitive, and the time-temperature margins for a thermal pasteurized liquid egg free of bacteria are narrow. Therefore, the use of a minimum heat treatment process that would ensure food safety is needed. In designing such process treatment, care must be taken to factor in even distribution of heat in a short period to minimize bacterial resistance. Thermal resistance of bacteria in food systems that use capillary tubes and thermal death time (TDT) glass tubes have been reported and were found to have limited use for food samples with low viscosity (23, 24, 27). Similarly, temperature could not be monitored accurately in the capillary tubes or the come-up time related to thermal lag in large glass tubes (25). In this study, we monitored inactivation
of *Escherichia coli* and *Salmonella Enteritidis* in liquid egg with a new aluminum TDT disk developed at Washington State University (Pullman, Wash.) (16). The TDT disk was attached with a type K thermocouple at the center to monitor treatment temperature within the disk. The volume of the TDT disk chamber is 1.2 ml and was constructed to allow rapid heating of samples in water or oil baths. The close, compact nature of this TDT disk provides near isothermal conditions necessary for the study of thermal death kinetics of microorganisms. In an earlier study from our laboratory, a thermal resistance of *E. coli* K-12 to *Salmonella Enteritidis* in liquid egg with the use of this new TDT disk device was investigated and reported (16). In this study, we concentrated our effort in understanding the thermal effect of the disk treatment on sublethal injury, viability loss, and leakage of intracellular materials from the bacterial cells. Leakage of bacterial intracellular ATP and UV-absorbing substances as a function of membrane damage was also investigated with a bioluminescence ATP assay and spectrophotometer. The bioluminescence ATP assay has been used to measure direct membrane damage of *Candida albicans* treated with ketoconazole and tioconazole (2) and *Listeria monocytogenes* treated with nisin, ampicillin, and streptomycin (34).

**MATERIALS AND METHODS**

Test strains and preparation of inocula. *E. coli* K-12 (ATCC 23716) and *Salmonella Enteritidis* (ATCC 13076) from the U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center culture collection, were used in this study. Individual cell cultures were maintained on trypticase soy agar (TSA) at 4°C. Before use, the cells were inoculated by loop in tryptic soy broth (TSB; Remel, Inc., Lenexa, Kans.) with incubation at 37°C for 16 to 18 h with shaking. A 0.1-ml cell aliquot was transferred to 20 ml of TSB and incubated at 37°C for 24 h. The overnight cell suspensions were centrifuged at 3,000 × g for 10 min at 5°C. The cell pellets were washed with an equal volume (100 ml) of sterile phosphate-buffered saline (PBS; pH 7.2) solution. Finally, the washed cells were resuspended in PBS and used as the inoculum. The inoculum was maintained at 5°C for less than 30 min until used to inoculate commercial pasteurized and laboratory-prepared liquid egg at 7.0 log CFU/ml as determined by plate count.

Preparation of liquid egg samples. Commercial pasteurized liquid egg white (PLEW), without preservatives, and fresh Grade A whole eggs were purchased from a local grocery store. Surfaces of the whole eggs were sanitized with 70% alcohol, rinsed with sterile denionized water, and allowed to dry inside a biosafety cabinet for 1 h. The shells were broken and the contents emptied into a Stomacher bag, and the contents were pumped into a Stomacher model 400 (Dynatech Laboratories, Alexandria, Va.) at 250 rpm for 30 s. The pH of the PLEW was 8.20, whereas the pH of our laboratory-prepared liquid whole egg (LWE) ranged from 7.50 to 7.65. To determine the initial bacterial load of each type of liquid eggs, 0.1 ml of the samples were plated in duplicate on TSA, and selective agar plates (sorbitol MacConkey agar [SMAC], xylose lysine sodium tetradecylsulfate [XLT4], and TSA plates containing 3 and 5% NaCl [TSA3 and TSA5]; Difco, Becton Dickinson, Sparks, Md.) incubated at 37°C for 24 h to determine the number of colony-forming units.

**Thermal inactivation of bacteria.** The inside and outside temperatures of the TDT disks were monitored and recorded with a Fluke 54 II thermometer (Everett, Wash.) for all experiments. Before each use, the TDT disks were dipped in sanitizer (Coverage Plus, E. R. Squibb & Sons, Inc., St. Louis, Mo.) for 2 min according to the manufacturer's instructions, rinsed five times with sterile water, and then air dried. An aliquot (1 ml) of PLEW and LWE inoculated with *E. coli* or *Salmonella Enteritidis* was added to each TDT disk. The disks were hermetically sealed and then submerged completely in a water bath (Isotemp 110, Fisher Scientific, Pittsburgh, Pa.), with temperatures set at 52, 54, 56, 58, or 60 ± 0.1°C. The samples were pulled from the water bath at 0 to 240 s and were immediately immersed in an ice water bath to cool before opening. Survival, injury, and inactivation of treated *E. coli* or *Salmonella Enteritidis* in liquid egg samples were determined by plating on a range of agar plates mentioned below. Leakage of bacteria, intracellular UV-absorbing materials, and ATP related to membrane damage were investigated. The controls were *E. coli* and *Salmonella Enteritidis* populations in liquid egg samples inside the TDT disks and placed in a water bath maintained at room temperature (~23°C) for the same amount of time.

**Enumeration of viable *E. coli* and *Salmonella Enteritidis.*** The surviving population of each bacterium and injury resulting from the TDT disk treatment was investigated with the use of selective plates: SMAC, XLT4, TSA3, and TSA5 (Difco, Becton Dickinson). Sublethal injury resulting from the TDT disk treatments was monitored with the use of differential plating methods on nonselective versus selective agar plates, and the difference in viable populations enumerated was considered injured cells. Percent injury was calculated with the formula (1 — colonies on selective agar/counts on nonselective agar) × 100.

Aliquots (0.1 ml) of inoculated liquid egg in TDT disks treated at different temperatures for a specific time were plated on a range of agar plates as stated above. Also, the number of colony forming units per milliliter on nonselective and selective agar media was used to calculate the viability loss, which is defined as the difference (log CFU per milliliter) in bacteria between control and heat-treated TDT disk samples (18). Treated and untreated inoculated liquid eggs were stored at 5°C for 24 h and then plated on nonselective and selective agar media to determine the effect of treatment and immediate cold storage on cell viability. Untreated inoculated liquid eggs were used as positive controls for each experiment, and uninoculated TDT heat-treated liquid eggs were used as the negative controls. All agar plates were incubated at 25 and 37°C for 48 h.

**Leakage of bacterial intracellular UV-absorbing materials.** To quantify the intracellular UV materials released from *E. coli* K-12 and *Salmonella Enteritidis*, the extracts from treated and untreated cells containing the UV materials were measured at 260 and 280 nm with a spectrophotometer (DUR 530, Beckman Coulter, Fullerton, Calif.) according to published reports (7, 33, 37).

**Bioluminescence ATP assay.** Extracellular (somatic) ATP content of commercial pasteurized and laboratory-prepared liquid egg before and after inoculation with *E. coli* and *Salmonella Enteritidis* inside TDT disks was determined. Portions (0.1 ml) of PLEW and LWE samples were mixed with 0.1 ml of luciferin-luciferase (Sigma, St. Louis, Mo.) and the somatic ATP content of the samples was measured in relative light units (RLUs) with an ATP bioluminescent assay kit (Turner Design, Sunnyvale, Calif.). The generated light signal (RLU) was measured with a TD-20/20 (DL Ready) luminometer (Turner Design) after 3 s of delay time and 14 s of integration time. Assays of standard amounts of
purified ATP were used to calculate ATP levels, and ATP concentrations in samples were expressed as log femtogram per milliliter (log fg/ml). Controls for background luminescence, consisting of 0.1 ml of the commercial pasteurized and laboratory-prepared liquid egg were run, and the readings were subtracted from readings for ATP determination. Possible inhibition of the luciferase reaction by any residues from the liquid egg homogenates was corrected by addition of known amounts of ATP standard to the reaction vial followed by addition of the luciferase enzyme (34).

Data analysis. All experiments were done in triplicate, with duplicate samples being analyzed at each sampling time. Data were subjected to analysis of variance (ANOVA) with SAS (SAS Institute, Cary, N.C.). The SAS program was used to determine significant differences in survival, viability loss, and inactivation and for correlation of ATP concentrations and intracellular UV-absorbing substances that leaked out during treatment. Significant differences (P < 0.05) between mean values for each variable tested were determined by the Bonferroni least significant difference method.

RESULTS

Survival of *E. coli* and *Salmonella Enteritidis* in liquid egg. Behavior of *E. coli* K-12 and *Salmonella Enteritidis* inoculated in PLEW and LWE samples without thermal treatment (23°C) for up to 240 s served as the control for this study. Populations of *Salmonella Enteritidis* and *E. coli* in TDT disks determined before thermal treatment averaged 7.7 and 7.4 log CFU/ml, respectively. These values were not significantly (P > 0.05) different in samples left at room temperature (~23°C) for up to 240 s. Also, the type of agar plates (TSA, TSA3, TSA5, SMAC, and XLT4) used for enumerating populations of untreated *Salmonella Enteritidis* and *E. coli* in PLEW and LWE determined at 240 s were not significantly (P > 0.05) different. Agar plates (TSA) supplemented with NaCl at 5% did not show more significant (P > 0.05) numbers than populations determined with SMAC and the XLT4 plates. Thermal treatment was increased to 54°C for up to 240 s to investigate the sensitivity of agar plates on recovery of treated cells, and the results are shown in Figure 1A and 1B. Thermal treatments at 54°C for up to 240 s, led to 1.3- and 1.8-log reductions of *E. coli* with TSA, TSA3 and TSA5, and SMAC plates, respectively (Fig. 1A). The results showed that addition of salts up to 3% did not affect the surviving populations, in that numbers determined by TSA and TSA3 were not significantly (P > 0.05) different. The trend in log reduction for *Salmonella Enteritidis* with TSA, TSA3 and TSA5, and XLT4 was the same, but the numbers determined were 0.66 and 1.64 log, respectively (Fig. 1B). The addition of 3% NaCl to the TSA plates did not affect the viability loss for *E. coli* and *Salmonella Enteritidis* populations, and the numbers of survivors recovered on plates without the salts were not significantly (P > 0.05) different. However, *E. coli* and *Salmonella Enteritidis* populations recovered on TSA plates with 5% NaCl were significantly (P < 0.05) different from TSA plates with or without 3% NaCl but not with selective plates (SMAC and XLT4) (data not shown). These findings were consistent throughout the study; therefore, we decided to report only the results determined with TSA, SMAC, and XLT4 plates.

Effect of thermal treatment. Survival of *E. coli* populations in PLEW thermally treated at different temperatures for up to 240 s and determined with TSA and SMAC plates are shown in Figure 2A. Viability loss for *E. coli* in PLEW determined with TSA was significantly (P < 0.05) different at 58 and 60°C for 240 s and averaged reductions of 2.5 log. Population reduction determined with SMAC plates was significantly (P < 0.05) different at temperatures above 54°C in samples treated for 120 s, and at 58 and 60°C for 180 s, the populations were below detection (<10 CFU/ml). Thermal treatment at 56°C for 240 s led to approximately 5-log reductions of *E. coli* populations with SMAC (Fig. 2A). In LWE samples inoculated with *E. coli*, viability loss at 54 and 56°C treatment for 240 s averaged 1.2 log, whereas viability loss at 58 and 60°C treatment averaged 2.3 log with TSA plates. Thermal treatment above 58°C for 180 s led to levels below detection (<10 CFU/
Survival of Escherichia coli K-12 (ATCC 23716) in pasteurized liquid egg white (PLEW) (A) and liquid whole egg (LWE) (B) thermally treated at 23, 54, 56, 58, and 60°C for 240 s and determined with the use of TSA and SMAC plates. Values are mean ± standard deviation of three determinations.

The viability loss for Salmonella Enteritidis in PLEW treated at different temperatures and determined with the use of TSA and XLT4 is shown in Figure 3A. All treatment showed a significant log reduction in population compared with the control. Thermal treatment at 54 and 56°C for 240 s did not show significant differences on the surviving population of Salmonella Enteritidis in PLEW, and the viability loss determined with TSA averaged 1.2 log. At higher thermal treatments (58 and 60°C), the average viability loss was 1.6 and 2.9 log, respectively. Surviving populations of Salmonella Enteritidis in PLEW determined at 240 s with XLT4 plates averaged 2 to 2.6 log at 56 and 58°C. For treatment at 60°C and 180 s, the numbers were below detection. The surviving populations in LWE treated at all temperatures and determined with TSA and XLT4 plates are shown in Figure 3B. Viability loss determined with TSA plates on LWE samples treated at 54 and 56°C were not significantly different, and the numbers averaged 1- to 1.2-log reductions, whereas treatment at 58 and 60°C at 240 s averaged 2.3 to 2.6 log, respectively. When the same samples with similar treatments were plated on XLT4, surviving populations were below detection, suggesting an approximately 7-log reduction (Fig. 3B).

Bacterial injury. The effects of thermal injury on E. coli and Salmonella Enteritidis in PLEW and LWE samples are shown in Figure 4A and 4B. All thermal treatments of E. coli and Salmonella Enteritidis in PLEW for up to 240 s led to significant amounts of injury. Treatment at 56°C for 240 s led to 64% and 58% injury in E. coli and Salmonella Enteritidis populations, respectively (Fig. 4A). Treatment at 58 and 60°C for 180 s led to minimal injury but to more inactivation of E. coli in PLEW than the results of Salmonella Enteritidis at 58°C. However, in all samples

Survival of Salmonella Enteritidis (ATCC 13076) in liquid whole egg (PLEW) (A) and pasteurized liquid egg white (LWE) (B) and thermally treated at 23, 54, 56, 58, and 60°C for 240 s and determined using TSA and XLT4 plates. Values are mean ± standard deviation of three determinations.
treated at 60°C for 240 s, percent injury was not determined because total inactivation was achieved. For *E. coli* and *Salmonella Enteritidis* populations in LWE thermally treated at all temperatures, again, more injured populations were determined at 120 s for treatments at 58 and 60°C, and by 180 s and above, injured populations were not detected. Unlike the PLEW samples, treatment at 56°C for 180 s led to significant injury for *E. coli* (40%) and *Salmonella Enteritidis* (76%) populations, and at 240 s, percent injury was not detected (Fig. 4B). The results of this study indicate that thermal injury for both *E. coli* and *Salmonella* bacteria at 60°C occurred at 120 s, whereas similar effects were determined for both bacteria treated at 56°C for 240 s. No injured populations were recovered in samples treated above 56°C and stored at 5°C for 24 h, suggesting that treatment at this temperature and storage will enhance the microbial safety of liquid eggs.

FIGURE 4. Percent injury of *Salmonella Enteritidis* (ATCC 13076) and *Escherichia coli* K-12 (ATCC 23716) in liquid whole egg (LWE) (A) and pasteurized liquid egg white (PLEW) (B), thermally treated at 23, 54, 56, 58, and 60°C for 240 s. Values are mean ± standard deviation of three determinations.

Leakage of intracellular ATP and UV-absorbing substances of bacteria. The initial ATP level determined immediately in PLEW and LWE without inoculated bacteria and with no thermal TDT disk treatment averaged 1.0 log fg/ml. This value did not change significantly when determined every 60 s for up to 240 s in samples inside TDT disks left at room temperature (~23°C). However, the initial ATP value in PLEW and LWE inoculated with *E. coli* and *Salmonella Enteritidis* increased from 1.0 to 2.4 log fg/ml after thermal treatment at 58 and 60°C for 120 s (Fig. 5). Treatment at 54 and 56°C for 180 s led to a similar amount of ATP, determined in PLEW and LWE inoculated with *E. coli* and *Salmonella Enteritidis*. Because more injured populations were determined at 120 s in samples treated at 58 and 60°C, we decided to investigate leakage of intracellular UV-absorbing substances of *E. coli* and *Salmonella Enteritidis* bacteria into the liquid egg samples as shown in Figure 6A and 6B. The results of intracellular UV-absorbing substances for *Salmonella Enteritidis* leaked into the PLEW (Fig. 6A) were much smaller than the amount determined for *E. coli* (Fig. 6B). Absorbance for intracellular UV-absorbing substances of *Salmonella Enteritidis* determined at 260 nm in PLEW was significantly (P > 0.05) different from the values determined at 280 nm. In samples inoculated with *E. coli*, values determined at 260 nm were significantly greater than values at 280 nm (Fig. 6B). Thermal treatment of PLEW samples at 56°C for 120 and 180 s clearly showed differences in the amount of substances leaked out from *E. coli* and *Salmonella Enteritidis*.

DISCUSSION

Plating of bacterial cells from TDT disks left at room temperature at zero time and up to 240 s on nonselective (TSA) and selective (TSA3, TSA5, and XLT4 or SMAC) agar plates revealed a nonsignificant reduction in the population of bacteria tested on all plates. The results seem obvious, in that treatment at 23°C was not enough to cause
membrane damage and the slight differences in populations determined with TSA, SMAC, and XLT4 agar plates could be the result of media composition. However, when heat (54 to 60°C) was applied to the TDT disks for up to 240 s and the samples were plated on nonselective and selective TSA and SMAC (E. coli) or XLT4 (Salmonella), the populations of both bacteria on SMAC and XLT4 were reduced considerably, indicating a high degree of injury relative to the media composition the injured populations can withstand.

The differences in viability loss for E. coli and Salmonella observed in LWE and PLEW can be attributed to water activity, pH, and nutrient composition between the two types of liquid egg. For example, PLEW has a higher pH value than LWE and was not tolerated by the bacteria compared with LWE. The heat transfer in PLEW was probably faster because of the absence of a lipid yolk component, and this might have contributed to the higher viability loss than with LWE (24). The E. coli and Salmonella investigated in this study might have used the lipid part of the egg yolk to stabilize their membranes and thus were able to withstand the thermal treatment against heat inactivation as previously reported (20, 21). Because of the possible protection by lipid, other researchers have recommended use of a higher pasteurization temperature for liquid whole egg than for liquid egg white (23).

The effect of thermal processing on injury or membrane damage and leakage of intracellular bacterial substances into the heating medium was investigated. Injury related to the release of lipopolysaccharide from the outer membrane of damaged E. coli K-12 cells sublethally heated in phosphate buffer at pH 7.0 has been reported (14). In this study, we investigated leakages of intracellular bacterial substances as a result of membrane damage. The increase of the initial UV-absorbing substances and their accumulation in thermally treated liquid eggs containing the injured bacterial cells suggest that these materials came from the bacteria. Similar accumulation of intracellular ATP in media containing yeast cells treated with ketoconazole and tioconazole (2) and L. monocytogenes in media containing nisin (34) was reported. In all these studies, the authors concluded that the accumulation of extracellular ATP in media containing Listeria cells treated with nisin, ampicillin, and streptomycin resulted from membrane damage caused by these compounds. The higher amount of UV-absorbing substances determined at 180 s during thermal treatment of samples at 58°C could be from leakage by dead cells, compared with the values determined at 120 s when bacteria injury was optimum.

In this study, we monitored bacterial ATP because ATP plays a key role in energy status of the cell and in regulating enzyme activity (10, 17); therefore, any perturbation that caused efflux/influx of substances in and out of bacterial membranes could affect the energy status and the enzymatic activity of the cell, leading to its death. The increase in extracellular ATP and the presence of UV-absorbing substances determined at A260 (nucleic acid) and A280 (protein) in liquid egg confirmed that heat treatment at 56°C or above for more than 120 s was enough to cause significant membrane damage in E. coli and Salmonella Enteritidis, leading to leakage of these substances. The substances that leaked from the injured bacteria cells diminished the capacity of the E. coli and Salmonella Enteritidis bacteria to maintain normal physiological activity, which ultimately led to their death. Other researchers have measured leakage of nucleic acid and protein of microwave-injured bacteria at 280 and 260 nm (33, 38). In these studies, the authors reported that the intracellular UV-absorbing substances that leaked from the bacteria into the cellular extracts contained protein with nucleic acids and that the most common contaminants in the extract were nucleic acids, which absorbed strongly at 280 nm (λ_max = 260 nm). Pyrimidines and purines, both nucleic acid compounds, are known to absorb UV light at a wavelength of 260 nm. We have reported similar leakage of a UV-absorbing substance from bacteria treated with radio frequency electric fields (33).
A higher percentage of bacterial injury was determined at 56°C for less than 240 s of treatment, which led to the leakage and accumulation of intracellular ATP from E. coli and Salmonella Enteritidis bacteria in the liquid egg samples. The choice of the Salmonella Enteritidis strain for this study was due to its frequent association with foodborne illness through consumption of contaminated egg, and the results obtained from this study clearly showed reductions of E. coli and Salmonella Enteritidis of more than 5 log. However, thermal processing of egg white under the minimum conditions specified by the USDA (35) led to a 1.4-log reduction of Salmonella Senftenberg 775 W (22) but was insufficient to eliminate L. monocytogenes strains (Scott A, PMM383, ATCC 19111, and V7) in egg white (20, 21). We are currently investigating the effect of this new TDT disk device on inactivation kinetics of Salmonella Senftenberg 775 W and L. monocytogenes strains (Scott A, PMM383, ATCC 19111, and V7) in egg white.

It is our conclusion that thermal treatment with TDT disks induced changes in the bacterial membrane. These changes led to injury or damage, which caused leakage of intracellular ATP, protein, or nucleic acids, or a combination of these substances, from E. coli and Salmonella, reducing the energy status and the enzymatic activity and leading to the death of the bacterial cells. Earlier pilot plant study on nonpathogenic surrogate E. coli strains were favorably compared with E. coli O157:H7 and Salmonella, and the authors concluded that E. coli K-12 can be used as a surrogate for Salmonella Enteritidis (12). Similarly, the results of this study showed a higher percentage of injury and leakage of intracellular bacterial substances in E. coli K-12 than Salmonella Enteritidis in PLEW and LWE, respectively. However, data from Jin et al. (16) on death kinetics for E. coli K-12 and Salmonella Enteritidis in PLEW and LWE appear to support the use of E. coli K-12 cells as a surrogate for Salmonella Enteritidis (12).

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REFERENCES


