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Research note

Thermal resistance of *Salmonella enteritidis* and *Escherichia coli* K12 in liquid egg determined by thermal-death-time disks ☆

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Abstract

A thermal-death-time (TDT) disk was designed to evaluate microbiological inactivation kinetics by heat. A first order inactivation kinetic model is described by the D value and the z value. These kinetic data are critical in the design, operation and regulation of thermal pasteurization. D and z values of *Salmonella enteritidis* strain 13076 and *Escherichia coli* K12 in liquid whole egg and liquid egg white were determined over the temperature range from 52 to 60 °C. In liquid whole egg, D_{54}, D_{56}, D_{58} and D_{60} values of *S. enteritidis* strain 13076 were 5.70, 0.82, 0.27 and 0.17 min, respectively, and D_{54}, D_{56}, D_{58} and D_{60} values of *E. coli* K12 were 9.10, 1.41, 0.67 and 0.22 min, respectively. In liquid egg white, D_{52}, D_{54}, D_{56} and D_{58} values of *S. enteritidis* strain 13076 were 6.12, 1.51, 0.42 and 0.19 min, respectively, and D_{52}, D_{54}, D_{56} and D_{58} values of *E. coli* K12 were 10.18, 1.82, 0.78 and 0.28 min, respectively. The z values for *S. enteritidis* strain 13076 and *E. coli* K12 ranged from 3.95 to 4.03 °C. The results showed that D values of *S. enteritidis* strain 13076 and *E. coli* K12 in liquid whole egg were higher than those in liquid egg white. *E. coli* K12 exhibited similar kinetic behavior, but higher thermal resistance than *S. enteritidis* strain 13076 in both liquid egg white and liquid whole egg. This study demonstrated that non-pathogenic *E. coli* K12 may serve as a surrogate for pathogenic *S. enteritidis* in liquid egg in the validation of a thermal pasteurization.

Keywords: Kinetics; TDT disk; Surrogate; Liquid egg; *E. coli*; *Salmonella*

1. Introduction

Microbial contamination of eggs is a well-known problem that has important economic implications for the poultry industry (Bruce & Drysdal, 1994; Wong & Kitts, 2003). The incidence of *Salmonella* infections caused by one serotype, *Salmonella enteritidis*, has increased steadily from 1976 to 1990 (Shah, Bradshaw, & Peeler, 1991). Salmonellosis is a leading cause of foodborne illness in the United States, resulting in an estimated 1.4 million infections, with more than 16,000 hospitalizations and nearly 600 deaths each year (CDC, 2001). In particular, the risk of illness increases when egg is used as an ingredient in prepared meals for the general public (Todd, 2001). As a result, the US Department of Agriculture (USDA) regulations mandate that commercial egg products must be subjected to pasteurization processes to reduce pathogens to a reasonably acceptable level.

According to the pasteurization standards for egg products by USDA, liquid egg white must be heated at 56.6 °C and liquid whole egg must be heated at 60 °C for minimum 3.5 min (USDA, 1969). The heat processes are designed to produce a salmonellae-free product and prolongs the shelf-life of the egg. Because the quality attributes of liquid egg product are very heat sensitive, it is therefore important

☆ Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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to use the minimum heat treatment that will still provide adequate safety in the final product.

Accurate determination of thermal resistance of microorganisms in liquid egg is important for designing thermal processes to ensure food safety and shelf-life. Pflug (2003) has reported procedures and methods that can be used to minimize the effect of potential test-system errors on microbial resistance data, and also listed heating and cooling lag-correction values for several commonly-used testing systems including glass TDT tubes and aluminium TDT tubes. Pflug (2003) showed that glass TDT tubes have longer temperature-response times, equivalent-times, and heating times for test units to reach 0.1 °F below test temperature than aluminium TDT tubes when 1 ml of water is heated in steam or oil to 121.1 °C. Therefore a new design of TDT device based on an aluminium TDT tube should be interesting to researchers in this area.

When using a surrogate microorganism for process validation, its thermal tolerance must be equivalent to or higher than the targeted pathogen. Its use, as opposed to using an actual pathogen, derives from the need to prevent the introduction of harmful organisms into the production facility. The safety and liability resulting from mishandling a pathogen to worker safety, food safety and safety of the processing environment could be devastating. Therefore, the use of surrogates by processing companies is of great value to validate a thermal process (FDA, 2000).

Escherichia coli K12 has well-defined characteristics, is nonpathogenic, and has been used extensively and could possibly serve as a surrogate for *S. enteritidis*. The objective of this study was to determine the thermal resistance of *E. coli* K12 and *S. enteritidis* strain 13076 in liquid egg white and liquid whole egg using a new type of aluminium TDT disk and to evaluate the suitability of *E. coli* K12 as a surrogate for *S. enteritidis* for liquid egg pasteurization. This study did not attempt to compare different TDT devices currently used by other researchers with the new type of aluminium TDT disk in thermal inactivation of *Salmonella*.

2. Materials and methods

2.1. Preparation of liquid egg samples

Commercial pasteurized liquid egg white without preservatives and fresh Grade A shell eggs were purchased from a local grocery store. Liquid whole egg was made in our lab by sterilizing the surface of eggs with 70% alcohol, breaking shells by hand into a stomacher bag and stomaching at 250 rpm for 30 s. The pH of liquid egg white was 8.20. The pH of liquid whole egg ranged from 7.50 to 7.65. Duplicate 1 ml liquid egg white samples or fresh prepared liquid whole egg samples were plated on tryptic soy agar (TSA: Difco, Detroit, MI) and incubated at 37 °C for 24 h for each experiment to determine the level of background microorganisms.

2.2. Thermal inactivation apparatus

A new aluminium TDT disk was developed at Washington State University (Pullman, WA) to allow rapid heating of samples in water or oil baths and provide close to ideal isothermal conditions in studies of thermal death kinetics of microorganisms. Fig. 1 shows the schematic diagram of the TDT disk. The aluminium test disk consisted of two parts: a base and a screwed-on cap to allow easy loading and unloading of the sample. A rubber o-ring between the two parts provided a hermetic seal. The disk-shaped cavity is 18 mm in diameter and 4.5 mm height, providing a sample space of 1.27 ml. A pre-calibrated type K thermocouple was installed in the center of the TDT disk. The inside center temperature and outside temperature of the TDT disk (water bath temperature) were monitored and recorded by a Fluke 54 II thermometer (Everett, WA) every second for all experiments. The time/temperature history was measured for each run using a non-inoculated sample, and downloaded to a computer. To record a temperature–time profile of a glass tube, 1 ml liquid egg sample was added into a glass tube (9 mm diameter by 125 mm high) and a thermocouple was placed and fixed in the center of liquid sample. The glass tube was then submerged in a water bath and temperature–time profiles were recorded.

Before each use, the TDT disks were sanitized using the following procedure: (1) washed with water, (2) soaked in a sanitizer (Coverage Plus, E.R. Squibb & Sons, Inc., St. Louis, MO) for 2 min according to the manufacturer's instructions, and (3) rinsed five times with sterile water, then air dried at room temperature under laminar flow.

2.3. Inoculation of liquid egg and heat treatment

E. coli ATCC 23716 (K12) and *S. enteritidis* ATCC 13076 were obtained from the culture collection of the US Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center. Cultures were

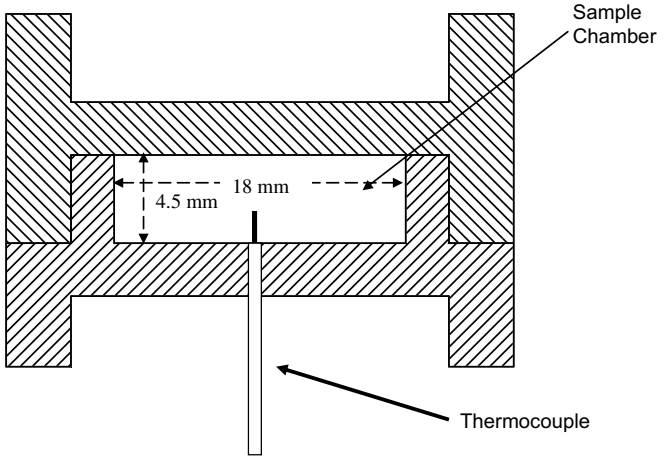

Fig. 1. Schematic diagram of aluminium TDT disk. Two halves are screwed together with an o-ring to provide a hermetic seal.

maintained on TSA at 4 °C. Prior to inoculation of product the organism was cultured in an Erlenmeyer flask with 250 ml tryptic soy broth (TSB: Remel, Inc., Lenexa, KS) with shaking at 37 °C for 16–18 h.

For thermal inactivation experiments, 50 ml of liquid egg white or liquid whole egg was thoroughly mixed with 1 ml of inocula containing approximately 1×10^9 cells of *E .coli* or *S. enteritidis* suspension/ml. One ml of inoculated egg sample was placed inside each TDT disk. Hermetically sealed TDT disks were then submerged completely in a water bath (Isotemp 110, Fisher Scientific, Pittsburgh, PA) at zero time. The temperature of the water bath was controlled at 52, 54, 56 or 58 ± 0.1 °C for liquid egg white, and 54, 56 or 58, or 60 ± 0.1 °C for liquid whole egg. At timed intervals of 0–4 min, duplicate TDT disks were removed from the water bath and immediately immersed in an ice-water bath. The sample from a TDT disk that was not exposed to heat served as an untreated control. An un-inoculated and unheated liquid egg sample was also enumerated to check for presence of background microflora.

2.4. Microbiological enumeration

A 0.1 ml sample was taken from a TDT disk and serially diluted in sterile Butterfield's phosphate buffer (pH 7.0, Hardy Diagnostics, Santa Maria, CA) with a minimum 1 ml transfer. Duplicate samples were then pour plated with TSA and the plates incubated at 37 °C for 24 h. Plates with 30–300 colonies were enumerated using a manual colony counter (Bantex, Burlingame, CA, Bantex Colony Counter 920).

2.5. Data analysis

The log numbers of the survivors at each time were used to determine *D* values. The SAS regression procedure (SAS software v.9, SAS Institute, Cary, NC) was used to describe the relationships between log survivors and thermal treatment times. *D* values were calculated from the reciprocal of the slopes of linear survivor curves. A linear regression was computed from log *D* values versus temperature, and an estimate of the *z* value was obtained from the absolute value of the inverse of the slope. All treatment conditions were performed in triplicate.

3. Results and discussion

Fig. 2 shows a typical come-up time of liquid egg in a TDT disk in water bath set at 60 °C. The come-up time was *ca.* 45 s for all the tested temperatures, which is 25 s shorter than that of the glass tube method. This could be explained by the design of the new TDT disk having a higher ratio of heated surface area to sample weight than that of a glass tube. In addition, the thermal conductivity properties of aluminium allow more rapid heating. For example, Al-Holy, Quinde, Guan, Tang, and Rasco (2004) used aluminium tubes for salmon caviar and found that the come-up times were significantly shorter in the aluminium TDT tubes (82.7 s) than in the glass tubes (181.7 s) at 60 °C. Short come-up time could reduce thermal lag time and increase accuracy for determining the heat resistance of *Listeria monocytogenes* (Donnelly, Briggs, & Donnelly, 1987).

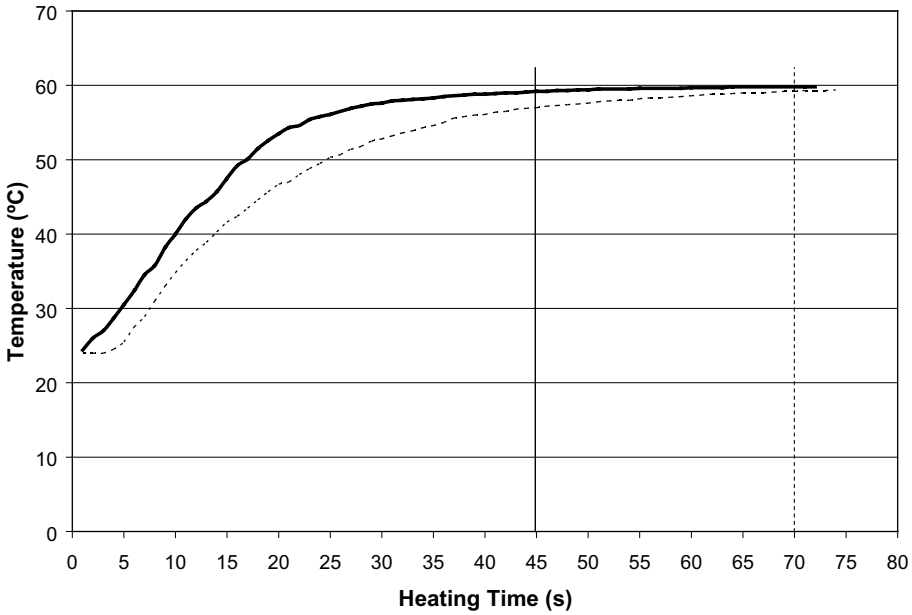

Fig. 2. A typical come-up time of liquid egg sample in a water bath at 60 °C. Solid line: Sample in a TDT disk; Dotted line: Sample in a glass tube (1 ml in 10 ml tube). The vertical lines represent the end of come-up time. The come-up time was determined when the center temperature of sample reached to 59.4 °C (99% of target temperature).

Figs. 3 and 4 illustrate survivor curves of *E. coli* and *S. enteritidis* in liquid whole egg and liquid egg white, respectively. Excluding the initial microbial counts, these survivor curves demonstrate a linear decline in the log number of surviving bacteria as a function of heating time. Some researchers reported that thermal destruction curves for *salmonellae* exhibit significant tailing due to the presence of two populations of cells, one more heat sensitive than the other (Humpheson, Adams, Anderson, & Cole, 1998; Moats, Dabbah, & Edwards, 1971; Peleg & Cole, 1998). Similar phenomena were also observed in this study when background microorganisms existed before the inoculation of *E. coli* or *Salmonella* (data not shown). Proper sanitization of the TDT disks also ensured that the observed survivor curves reveal the true heat resistant characteristics of the bacteria in consideration.

Based on the linear portion of these survivor curves, D values were calculated and z values were obtained. Table 1 summarizes the D values and z values for *E. coli* and *S. enteritidis* in liquid whole egg or liquid egg white.

The D_{54}, D_{56}, D_{58} values of *S. enteritidis* in liquid whole egg were 5.70, 0.82, 0.27 min, respectively, and D_{54}, D_{56}, D_{58} values of *S. enteritidis* in liquid egg white were 1.51, 0.42, 0.19 min. It appears that the thermal resistance of *Salmonella* in liquid whole egg was higher than that in liquid egg white. This is probably due to the products' different pH, water activity, and nature of constituents (Michalski, Brackett, Hung, & Ezeike, 1999). For instance, *S. enteritidis* may not be able to tolerate the high pH found in egg white as opposed to the neutral pH of whole egg (Palumbo, Beers, Bhaduri, & Palumbo, 1996), and *S. ente-*

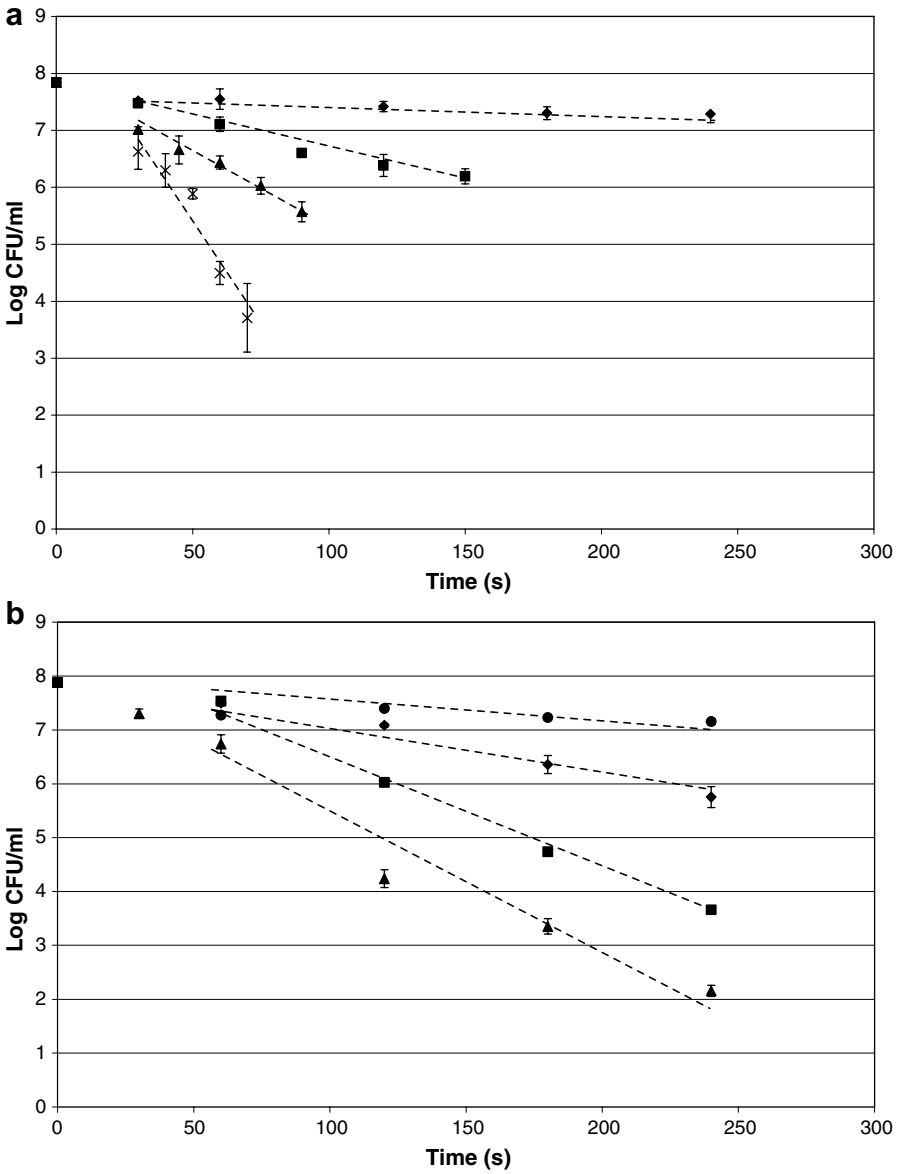

Fig. 3. Survivor curve of *E .coli* K12 in liquid whole egg (a) and liquid egg white (b). Error bars indicate standard deviation. Data points were from the average of four measurements ($n = 4$). ●: 52 °C; ♦: 54 °C; ■: 56 °C; ▲: 58 °C; ×: 60 °C. Test temperatures for liquid whole egg were from 54°C to 60°C; test temperatures for liquid egg white were from 52 °C to 58 °C.

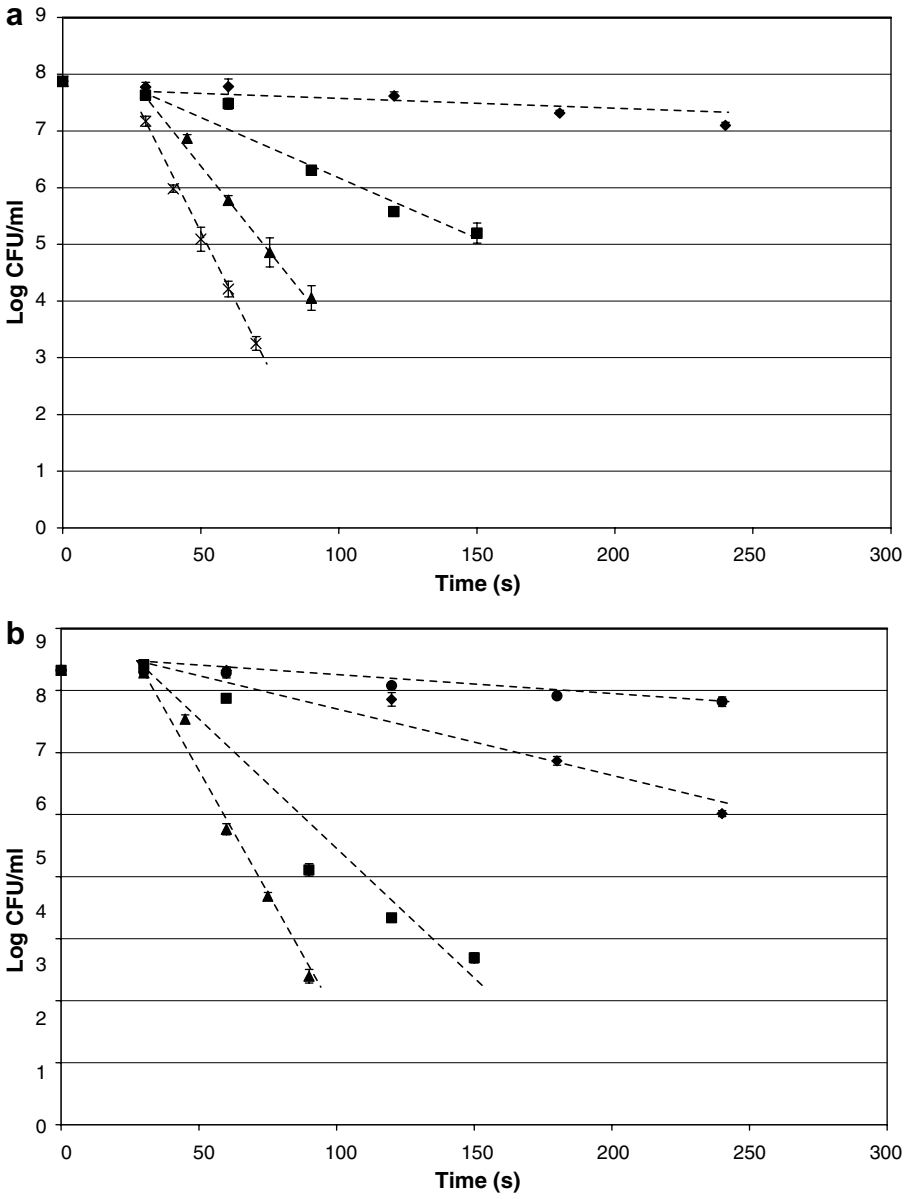

Fig. 4. Survivor curve of *S. enteritidis* in liquid whole egg (a) and liquid egg white (b). Error bars indicate standard deviation. Data points were from the average of four measurements ($n = 4$). ●: 52 °C; ◆: 54 °C; ■: 56 °C; ▲: 58 °C; ×: 60 °C. Test temperatures for liquid whole egg were from 54°C to 60°C; test temperatures for liquid egg white were from 52 °C to 58 °C.

Table 1
D values and *z* values of *E. coli* K12 and *S. enteritidis* 13076 in liquid egg white and liquid whole egg

Medium	Pathogens	*D* values (min)[a]					*z* values (°C)
		52 °C	54 °C	56 °C	58 °C	60 °C	
LWE[b]	*E.coli* K12		9.10 (5.26)	1.41 (0.14)	0.67 (0.065)	0.22 (0.001)	3.95
	S. enteritidis		5.70 (3.15)	0.82 (0.37)	0.27 (0.02)	0.17 (0.003)	4.08
LEW[c]	*E.coli* K12	10.18 (1.30)	1.82 (0.73)	0.78 (0.01)	0.28 (0.004)		3.98
	S. enteritidis	6.12 (2.13)	1.51 (0.17)	0.42 (0.07)	0.19 (0.02)		4.03

[a] Values in parentheses are standard deviations.
[b] Liquid whole egg.
[c] Liquid egg white.

ritidis can utilize the nutritious proteins and lipids found in egg yolk to help stabilize the bacterial cell membrane against heat inactivation (Muriana, Hou, & Singh, 1996). Our observation is also in agreement with other publications (Jung & Beuchat, 2000; Osborne, Straka, & Linewe-aver, 1954), and is consistent with the regulatory

requirement of higher pasteurization temperature for liquid whole egg than that for liquid egg white.

The D_{60} value of *S. enteritidis* in liquid whole egg was 0.17 min. The z value of *S. enteritidis* in liquid whole egg was 4.08 °C. These values were similar to previous data summarized by Doyle and Mazzotta (2000) on different serotypes of *Salmonella* in liquid whole egg. Breeuwer, Lardeau, Peterz, and Joosten (2003) also found that D_{60} of *S. enteritidis* in phosphate buffer was 0.2 min and z value was 4.3 °C.

As summarized in Table 1, *E. coli* K12 exhibited a slightly higher thermal resistance at each tested temperature than *S. enteritidis* in liquid whole egg or liquid egg white. A similar phenomenon was also observed by other researchers. From tests in pH 7 phosphate buffer, Breeuwer et al. (2003) found that *E. coli* 234 had a higher D_{60} than two strains of *S. enteritidis* (*S. enteritidis* 331 and 495). Eblen, Annous, and Sapers (2005) also revealed that both pathogenic and non-pathogenic *E. coli* strains were more thermally resistant than two strains of *Salmonella* (*Salmonella* Montevideo G4639 and *Salmonella* Poona RM 2350).

US regulations require that liquid egg white must be heated at 56.6 °C and liquid whole egg must be heated at 60 °C for minimum 3.5 min (USDA, 1969). Liquid whole egg and liquid egg white pasteurized in holding tube with an average residence time of 3.5 min would have true residence times of 2.83 min and 2.65 min, respectively, based on the fastest particle (Scalzo, Dickerson, Read, & Parker, 1969). Using these pasteurization conditions and D_{60} values from this study, the liquid whole egg pasteurization at 60 °C would result in a 16.6 D process for *S. enteritidis* and 12.8 D process for *E. coli* K12. In the case of liquid egg white, $D_{56.6}$ is calculated as 0.37 min for *S. enteritidis* and 0.75 min for *E. coli* K12. The pasteurization process (a true residence time of 2.65 min at 56.6 °C) would obtain a reduction of 7.2 log cycles of *S. enteritidis* and 3.6 log cycles of *E. coli* K12. Therefore, a process design based on *E. coli* K12 would generate a satisfactory margin of safety for liquid egg pasteurization when *S. enteritidis* is concerned, based on data for the strains used in this study.

The heat resistance of *Salmonella* is highly influenced by the strain tested, the type of experiment method used, culture conditions prior to the experiment, heating menstruum, and recovery conditions (Doyle & Mazzotta, 2000; Pflug, 2003). Breeuwer et al. (2003) reported that *S.* Senftenberg had a higher D_{60} value (2.4 min) with a higher z value (5.7 °C) than *S. enteritidis*. Using a flow-injection system, Muriana et al. (1996) found that *S. enteritidis* grown in egg yolk medium before inoculation into liquid whole egg had higher D values than those grown in tryptic soy broth. When a five-strain cocktail of *S. enteritidis* was inoculated into liquid whole egg (pH 7.36) and a capillary tube method was used, D_{58} value of 1.50 min was observed by Michalski et al. (1999). Schuman and Sheldon (1997) obtained $D_{58.3}$ value of 1.00 min for a five-strain cocktail of *Salmonella* in liquid egg white (pH 8.2). Palumbo et al. (1996) indicated that a six-strain mixture of *S. enteritidis*, *S. typhimurium* and *S. senftenberg* in liquid egg white (pH 8.8) had a $D_{57.7}$ value of 0.78 min as determined by a 9 ml glass vial method. It is difficult to compare data from different experiment, because of differences in the serotypes of *Salmonella* used, in protocols for testing, in growth media, in procedures for enumerating survivors, etc. Therefore, a side by side comparison of *Salmonella* with *E. coli* within the same experiment would help to determine their similarity in heat resistance.

This study was a first attempt to match the thermal resistance of a nonpathogenic surrogate organism *E. coli* K12 to pathogenic *S. enteritidis* side by side using a newly designed TDT device. The results of the present study revealed that *E. coli* K12 exhibited similar kinetic behavior, but higher thermal resistance than *S. enteritidis* strain 13076 in both liquid egg white and liquid whole egg. Thus, *E. coli* K12 should be an appropriate surrogate for use in evaluating the efficacy of thermal pasteurization for reducing and/or eliminating *S. enteritidis* in liquid egg in a pilot plant environment. However, there was only one strain of *S. enteritidis* tested in this study. Another strain or a cocktail of *S. enteritidis*, or other serotype of *Salmonella* may possess higher thermal resistance than *S. enteritidis* strain 13076. Therefore, research with additional strains of *Salmonella* is recommended to identify an effective all-purpose surrogate organism for *Salmonella* spp.

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