Food Control 94 (2018) 1–6

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Heat resistance of *Salmonella* Enteritidis in four different liquid egg products and the performance and equivalent conditions of Ministry of Food and Drug Safety of South Korea and US Department of Agriculture protocols

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ARTICLE INFO

Keywords:
Liquid egg
Salmonella Enteritidis
Heat
D-value
Capillary tube

ABSTRACT

Heat treatment is one of the most effective methods for eliminating undesirable microorganisms from foods. *Salmonella* Enteritidis has long been known to be strongly associated with liquid egg products. To investigate the influence of the liquid egg matrix on the heat tolerance of residual bacteria, we evaluated the thermal inactivation D-values of *S.* Enteritidis in four different liquid egg products (egg white, whole egg, egg yolk, and egg yolk plus 10% NaCl). Additionally, the performance and science-based equivalent conditions of the Ministry of Food and Drug Safety (MFDS) and US Department of Agriculture (USDA) protocols for liquid egg products were investigated using calculated D-values. Liquid egg products inoculated with *S.* Enteritidis were heated in capillary tubes to temperatures ranging from 51 to 59 °C and then spread onto tryptic soy agar plates, followed by incubation at 37 °C for 24 h. *S.* Enteritidis showed the lowest D-values in egg whites, followed by whole eggs and egg yolks. The addition of NaCl to egg yolks increased the heat resistance of *S.* Enteritidis. MFDS protocols for egg whites and whole eggs showed higher performance, whereas MFDS protocols for egg yolks and egg yolks plus 10% NaCl showed lower performance than USDA protocols. Overall, our findings may facilitate greater flexibility in egg pasteurization.

1. Introduction

Liquid egg is included as an ingredient in hundreds of food products (Goulter, Dykes, & Small, 2008; Gurtler, Marks, Jones, Bailey, & Bauer, 2011). Liquid egg products pass through various processing steps, including egg washing, egg opening, classification, and heat treatment (Froning et al., 2002; Michalski, Brackett, Hung, & Ezeike, 1999). Liquid egg products are largely categorized as whole eggs, egg whites, and egg yolks, and these liquid egg types retain different food matrix components, such as pH, solids, viscosity, and nutrient compositions (Gurtler, Marks, Bailey, Juneja, & Jones, 2013; Schuman & Sheldon, 1997). For example, egg whites are generally alkaline, with a pH of 8–10, whereas whole eggs and egg yolks are mild acidic or neutral, with a pH of 6–7 (Froning et al., 2002). Such food matrix differences in liquid egg products influence the survival of foodborne bacteria during food processing (Acosta, Usaga, Churey, Worobo, & Padilla-Zakour, 2017; Froning et al., 2002; Gurtler et al., 2013; Gutler et al., 2011).

Salmonella spp. are a leading cause of foodborne diseases worldwide, causing various illnesses, such as typhoid fever, gastroenteritis, and septicemia (Favier, Escudero, De Guzmen, & Stefanini, 2008; Gurtler & Jin, 2012; Humphrey, Chapman, Rowe, & Gilbert, 1990; Schuman & Sheldon, 1997). In particular, *Salmonella* Enteritidis has long been known to be strongly associated with egg or liquid egg products (Jin, Zhang, Boyd, & Tang, 2008; Michalski et al., 1999; Peña-Meléndez, Perry, & Yousef, 2014). *S.* Enteritidis has been used as an indicator bacterium to evaluate the performance of pasteurization protocols for liquid egg products (Favier et al., 2008; Froning et al., 2002). Thus, the aim of egg pasteurization is now to reduce the target amount of *S.* Enteritidis, rather than to comply with specific temperature/time combinations (Froning et al., 2002).

Heat treatment is a major pasteurization method used to eliminate foodborne pathogens in liquid egg products (Gurtler et al., 2011; Manas et al., 2001). However, high temperature treatment can decrease albumen (egg white) quality by increasing viscosity, aggregation, and coagulation because major albumen proteins (AP) are sensitive to heat (Froning et al., 2002; Robertson & Muriana, 2004). Ovalbumin (54% of AP), ovotransferrin (12% of AP), and ovomucoid (11% of AP) have low denaturation temperatures of 84, 61, and 79 °C, respectively (Gurtler & Jin, 2012). Thus, liquid egg pasteurization is conducted at a lower temperature than other pasteurized foods, requiring specific attention

Abbreviations: PBS, phosphate-buffered saline; MFDS, Ministry of Food and Drug Safety; USDA, US Department of Agriculture

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https://doi.org/10.1016/j.foodcont.2018.06.024
Received 23 February 2018; Received in revised form 12 June 2018; Accepted 13 June 2018
Available online 14 June 2018
0956-7135/

to prevent the growth and persistence of undesirable microorganisms in the final egg product (Froning et al., 2002; Michalski et al., 1999). For these reasons, food-associated government agencies, such as the Ministry of Food and Drug Safety (MFDS) of South Korea and US Department of Agriculture (USDA), have established low-temperature pasteurization protocols detailing the minimum temperatures and times (Froning et al., 2002; MFDS, 2016). In the USDA protocols, egg whites must be heated at 55.6 °C for 372 s or 56.7 °C for 210 s, whole eggs must be heated at 60 °C for 210 s, and egg yolks must be heated at 60 °C for 372 s or 61.1 °C for 210 s (Froning et al., 2002; Gurtler et al., 2011).

Current liquid egg pasteurization guidelines allow not only prescribed methods, but also science-based equivalent methods, which show equal performance under heating conditions for reducing *S.* Enteritidis (Froning et al., 2002; MFDS, 2016). However, specifications or related studies for equivalent methods are limited, despite the high demand for liquid egg products. In addition, some problems also occur when liquid egg products are traded with other countries in which different pasteurization methods are used (Froning et al., 2002; MFDS 2016; Michalski et al., 1999). For example, MFDS protocols for whole eggs include higher temperature criteria (64 °C), but lower time criteria (150 s) than USDA protocols (60 °C for 210 s) (Froning et al., 2002; MFDS, 2016). Evaluating the performance of such protocols for reducing *S.* Enteritidis should apply appropriate pasteurization methods; however, such quantitative evaluation methods have not yet been established. D- and Z-values are used to evaluate the effects of heat treatment on reducing microorganisms (McCormick, Han, Acton, Sheldon, & Dawson, 2003). The D-value (or decimal reduction time) is defined as the time required for a 1 log or one decimal reduction in the number of microorganisms, and the Z-value refers to the degrees Celsius required to reduce D by a factor of 10 (Silva & Gibbs, 2012).

In the current study, we investigated the influence of the food matrix on heat tolerance of residual bacteria by estimating the thermal inactivation D- and Z-values of *S.* Enteritidis in four different liquid egg products (egg whites, whole eggs, egg yolks, and egg yolks plus 10% NaCl). Additionally, the performance and science-based equivalent conditions of MFDS and USDA protocols for the four liquid egg products were investigated using calculated D- and Z-values.

2. Materials and methods

2.1. Culture and culture media

One strain of *S.* Enteritidis was used in the current study. The strain was maintained on tryptic soy agar (TSA; Difco, Detroit, MI, USA) at 4 °C and subcultured once a month until used in the experiment. Single colonies on TSA were grown in 10 mL tryptic soy broth (TSB; Difco) at 37 °C for 24 h, and 50 μL of culture was then subcultured in 10 mL TSB at 37 °C for 24 h. Next, 1 mL of culture was centrifuged at 14,000 rpm for 3 min and washed with 1 mL phosphate-buffered saline (PBS). The washed cultures were added to 9 mL liquid egg products (whole eggs, egg whites, egg yolks, and egg yolks plus 10% NaCl) resulting in 10^8–10^9 CFU/mL in the unheated product. Commercial pasteurized whole eggs, egg whites, and egg yolks were purchased from a local grocery store. Samples of egg yolks plus 10% salt were prepared by adding 10% NaCl to egg yolks. The pH, solids (%), and viscosity of the four liquid egg products are indicated in Table 1. The inoculated egg products were kept on ice water for a maximum of 1 h before use.

2.2. Thermal inactivation of S. Enteritidis in capillary tubes

Fifty microliters of inoculated egg products were injected into 1.1-mm diameter capillary tubes (Kimble Chase, Vineland, NJ, USA) using a micropipette, and the capillary tubes were sealed using Cha-seal tube wax sealing compound (Kimble Chase), which is typically used to seal hematocrit capillary blood collection tubes before centrifugation. The capillary tubes were submerged into a water bath set to 51, 53, 55, 57, or 59 °C. Liquid whole eggs, egg yolks, and egg yolks plus 10% salt were heated in capillary tubes at 53 °C for 90, 180, 270, or 360 s; 55 °C for 60, 120, 180, or 240 s; 57 °C for 30, 60, 90, or 120 s; or 59 °C for 15, 30, 45, or 60 s. Liquid egg whites were heated in capillary tubes at 51 °C for 90, 120, 180, or 270 s; 53 °C for 90, 120, 180, or 270 s; 55 °C for 45, 90, 135, or 180 s; or 57 °C for 20, 40, 60, or 80 s. Each 50 μL of unheated cell suspension was serially diluted in PBS and plated in duplicate on TSA to confirm initial populations. At the end of each heating time, capillary tubes were immediately immersed in an ice water bath (maximum 1 h) until microbiological analysis. The ends of the capillary tubes were crushed using a capillary tube cutter to obtain heated cell suspensions with a micropipette. The population of surviving microorganisms was evaluated by dilution of heated cell suspensions in 450 μL PBS, and the serial dilutions were spread onto TSA plates in duplicate. Plates were incubated for 48 h at 37 °C. All treatment conditions were performed in 10 times.

Table 1
pH, solids (%), and viscosity of four liquid egg products.

Egg quality	Egg products			
	Egg white	Whole egg	Egg yolk	Egg yolk + 10% NaCl
pH	9.02	7.36	6.48	6.48
Solid (%)	27.78	14.97	41.60	41.60
Viscosity (mPa.S)	12.31	7.96	74.78	74.78

2.3. Data analysis (D-values, Z-values, and log reductions)

Survival curves (the population of surviving microorganisms [log cfu] versus heating time [s]) were plotted for each heating temperature, and the linear regression equation was obtained for each case using Microsoft Excel XP software (Microsoft Corporation, Redmond, WA, USA). D-values were calculated as the negative reciprocal of the slope of the thermal inactivation curves. The correlation coefficient (r^2) was 0.92 ± 0.04 for all *S.* Enteritidis trials, indicating that the inactivation curves were linear and that D-values could be calculated directly from them. The log D values versus heating temperatures were also plotted for each liquid egg product using the same software, and Z-values (°C) were calculated as the negative reciprocal of the slope of the linear regression curve. Based on calculated Z-value, the performance of the pasteurization protocol was evaluated.

3. Results

3.1. D-values and Z-values of S. Enteritidis in four liquid egg products

Fig. 1 illustrates thermal inactivation curve of *S.* Enteritidis in four liquid egg products. The survival curves demonstrated linear declines in the log number of surviving *S.* Enteritidis as a function of heating time. As expected, the population of *S.* Enteritidis decreased with increasing temperature in all liquid egg products. The D-values were calculated from the thermal inactivation curve for *S.* Enteritidis in the four different liquid egg products at various temperatures, as shown in Table 2. The results across the entire temperature range showed the lowest D-values in egg whites, followed by whole eggs and egg yolks (Table 2). The $D_{51°C}$, $D_{53°C}$, $D_{55°C}$, and $D_{57°C}$ values of *S.* Enteritidis in liquid egg whites were 151.52, 66.23, 36.63, and 16.75 s, respectively. In particular, *S.* Enteritidis in egg yolks plus 10% NaCl ($D_{53°C}$ = 370.37 s; $D_{55°C}$ = 204.08 s; $D_{57°C}$ = 94.34 s; $D_{59°C}$ = 54.05 s) showed higher D-values than in egg yolks ($D_{53°C}$ = 312.50 s; $D_{55°C}$ = 136.99 s; $D_{57°C}$ = 62.50 s; $D_{59°C}$ = 32.57 s). The log D-value (s) versus temperature (°C) curves of *S.* Enteritidis in four liquid egg products are shown in Fig. 1E. The Z-values in egg whites, whole eggs, egg yolks, and egg yolks plus 10% salt were 6.40, 6.08, 6.09, and 6.29 °C, respectively (Table 2).