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Heat Treatment of *Listeria monocytogenes* in Liquid Egg Products with Low Temperature

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ABSTRACT

This study was done in order to express a method for thermal destruction of *Listeria monocytogenes* as a psychrotrophic bacterium with ability to grow at refrigerator temperatures in liquid egg products including egg white, egg yolk and homogenized liquid whole egg at lower temperature and longer than normal pasteurization to decrease the damages upon nutrient compounds of egg. Thermal destruction was done in the temperatures of 53, 55 and 57.5°C. Application of thermal process was done by glass tube method. In present study, D-value and Z-value of *L. monocytogenes* in egg white, egg yolk and homogenized liquid whole egg at 53, 55 and 57.5°C was determined. Thermal resistance was highest in egg yolk. The lowest thermal resistance was observed in egg white and homogenized liquid whole egg had a medium thermal resistance. For *L. monocytogenes*, D-values ranged from 6.69 min in egg white at 57°C to 24.99 min in egg yolk at 53°C. This study indicated that the temperatures of 53, 55 and 57.5°C, respectively can be used to remove *L. monocytogenes* to maintain the egg quality.

Key words: Thermal resistance, *Listeria monocytogenes*, egg

INTRODUCTION

Listeria monocytogenes is an important foodborne pathogen that causes listeriosis in humans and animals. Foodborne listeriosis often associated with severe illness mainly in unborn children, infants and the elderly, as well as in immunocompromised persons. In the recent 25 years, food has been an important factor in the transmission of human listeriosis in developed countries (Tompkin, 2002). *L. monocytogenes* leads to meningitis or meningo-encephalitis, bacterimia, septicaemia and serious bacterial infection in human (De Valk *et al*., 2005). Milk and milk products, meat and meat products, plant products and fish and fish products can considered as a source of *Listeria* spp., (Rivoal *et al*., 2010). In epidemiological studies, egg and egg products have never been caused listeriosis but is most frequently isolated from egg shells and in the environment of laying hens (Gray, 1958). Chemaly *et al*. (2008) sampled from dust and faeces of 200 laying hen farms and showed that 15% of samples were contaminated with *Listeria* and he reported which bacterium is resistant under storage and handling conditions of shell eggs (Chemaly *et al*., 2008). *L. monocytogenes* is a psychrotrophic bacterium with the ability to grow at low temperatures. Slow growth of some *L. monocytogenes* strains was observed at a temperature of 1.5-0.1°C and may tolerate high salt concentrations (23.8% NaCl) (Larson *et al*., 1999). This organism can survive 90 and >14 days on egg stored at 5 and 10°C, respectively, persist on inoculated eggs treated with

sodium hypochlorite containing 100 ppm available chlorine (Gandhi and Chikindas, 2007; Bartlett, 1993). It can grow in infected egg which stored at refrigeration and ambient temperatures (Gandhi and Chikindas, 2007). Eggs contain nutrients that form a suitable substrate for the growth and multiplication of microbes (St. Louis *et al*., 1988). The best method for controlling these pathogens in egg products (liquid whole egg, liquid egg white and liquid egg yolk) is use of heat of pasteurization (Tompkin, 2002). For the standard pasteurization of egg products suggested by USDA, it is necessary 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). The heat resistance of *L. monocytogenes* depends on the age, pH, growth culture, salt, acid content, water activity and the presence of potential inhibitors (Palumbo *et al*., 1995; Palumbo *et al*., 1996). Usually the D-value (decimal reduction time or time required to inactivate 90% of the population), characteristic to the heat sensitivity of the bacterium (Doyle *et al*., 2001). Another factor is Z-value (negative reciprocal of the slope of the regression line between decimal logarithms of D-values), that is important in heat treatment (Murphy *et al*., 2002). To determine the heat resistance of bacteria, various procedure are used such as thermal-death-time disks (Jin *et al*., 2008), sealed tubes, flow heat exchanger (Mackey *et al*., 1990), glass TDT tubes (Pflug, 2003) and aluminium TDT disk (Jin *et al*., 2008). In the present study, glass TDT tubes method for heat injury at 53, 55 and 57.5°C were used. Our purpose is to develop a model for a deletion *L. monocytogenes* in egg products at 53, 55 and 57.5°C, respectively.

MATERIALS AND METHODS

Collection of samples: Samples (fresh eggs) were collected from a Shahrekord egg processing plant 24 h before the experiments and were stored in a refrigerator until testing. The eggshells washed with 70% ethanol and allowed to air dry (Monfort *et al*., 2012). The eggs were broken in the aseptic laboratory condition and egg products were separated in three samples, containing homogenized liquid whole egg, egg yolk and egg white and transferred to a sterile glass bag. These samples were cultured on tryptic soy agar as enrichment media for 24 h at 37°C and then on palcam agar as selective media to examine *Listeria* contamination. As heat resistance of *L. monocytogenes* can be variable with pH of food matrix (Juneja and Eblen, 1999), pH of samples were detected.

Microbial strain: We prepared inoculum with concentration of approximately 5×10^8 CFU mL^{-1} of 24 h fresh culture of the *L. monocytogenes* ATCC 19114 grown on tryptic soy broth media at 37°C for 24 h. One mL of this media (TSB containing bacteria) or 5×10^8 CFU of *L. monocytogenes* was inoculated into 100 mL of each liquid egg samples (5×10^6 CFU mL^{-1} of egg samples).

Heat process: Infected samples were incubated in water bath (model: LWB30T) at 53°C and sampling was done at different times (every 5 min). For reisolation of bacteria, serial dilution and culture on Tryptic Soy Agar with 0.6% Yeast Extract (TSAYE) media with surface plating method was done (Jayamanne and Samarajeewa, 2010). After 48 h incubation at 37°C, counting of bacterial colonies on TSA was done with a colony counter. These studies were also done at 55 and 57.5°C. All the experiments repeated three times.

Parameters to evaluate lethality of treatments: To evaluate lethality of treatments, the glass TDT tubes system (Pflug, 2003) were used. At each temperature (53, 55 and 57.5°C), time and temperature heating data were recorded. D-value and Z-value of *L. monocytogenes* for each sample

were determined. D-values derived from negative inverse slope of the linear portion of survivor curves. The Z-values were determined as the negative inverse slope of the $\log_{10}D$ vs. temperature plot (Murphy *et al*., 2002). Using the destruction time of *L. monocytogenes* in homogenized liquid whole egg, egg yolk and egg white were compared.

RESULTS AND DISCUSSION

In the present study, inactivation kinetics of *L. monocytogenes* was plotted by log-linear decline in surviving cells with time. Results are shown in the Fig. 1. Based on the linear portion of these survivor curves, D-values were calculated and Z-values were obtained. Time required to thermal destruction of all bacteria (5×10^8 CFU mL^{-1}) at 53°C in egg yolk, egg white and whole egg were 125, 65 and 95 min, respectively.

At 55°C scale down rate of bacteria was observed that like to 53°C but thermal destruction of *L. monocytogenes* was happened faster than 53°C. At the temperature of 53°C for 40 min thermal destruction in egg yolk, egg white and homogenized liquid whole egg were 4.59, 1.45 and 2.97 logarithmic cycle, respectively (Fig. 2). Thermal destruction of all liquid egg products were occurred very fast in this temperature. In fact the time required for deletion total of bacteria (5×10^6 CFU mL^{-1}) at 57.5°C for egg yolk, egg white and homogenized liquid whole egg were 40, 50 and 70 min, respectively (Fig. 3).

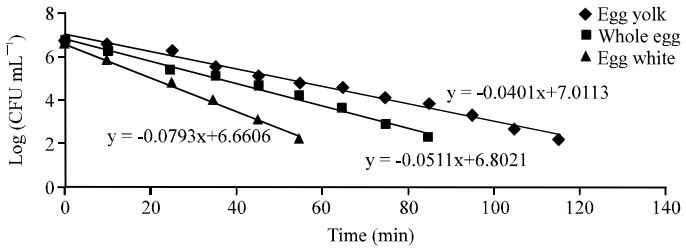

Fig. 1: Heat destruction of *L. monocytogenes* in egg yolk, egg white and whole egg at 53°C

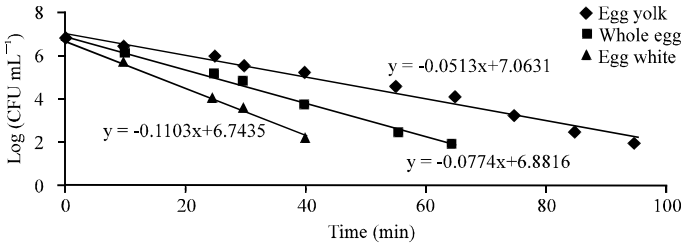

Fig. 2: Heat destruction of *L. monocytogenes* in egg yolk, egg white and whole egg at 55°C

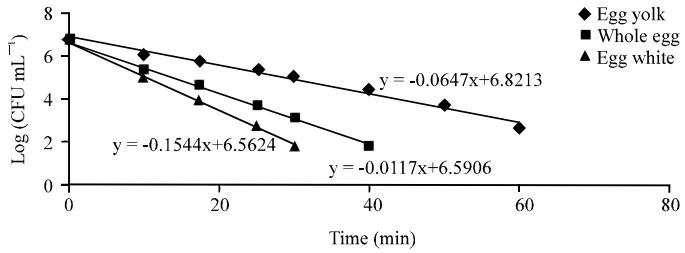

Fig. 3: Heat destruction of *L. monocytogenes* in egg yolk, egg white and whole egg at 57.5°C

Table 1: D-value of *L. monocytogenes* in egg yolk, egg white and whole egg

Sample	D_{53} (min)	D_{55} (min)	$D_{57.5}$ (min)
Egg yolk	24.99 (R = 0.997)	19.60 (R = 0.997)	15.04 (R = 0.998)
Egg white	12.65 (R = 0.998)	9.09 (R = 0.989)	6.69 (R = 0.996)
Whole egg	19.60 (R = 0.991)	12.98 (R = 0.989)	8.54 (R = 0.99)

Table 2: Z-value of *L. monocytogenes* in egg yolk, egg white and whole egg

Sample	Z-value (°C)
Egg yolk	20.83
Egg white	16.94
Whole egg	12.65

In this study, D-value for *L. monocytogenes* at 53°C was acquired highest amount (24.99 min) in the egg yolk and lowest amount (6.69 min) was acquired for egg white at 57.5°C (Table 1).

The Z-value was obtained from the slope of log10(D) vs. temperature plot and was 20.83, 16.94 and 12.65°C for *Listeria* in egg yolk, egg white and whole egg, respectively (Table 2).

Our result showed that in all temperature reduction of live germ count most rapidly in the liquid egg white. Heat destruction in egg white was fastest and *Listeria* in homogenized liquid whole egg had medium thermal resistance. Significant ($p<0.05$) differences were observed among D-values in all temperature (53, 55 and 57.5°C, respectively) between egg white and egg yolk. This is maybe due to the difference between pH, water activity and nature of constituents in two samples (Yang *et al*., 2000). In addition to the alkaline pH of egg white (8.9±0.3), it can be due to the proliferation inhibiting and cell destroying effects of lysozyme, conalbumin and avidin (Ibrahim *et al*., 2000; Castellano *et al*., 2001; Park *et al*., 2006; Board and Fuller, 1974). On the other hand, the absence of antimicrobial substances and the presence of lecithin in yolk egg causes the bacterial egg yolk resistant to test temperature (Chhabra *et al*., 2002; Muriana *et al*., 1996). Egg yolk contains 7.87% fat but egg white does not have any fat (Jorgensen *et al*., 1995). This fat can be protective for *L. monocytogenes* from heat at pasteurization process.

CONCLUSION

This study suggests that the lower thermal process is applied to liquid egg white than the liquid egg yolk. Hank *et al*. (2001) used low temperatures for shell pasteurization process and showed that pasteurization of egg at 55°C does not cause significant damage in total or soluble egg protein and reported that 55°C had no effect on the protein quality of albumen Hank *et al*. (2001). Hence, the data from this study will be useful for pasteurization of eggs at temperatures of 53, 55 and 57.5°C, respectively. This temperature can eliminate *L. monocytogenes* with maintenance of nutrients fragment in egg products. This study could be done for other bacteria, especially food-borne pathogens.

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