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Growth and survival kinetics of *Listeria monocytogenes* in cooked egg whites

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ABSTRACT

Peeled hard-boiled eggs (HBE) are ready-to-eat products susceptible to surface contamination by *Listeria monocytogenes*. This study investigated the growth and survival of *L. monocytogenes* between 4 and 43 °C in egg whites cooked under different conditions (70 °C for 15 min, 80 °C for 20 min, and 100 °C for 10 min). *L. monocytogenes* inoculated to samples cooked at 100 °C could grow uninhibitedly between 4 and 40 °C, exhibiting no lag phases, but failed to grow at 43 °C. The growth process was described by a 3-parameter logistic primary model, with the specific growth rates fitted equally well to the Ratkowsky square-root and Cardinal models. According to the Ratkowsky square-root model, the estimated minimum (nominal) and maximum growth temperatures were −0.3 and 47 °C, which were 1.6 and 44.3 °C, respectively, according to the Cardinal model.

L. monocytogenes did not grow well when inoculated to egg white samples cooked at 70 and 80 °C. Images of scanning electron microscopy showed that *L. monocytogenes* was damaged in samples cooked at these temperatures. Although experiencing a <2 log cfu/g initial growth, *L. monocytogenes* was inhibited in these samples at all storage temperatures, probably due to the antimicrobial activities of heat-denatured and polymerized lysozyme formed at 70 and 80 °C, which were absent in samples cooked at 100 °C.

The results of this study showed that cooking temperature affected the survival and growth of *L. monocytogenes* in cooked egg whites, suggesting that HBEs may be cooked at a lower temperature in order to retain the antilisterial activities. The mathematical models developed in this study can be used to predict the growth and survival of *L. monocytogenes* in BHEs and for conducting risk analysis of this type of products.

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1. Introduction

According to the statistics from the USDA Economic Research Service (USDA ERS, 2012), the United States is the second-largest egg producer in the world with over 90 billion eggs produced annually. Over 75% of the U.S. egg production is for human consumption with a per capita consumption of around 250 eggs. Many types of egg products are produced, including raw or pasteurized whole eggs, whites, yolks, and various blends with or without non-egg ingredients (Mukhopadhyay, Tomasula, Luchansky, Porto-Fett, & Call, 2010). Hard-boiled eggs (HBEs) are fully cooked, usually peeled ready-to-eat (RTE) products, sold as whole eggs in pouches or trays, or used as an ingredient in other value-added products, such as salads and sandwiches.

As with all egg-containing products, *Salmonella* is a major pathogen of concern and must be destroyed prior to consumption. According to the recommendation from the U.S. Food and Drug Administration (U.S. FDA), raw shell eggs broken for immediate preparation and service must be cooked to 63 °C for 15 s and foods prepared with raw shell eggs not broken for immediate preparation and service must be cooked to 68 °C for 15 s to prevent egg-borne Salmonellosis (U.S. FDA, 2002). According to the advice from the USDA Food Safety and Inspection Service (USDA FSIS), casseroles and other dishes containing eggs should be cooked to a safe minimum internal temperature of 71.1 °C (USDA FSIS, 2011). Based on these recommendations, HBE products should be cooked to an internal temperature of 68 °C for at least 15 s or 71.1 °C to ensure microbial safety. According to Humphery, Greenwood, Gilbert, Rowe, and Chapman (1989), boiling eggs for 10 min could achieve >7 log-reductions of *Salmonella* Enteritidis PT4. After cooking, HBEs should be

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free of foodborne pathogens and are ready-to-serve and RTE. In the industry, the boiling process can take as much as 18 min in 97.5 °C hot water to achieve uniform heating in a commercial egg cooker (Sanovo Technology Group, 2013).

As with all RTE foods, *Listeria monocytogenes* is a major foodborne pathogen of concern. Thermally processed HBEs, free of *Salmonella* spp. immediately after cooking, can be re-contaminated with *L. monocytogenes* in the post-processing environments. A study reported by Claire et al. (2004) showed that *L. monocytogenes* can grow in HBEs packaged under various gas atmospheres at both refrigerated (4 °C) and temperature abuse storage conditions (8 and 12 °C). Recently, contamination occurred and a major U.S.-based egg manufacturer recalled HBEs from the market due to discovery of *L. monocytogenes* (U.S. FDA, 2012a). Products made from contaminated HBEs were also recalled (U.S. FDA, 2012a, 2012b) across 34 states in the United States. More than a million HBEs were recalled as a result.

While most research of HBEs focuses on the safety of products concerning *Salmonella*, the objectives of this research were directed to investigate the growth kinetics of *L. monocytogenes* in HBEs, in view of the recent incidence of contamination of *L. monocytogenes* in these products (U.S. FDA, 2012a). Since the contamination of *L. monocytogenes* in HBEs primarily occurs on egg white surfaces, this study was conducted specifically in cooked egg whites at different storage temperatures and attempted to describe its growth behaviors via predictive mathematical modeling. The mathematical models developed from this study may serve as a scientific basis and assist the egg industry to produce safer HBE products and for regulatory agencies to conduct risk assessments of HBEs exposed to various temperature-abuse conditions.

2. Materials and methods

2.1. Preparation of bacteria

Five rifampicin-resistant *L. monocytogenes* strains, including three strains of *L. monocytogenes* 4b (F2365, H7858, and ATCC 19115), one strain of *L. monocytogenes* 1/2b (F4260), and one strain of *L. monocytogenes* 1/2a (V7), were obtained from the stock culture collection of the Eastern Regional Research Center (ERRC) of the USDA Agricultural Research Service (ARS) located in Wyndmoor, PA (Fang, Liu, & Huang, 2013). Each strain of *L. monocytogenes* was resistant to 100 mg/L of rifampicin (Sigma, R 3501-5G, Sigma–Aldrich Co., MO) in Brain Heart Infusion broth (BHI broth, BD/Difco Laboratories, Sparks, MD). Fresh cultures were prepared for each experiment. Stock cultures were prepared by streaking each strain of the overnight culture onto Tryptic Soy agar (TSA BD/Difco) plates containing 100 mg/L rifampicin (TSA/R). The stock cultures were kept at 4 °C in a refrigerator and transferred every 2–3 weeks to maintain viability.

One day prior to an experiment, each strain (a 1-μl loop) was inoculated to 10 ml of BHI broth supplemented with 100 mg/L rifampicin and held at 37 °C in an orbital shaker (~100 rpm) for approximately 22–24 h. Each overnight culture was harvested by centrifugation (2400 × *g*, 15 min, 4 °C), washed once with 10 ml of 0.1% peptone water (PW, BD/Difco), and re-suspended in 5 ml PW. The five individual strain bacterial cultures were combined to form a cocktail, which contained approximately $10^{9.5-9.7}$ cfu/ml of *L. monocytogenes*.

The results of a preliminary study showed that there was no difference in the growth behaviors of natural and rifampicin-resistant strains of *L. monocytogenes* in cooked egg whites and therefore, the antibiotic resistant strains of *L. monocytogenes* were used to inoculate cooked egg white samples.

2.2. Egg whites and sample preparation

For HBEs, the contamination of *L. monocytogenes* occurs primarily on the surfaces of the coagulated egg white layer, which may prevent *L. monocytogenes* from entering egg yolks. Therefore, this investigation was conducted using egg whites as test samples. Pasteurized 100% all natural liquid egg whites in 32 oz cartons (907 g) purchased from a local grocery store were used in the experiments. Although the egg white was previously pasteurized and free of foodborne pathogens when purchased, all samples were tested for potential presence of *L. monocytogenes* by direct plating (0.1 ml) onto PALCAM *Listeria* agar (PALCAM agar, BD/Difco) plates (37 °C, 48 h). The opened egg white cartons were refrigerated at 4 °C and used within two weeks of purchase. All samples were tested negative for *L. monocytogenes* prior to being used in the experiments.

Pasteurized liquid egg whites (5 ± 0.1 g) were aseptically weighed into sterile sampling bags (Whirl-Pak®, 207 ml, 95 mm × 180 mm × 0.08 mm, NASCO – Fort Atkinson, Fort Atkinson, WI). The water vapor and oxygen transmission rates of the filter bags were 7.8 g/m^2/day and 3100 ml/m^2/day, respectively, according to the manufacturer. Thirty six (36) egg white samples were prepared and divided into three groups, each containing 12 samples.

To evaluate the severity of heat treatment during cooking of shell eggs on the survival of *L. monocytogenes* in cooked eggs, egg white samples were coagulated at different temperatures and times in a hot water bath. The first group of egg white samples was heat-treated at 100 °C for 10 min (Humphery et al., 1989). The second group was heat-treated at 80 °C for 20 min. The third group received a treatment at 70 °C for 15 min. The first group received the most severe cooking and the third group the least.

After heat treatment, each sample was spread-inoculated with a 0.1-ml aliquot of appropriately diluted *L. monocytogenes* cocktail. After inoculation, all bags were labeled and hand-shaken for 1 min to disperse the bacterial cells. The initial concentration of *L. monocytogenes* in cooked egg whites was ca. 10^{2-3} cfu/g, determined by direct plating onto PALCAM agar plates.

2.3. Growth study

The inoculated cooked egg white samples were placed in incubators maintained at 4, 8, 12, 16, 20, 25, 30, 33, 37, 40, or 43 °C. Samples were removed from the incubators at pre-determined time intervals according to incubation temperatures. On average, 10–12 sampling points were used for each growth curve at each incubation temperature. For enumeration of bacteria after incubation, 10 ml PW was added to each sample bag. The samples were stomached for 2 min each side at the maximum speed in a stomacher (Model BagMixer® 100W, Interscience Co., France). A small volume (0.1 or 1 ml) of the liquid portion of the stomached samples was plated, either directly or after serial dilution, onto freshly prepared TSA/R and TSA. The TSA plates were used to enumerate total aerobic bacterial counts (TAB) and TSA/R plates for *L. monocytogenes* in the cooked egg white. Both TSA/R and TSA plates were held in an incubator maintained at 37 °C for 22–24 h. The bacterial colonies were counted and converted to the logarithm of base 10 and recorded log cfu/g. Two replicates of growth experiments were conducted for each temperature condition. As a quality control procedure, the control and inoculated samples were plated periodically onto PALCAM *Listeria* agar plates (BD/Difco) to check for recovery of *Listeria* cells.

2.4. Growth models

For egg white samples heat-treated at 100 °C, the inoculated *L. monocytogenes* began to grow immediately without experiencing