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Salmonella survival during soft-cooked eggs processing by *t*emperature-controlled water circulator

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ABSTRACT

Soft-cooked eggs have been cooked and served worldwide, however concerns frequently raise about the safety of these preparations, assuming the possibility of eggs be contaminated by *Salmonella*. Temperature-controlled water circulators at low temperature (62 °C–65 °C) for long periods (at least 1 h) has been used to thermally process eggs, aiming to modify its textures. However, time and temperature patterns are not in agreement with some recommendations for processing food preparations at least 70 °C. This study was undertaken to analyze the survival of *Salmonella* spp. during soft-cooked eggs processing by temperature-controlled water circulator. A pool of *Salmonella* spp. was inoculated in egg yolks and were incubated at 37 °C, for 18 h, reaching 7.7 ± 0.1 $\log_{10}$ CFU/g. Contaminated eggs were processed at 62 °C for 60 min and samples were collected in order to investigate *Salmonella* survival. Results indicated that the egg center temperature reached 61.7 ± 0.4 °C after 30 min, completely inactivating 7.7 log of *Salmonella* spp. After 30 min of cooking, yolk remained liquid and the egg white slightly opaque, demonstrating that the *Salmonella* inactivation was not related with the solidification of egg white or yolk. The survival curve did not follow first order kinetic and Double Weibull model was used to estimate inactivation kinetic parameters. In summary, the results of this study can be used by food processors in order to validate soft-cooked eggs processing by temperature-controlled water circulator.

1. Introduction

Salmonella has caused an expressive impact on foodborne illnesses worldwide. In the United States (U.S), the incidence of *Salmonella* infection was 16 per 100,000 in 2017 (Marder et al., 2018). In 2015, *Salmonella* outbreaks, cases and hospitalizations were all ranked the highest compared to food diseases caused by other pathogens (CDC, 2017). Salmonellosis remains the second most common zoonosis in humans in the Europe Union (EU), being that the number of confirmed cases was 94.53, with a notification rate of 20.4 per 100,000 population (EFSA, 2017). In Brazil, 30% of the pathogens identified in foodborne outbreaks were associated with *Salmonella* in the last decade (BRAZIL, 2018).

Salmonellosis has traditionally been associated with egg consumption and represents one of the highest risk agent/food combinations. The prevalence of *Salmonella* in eggs is normally low, 0.005% in the US (Ebel & Schlosser, 2000). In the EU, 0.29% of the 5782 tested table egg units were *Salmonella* positive, in 2016 (EFSA, 2017). In Brazil, several studies reported different incidences of this pathogen inside eggs and ranged between absence and 33% (Baú, Carvalhal, & Aleixo, 2001; Kottwitz et al., 2013; Oliveira & Taham, 2011; Wolschick & Bosco, 2015). Although the low prevalence, the number of Salmonellosis human cases associated with eggs can still be large, especially because eggs are highly consumed and used in many dishes that frequently are not well heat-treated (EFSA, 2017; Windhorst, Grabkowsky, & Wilke, 2013).

Thermal processing is still one of the most common and effective methods to inactivate *Salmonella* inside eggs. Based on this fact, different regulatory agencies worldwide recommend or require that eggs that will be prepared and served should be cooked to an internal temperature of at least 70 °C (BRAZIL, 2004; CDC, 2011; Canada, 2013; FDA, 2016). Some regulation bodies set higher specifications, requiring both yolk and egg white should be solid before serving (FDA, 2016; RIO GRANDE DO SUL, 2009; SÃO PAULO, 2013). When the temperature reaches 70 °C the egg yolk coagulates and the protein ovomucoid denatures, the consistency of the egg white becoming harder (Baldwin, 2012; This, 2016). However, in several restaurants around the world, especially those linked to fine gastronomy, eggs are cooked at relatively low temperatures, around 60 °C (Vega & Mercadé-Prieto, 2011), in order to keep the white and yolk soft or slightly modifying their texture.

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Actually, different textures can be created using the slow cooking of the eggs. The new approach used in restaurants consists of cooking eggs at constant low temperature for long periods of time (at least 1 h), through the use of temperature-controlled water circulators. Eggs cooked through this technique are described as “6× °C egg”, the “X” usually varies from 0 to 5 °C, depending on the restaurant. However, the most common thermal processing pattern is the use of temperatures starting from 62 °C, because the use of temperatures below 61 °C requires longer cooking periods to increase the viscosity, becoming unusual. Cooking an egg using the same temperature at different times, or different temperatures with a fixed time can form textures going from viscous fluid to gelled yolk (Vega & Mercadé-Prieto, 2011). Therefore, the “6× °C egg” disagrees with official recommendations of texture and temperature. Thus, restaurants may need to validate this cooking practice for eggs.

Studies have demonstrated that shell eggs immersed in water at low temperatures (52–58 °C) reduced the *Salmonella* population, therefore this method is particularly useful for the egg industry (Geveke, Gurtler, Jones, & Bigley, 2016; Park & Cho, 2006). However, no study has reported the use of this technique as a method to control *Salmonella* in foodservices. Thus, the objective of this study was to evaluate the inactivation of *Salmonella* spp. in eggs cooked at low temperature (62 °C) through the use of temperature-controlled water circulator, in order to validate the safety of these eggs served in restaurants.

2. Material and methods

2.1. Bacterial strains and inoculum preparation

Five strains of *Salmonella* were used as a pool: *S.* Enteritidis SE86, S. Enteritidis 55507, *S.* Typhimurium L12031 (isolated from food outbreaks reported in Rio Grande do Sul State, Brazil), *S.* Minnesota and *S.* Heidelberg, isolated from poultry products. All strains were grown separately on 5 ml of Brain Heart Infusion broth (BHI; Merck, Darsmtadt, Germany) at 37 °C, for 18–24 h. After incubation, 2 ml of BHI broth containing each strain were used in order to compose 10 ml of *Salmonella* pool. The pool was centrifuged (3500 RPM, 10 min, 4 °C) (CIENTEC CT-5000 R, Brazil), the supernatant was discharged and pallet was washed three times with 0.1% peptone water (w/w) (Merck, Darsmtadt, Germany). Finally, cells were re-suspended 0.1% in peptone water (w/w) and final cell concentration was adjusted through optical density (OD_{630nm}) and plate counts at 10^8 CFU/ml. Decimal serial dilutions in 0.1% peptone water (w/w) were prepared and used as inoculum in eggs as described below.

2.2. Growth of Salmonella pool in eggs

Eggs were inoculated according to the method report by (De Paula, Mariot, & Tondo, 2005) with some modifications. Eggs (Filippsen Eggs, Brazil), weighing 60–78 g were chosen and the yolk was inoculated using an egg candler and a sterile needle of 25 mm × 0.80 mm (Descarpack, Jiangsu Jichun Medical Devices Co., China) attached to a 10 ml syringe (Plastipak TM Becton Dickinson). A hole was punched through the shell of each egg and the needle was inserted about 2.5 cm until reached the yolk. After the yolk inoculation with approximately 100 cfu of *Salmonella* pool, a drop of quick-drying glue (Scotch, 3M, Brazil) was used to close the hole of the shell egg. The eggs were incubated at 37 °C for 18–24 h. After incubation *Salmonella* spp. population was counted and ranged from 7 to 8 log CFU/g.

2.3. Egg preparation

After 18–24 h incubation, artificially contaminated eggs were stored for 60 min at room temperature, and then were immersed in a temperature-controlled water circulator (TermoHobby, H2heater, Brazil) at 62 °C for 60 min. Three eggs were removed from random positions at each set time points (5, 8, 10, 12, 14, 15, 16, 20, 25, 30 and 60 min) in order to quantify *Salmonella*. Post-treated eggs were immediately immersed into ice-water bath to stop the cooking process. Inoculated and non-heat treated shell eggs were used as controls. The experiment was performed three times and all counts were done in triplicate.

2.4. Internal temperature

The temperature of the center of eggs was monitored using egg samples (not inoculated) previously stored at room and refrigeration temperature. A hole was punched through the shell egg and K-type thermocouples were inserted through *sous vide* foam tape and the temperature was recorded using a data logger (Tenmars, Taiwan).

2.5. Microbiological analysis

Each egg was aseptically opened and the content was placed in a sterile plastic bag and then mixed using a stomacher (Stomacher® 400, Seward, England) for 1 min. To obtain the 10^{-1} dilution, 25 g were blended with 225 ml of sterile 0.1% peptone water (w/w) for 2 min in a sterile plastic bag using a stomacher. Subsequent dilutions were obtained by mixing 1 ml aliquots with 9 ml of 0.1% peptone water (w/w), and 100 μl these dilutions were spread on agar plates. Agar plates were prepared using Lysine Desoxycholate (XLD, Merck, Darsmtadt, Germany) plus a thin layer of Tryptic Soy Agar (TSA, KASVI, Italy) according to the one-step TAL method report by Kang and Fung (2000). The plates were incubated at 37 °C for 24 h and typical *Salmonella* colonies were counted. The detection limit of plate counts was 100 CFU/g. When increased sensitivity was required, 1000 μl of the undiluted suspension were plated on four XLD-TSA agar plates (250 μl for plate).

2.6. Complete inactivation test

Complete inactivation test was conducted to detect ≤1 CFU/g of *Salmonella*. Three post-treated eggs removed from random positions at each set time points (20, 25, 30 and 60 min) were immediately immersed into ice-water bath and then incubated at 37 °C for 24 h. Presence/absence testing per 25 g was performed as described above (item 2.5).

2.7. Data analysis

The inactivation kinetics was modeled with GInaFiT (Geeraerd and Van Impe Inactivation Model Fitting Tool) version 1.6, a freeware Tool for Microsoft® Excel. The Root Mean Sum of Squared Errors (RMSE), Mean Sum of Squared Errors, coefficient of determination (R^2) and adjusted coefficient of determination (R^2_{adj}) were evaluated and the Double Weibull model was chosen as best fit. In addition, the time needed for a 4 log reduction of the initial microbial population (4D) was also automatically estimated (Coroller, Leguerinel, Mettler, Savy, & Mafart, 2006).

3. Results and discussion

3.1. Temperature profile

Raw eggs have been stored in two ways before cooking, kept them at room temperature or refrigerated. In order to validate eggs submitted at different storage conditions, both methods of storage were analyzed.

The temperature profile of the center of eggs previously stored at room and refrigeration temperature, during cooking at 62 °C is presented in Fig. 1. The heating profile did not show differences between room and refrigeration temperature. The come-up time (time that the center of an egg reaches within 1 °C of the final temperature) for both storing conditions was 21 ± 2 min (Fig. 1). This result is in agreement with others studies. For example, according to Geveke et al. (2016) eggs

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