Inactivation of *Salmonella* during cocoa roasting and chocolate conching

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A B S T R A C T

The high heat resistance of *Salmonella* in foods with low water activity raises particular issues for food safety, especially chocolate, where outbreak investigations indicate that few colony-forming units are necessary to cause salmonellosis. This study evaluated the efficiency of cocoa roasting and milk chocolate conching in the inactivation of *Salmonella* 5-strain suspension. Thermal resistance of *Salmonella* was greater in nibs compared to cocoa beans upon exposure at 110 to 130 °C. The D-values in nibs were 1.8, 2.2 and 1.5-fold higher than those calculated for cocoa beans at 110, 120 and 130 °C. There was no significant difference ($p > 0.05$) between the matrices only at 140 °C. Since in the conching of milk chocolate the inactivation curves showed rapid death in the first 180 min followed by a lower inactivation rate, and two D-values were calculated. For the first time interval (0–180 min) the D-values were 216.87, 102.27 and 50.99 min at 50, 60 and 70 °C, respectively. The other D-values were determined from the second time interval (180–1440 min), 1076.76 min at 50 °C, 481.94 min at 60 °C and 702.23 min at 70 °C. The results demonstrated that the type of matrix, the process temperature and the initial count influenced the *Salmonella* resistance.

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1. Introduction

Outbreaks of salmonellosis associated with consumption of chocolate have been known since the '70s and despite technological development (D'Aoust, 1977, Werber et al., 2005), continue to occur today. The low water activity (0.3 to 0.5) and high fat content (>20%) observed in most chocolate result in an increase in the heat resistance of *Salmonella* (D'Aoust, 1977; Krapf and Gantenbein-Demarchi, 2010; Podolak et al., 2010). Furthermore, the high fat content also protects the pathogen against gastric acidity resulting in a reduction of dose–response curve with a low infectious dose. In Canada, a very small number of *Salmonella* viable cells (0.043 MPN/g) were recovered from chocolate associated with an outbreak caused by *S.* Nima (Hockin et al., 1989). Another important characteristic to be considered is that although *Salmonella* cannot multiply in this kind of product, it can remain viable for long periods of time (Tamminga et al., 1976).

Cocoa-based ingredients are not solely responsible for the possible introduction of *Salmonella* in chocolate, but they have been identified as a source of contamination in certain outbreaks (Werber et al., 2005). The contamination of almonds, nuts and cocoa occurs mainly during pre-processing (fermentation, drying and storage) (Nascimento et al., 2010; Beuchat and Mann, 2011). According to ICMSF (2005), cocoa roasting is considered to be the main step in the process responsible for the reduction of *Salmonella* in the product. It can be performed in

whole cocoa beans or in nibs (cotyledons split hulls and germ-free), using temperatures between 110 and 140 °C (Beckett, 2008). The temperature and process time vary according to factors such as equipment used, origin and harvest period of cocoa, moisture, and flavor desired in the final products (Schwan and Wheals, 2004). A reduction of natural microbial contamination of the cocoa beans between 1 and 2-log was obtained in some studies using temperatures up to 150 °C (Barrile et al., 1971 Stobinska et al., 2006). There are few published data on the efficiency of this type of thermal process in the elimination of *Salmonella* from products of low water activity. Abd et al. (2008) observed a reduction of 5 log CFU/g of *Salmonella* in almonds after 2 min at 126.7 °C. Beuchat and Mann (2011) demonstrated that pecan nuts achieved reductions between 1 and 2 log CFU/g after treatment at 120 °C for 20 min. Izurieta and Komitopoulou (2012) found reductions between 0.12 and >6.93 log CFU/g in the shells of cocoa beans and hazelnuts after 15 min at 100 °C.

Regarding chocolate making, the only step that employs heat treatment is the conching. At this stage, the product is submitted for several hours to agitation and shear for several hours under controlled temperatures usually between 50 °C and 70 °C (Beckett, 2008). The processing time depends on the type of equipment, and moisture and flavor desired in chocolate (Beckett, 2008). Some studies especially in the '60s and '70s showed high thermal resistance of *Salmonella* to the conching process in different chocolate. In milk chocolate, Goepfert and Biggie (1968) obtained a reduction of 1-log at 71.1 °C after 440 min for *Salmonella* Senftenberg 775 W and 816 min for *Salmonella* Typhimurium. Barrile and Cone (1970)

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observed the same reduction after 11 min at 90 °C for *S.* Anatum. In dark chocolate, a reduction of 1-log was achieved after 600 min at 70 °C (Krapf and Gantenbein-Demarchi, 2010).

Historically, thermal inactivation of microorganisms in food has been described by first order kinetics. This model allows the establishment of the appropriate heat treatment by determination of the decimal reduction time (D-value) and the temperature range necessary to change the D-value tenfold (z-value) (Tomlins and Ordal, 1976). According to van Asselt and Zwietering (2006), the $D_{70\,°C}$ value for *Salmonella* in chocolate is 3-log higher than that established for most foods. Because of the context considered herein, it is clear that the control of raw material and thermal process is essential in preventing *Salmonella* contamination in chocolate (Cordier, 1994; ICMSF 2005). For this reason, more data on the resistance of *Salmonella* to thermal treatments used in the chocolate production chain are required. Based on this, the objective of this study was to evaluate the thermal inactivation of *Salmonella* during roasting of cocoa beans and nibs, and conching of milk chocolate.

2. Material and methods

2.1. Salmonella strains

A pool of five *Salmonella enterica* serotypes was used as an inoculum (*S.* Typhimurium ATCC 14028, *S.* Oranienburg IAL 1203, *S.* Senftenberg IAL 1235, *S.* Eastbourne IAL 1131 and *S.* Enteritidis ATCC 13076). The choice of the serotypes used in this study was based on the following criteria: *S. typhimurium* and *S.* Senftenberg because they have a high thermal resistance; *S.* Oranienburg and *S.* Eastbourne, although not being the same strains, are serotypes that have already been involved in outbreaks resulting from consumption of chocolate; and *S.* Enteritidis because it is isolated more often from food. All strains were stored at −80 °C, and maintained on tryptic soy agar (TSA, Difco) slants at 4 °C for use as initial starter culture for preparation of the inoculum.

2.2. Preparation of inocula

Each *Salmonella* strain was cultivated twice in tryptic soy broth (TSB, Difco) at 37 °C for 18–24 h. Then, a pool of the 5 strains was prepared by mixing an equal volume of each culture in the same tube, which was centrifuged at 3000 *g* for 10 min at 20 °C (centrifuge PK 121R, ALC, Italy). The supernatant was discarded after centrifugation and the biomass obtained was rinsed twice in phosphate-buffered saline (PBS), after which it was resuspended in PBS at a final concentration of 10^8 CFU/ml. Cell numbers in each cell suspension were determined by plating appropriate dilutions on TSA. The inoculum level of each serotype was $10^{7–8}$ cfu/g.

2.3. Evaluation of thermal resistance of Salmonella

2.3.1. Cocoa roasting

Cocoa beans in shell and nibs obtained from Brazillian producers were used in this experiment. Fifty grams (50 g) of each matrix were inoculated with 1% (0.5 ml) of the *Salmonella* suspension (ca. 10^8 MPN/g), plus 1.5% Tween 80 (Merck) to help to reduce the surface tension. After homogenization by hand for 1 min, with the purpose of ensuring a maximum adherence of the inoculum and no change in the initial water activity of the product, the samples were kept in a biosafety cabinet (Vecco, Brazil) at 35 °C for 10 min to dry. Immediately after, the water activity was determined using Aqualab Water Activity Meter (Decagon Devices, Inc., Pullman, WA), and the samples were arranged in aluminum screen trays (12 cm × 8 cm × 2 cm). The roasting process was carried out in a forced-air oven (Marconi, Brazil) at four temperatures (110, 120, 130 and 140 ± 1.0 °C) for five different time periods (10, 20, 30, 40 and 50 min). Once the target temperatures were achieved, five samples were placed in the oven. After each 10 min period one sample

was removed as quickly as possible to minimize changes in the temperature of the oven air. Four thermocouples (Pt 100), three placed on the sample surface and one in the center position of the oven, were used to measure the temperature in real-time (each 2 s). Thermocouples were set to give an accuracy of ± 0.1 °C. The temperature data were recorded on data logging equipment (MyPCLab, Novus, Brazil) connected to a computer. After each process, the samples were immediately transferred to sterile bags and cooled in running water to 35 °C. *Salmonella* enumeration was performed by the Most Probable Number (MPN) before and after heat treatment. Each roasting process was replicated three times.

2.3.2. Conching of milk chocolate

The conching process was performed in batches of 500 g in a jacketed glass reactor, which was combined with a digital mechanical stirrer TE 039/1 (Marconi, Brazil), maintained at 380 rpm. The process temperature was controlled by an ultra thermostatic bath MA 184 (Marconi, Brazil) coupled to the reactor. The formulation used for preparing the milk chocolate was sucrose (43%), cocoa butter (21.5%), cocoa liquor (14%), milk powder (12%), skimmed milk powder (9%) and soybean lecithin (0.5%) (Melo et al., 2007). The powdered ingredients (milk, skimmed milk and sucrose) and part of the cocoa butter (20%) were previously refined in a three-roll mill internally cooled to 15 °C (Drais Werke, Germany). This refined mass was then transferred to the jacketed glass reactor, and the remainder of the molten cocoa butter and lecithin was added. After homogenization, cocoa liquor inoculated with *Salmonella* was added. For inoculation, seventy grams (70 g) of the cocoa liquor were previously melted in a water bath at 60 °C, cooled to 35 °C, and then inoculated with 0.4% (0.3 ml) of the *Salmonella* suspension. Thus, the initial concentration of the inoculum in the chocolate mass was approximately 10^4 MPN/g. The samples were treated at three temperatures (50, 60 and 70 ± 0.5 °C) for four different time periods (180, 480, 900 and 1440 min, respectively). A digital thermometer (Testo, Brazil) was used to monitor the temperature of the chocolate mass every 15 min. Immediately after each process, the samples were transferred to sterile bags and cooled in running water to 35 °C. The enumeration of *Salmonella* was carried out by Most Probable Number (MPN) technique immediately after the addition of the contaminated liquor and at the end of each process. *Salmonella* detection in 100 g of chocolate was performed in samples that had counts below the detection limit for enumeration. Each conching process was replicated three times on separate occasions.

2.4. Detection and enumeration of Salmonella

The determination of *Salmonella* count was performed by the Most Probable Number technique (MPN) and the detection method was carried out according to Andrews and Hammack (2005). Decimal dilutions were prepared using 0.1% peptone water. The first dilutions of cocoa bean and nib samples were mixed manually, and the other dilutions were homogenized in a stomacher. For pre-enrichment of cocoa beans, Buffered Peptone Water (BPW, Difco) was used, while for cocoa nibs and chocolate, the medium was reconstituted 10% skimmed milk (Nestlé, Brazil) supplemented with 1% Brilliant Green (Merck). After incubation at 35 °C for 18–24 h, 0.1 ml of each portion was added to 10 ml of Rappaport-Vassiliadis Modified broth (Difco) and 1.0 ml to 10 ml Tetrathionate broth (Difco). Enrichment broths were incubated for 24 h at 42 °C and 35 °C, respectively. After that, cultures were streaked on Xylose Lysine Deoxycholate agar (XLD, Difco) and Bismuth Sulfite agar (BS, Difco) and the plates were incubated at 35 °C for 24–48 h. Presumptive-positive colonies were subjected to confirmation by biochemical and serological tests. The detection limit for enumeration by MPN technique was 0.03 MPN/g (−1.52 log MPN/g). The detection limit by enrichment was 1 cell per 100 g of chocolate.