International Journal of Food Microbiology 159 (2012) 225–229

Contents lists available at SciVerse ScienceDirect

International Journal of Food Microbiology

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

Inactivation of *Salmonella* during cocoa roasting and chocolate conching

Maristela da Silva do Nascimento [a,*], Daniela Merlo Brum [a], Pamela Oliveira Pena [a], Maria Isabel Berto [a], Priscilla Efraim [b]

[a] Institute of Food Technology, Avenida Brasil, 2880, Campinas, São Paulo, 13070-178, Brazil
[b] Department of Food Technology, University of Campinas, Rua Monteiro Lobato, 80, Campinas, São Paulo, 13035-388, Brazil

ARTICLE INFO

Article history:
Received 24 April 2012
Received in revised form 8 August 2012
Accepted 24 August 2012
Available online 30 August 2012

Keywords:
Salmonella
Cocoa
Chocolate
Heat resistance
D-value

ABSTRACT

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.

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)

* Corresponding author. Tel.: +55 19 37431819; fax: +55 19 32424585.
E-mail address: mnascimento@ital.sp.gov.br (M.S. Nascimento).

0168-1605/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijfoodmicro.2012.08.017

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 *Salmonell*a 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.

2.5. Calculation of D- and z-values

The lethal effect at a given temperature was estimated by calculating the decimal reduction time (D-value) that represents the time to reduce the microbial population by one log cycle. D-value was determined by Eq. (A1):

$$D = t/\left(log\ N_0 - log\ N_f\right) \quad \text{(A1)}$$

where:

N_0 initial number of microorganisms
N_f number of surviving cells after heat treatment
t time (in minutes) of heat treatment.

The z-value (temperature range required to change the D-value by tenfold) was determined by Eq. (A2):

$$z = T1 - T2/(log\ D_{T1} - log D_{T2}) \quad \text{(A2)}$$

where:

T1 Temperature 1
T2 Temperature 2
D_{T1} D-value at temperature 1
D_{T2} D-value at temperature 2.

2.6. Statistical analysis

The analysis of variance (ANOVA) and the Tukey test at the 5% level of significance were used to compare means among experimental groups and were analyzed using SAS software (version 9,1, SAS Institute, Cary, NC).

3. Results

3.1. Roasting of cocoa beans and nibs

Thermal inactivation curves for *Salmonella* in cocoa beans and nibs heated at 110 to 140 °C for up to 50 min are shown in Figs. 1 and 2, respectively. In cocoa beans, the target temperature was achieved after 12 min at 110 °C, 15 min at 120 or 130 °C and after 19 min at 140 °C. After 10 min of heating, an initial population of 6.00 log MPN/g was reduced by 1.79 log MPN/g at 110 °C, 3.05 log MPN/g at 120 °C, 3.59 log MPN/g at 130 °C and 5.28 log MPN/g at 140 °C. Only the treatments at 120 and 130 ° C showed no statistically significant difference ($p > 0.05$) on the *Salmonella* inactivation during this 10 min period. At 110 and 120 °C for 20 min, reductions of 3.25 and 6.83 log MPN/g were observed, whereas at 130 or 140 °C the *Salmonella* count decreased below the detection limit (<0.03 MPN/g). The same reduction level was achieved after 30 min at 120 °C and 40 min at 110 °C (Fig. 1).

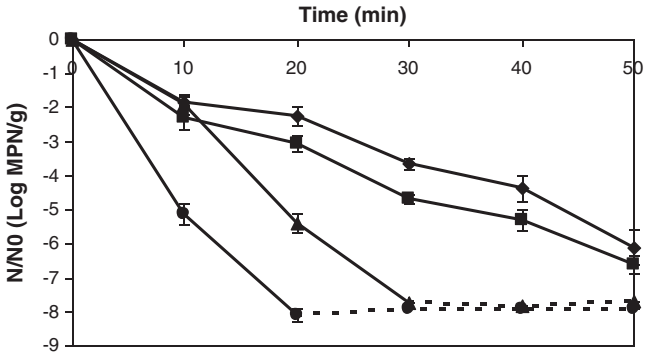

Fig. 2. Inactivation of *Salmonella* in cocoa nibs during roasting at different temperatures. (♦) 110 °C, (■) 120 °C, (▲) 130 °C, (•) 140 °C. (—) Count below the detection limit.

In cocoa nibs, the target temperature was achieved after 11 min at 110 °C, 13 min at 120 °C, 14 min at 130 °C and after 19 min at 140 °C. The reductions observed in the treatments at 110, 120 and 130 °C for 10 min (1.83, 2.30 and 1.89 log MPN/g, respectively) were not significantly different ($p > 0.05$), whereas at 140 °C a significant reduction of 5.14 log MPN/g was obtained. After 20 min of heating, all treatments showed statistically different reductions ($p < 0.05$), 2.25 log MPN/g at 110 °C, 3.06 log MPN/g at 120 °C, 5.39 log MPN/g at 130 °C and counts below the detection limit at 140 °C. Reductions of 3.66 and 4.68 log MPN/g were observed when the samples were heated for 30 min at 110 and 120 °C, respectively; at 130 °C the initial viable cells were reduced to below the detection limit. Treatment at 110 or 120 °C for up to 50 min failed to reduce *Salmonella* population below the detection limit; the highest reductions observed were around 6 log MPN/g (Fig. 2). Results indicated that the experimental matrix (cocoa beans or nibs) significantly influenced ($p < 0.05$) the resistance of *Salmonella* when the following temperatures were used: 110 °C and 120 °C up to 50 min and 130 °C for only the first 20 min.

The D-value was calculated by linear regression curves based on thermal inactivation of *Salmonella* in cocoa beans and nibs (Table 1). The correlation coefficient (r^2) was between 0.948 and 0.998. The tails (dotted line) observed in Figs. 1 and 2 represent counts below the detection limit, consequently they were not used to calculate the D-values. For cocoa beans, D-values at 110 and 120 °C were significantly different ($p < 0.05$) than those obtained at 130 and 140 °C, which show the same D-value. The D-values were 4.79 min for 110 °C, 3.62 min for 120 °C and 2.55 min for both 130 and 140 °C.

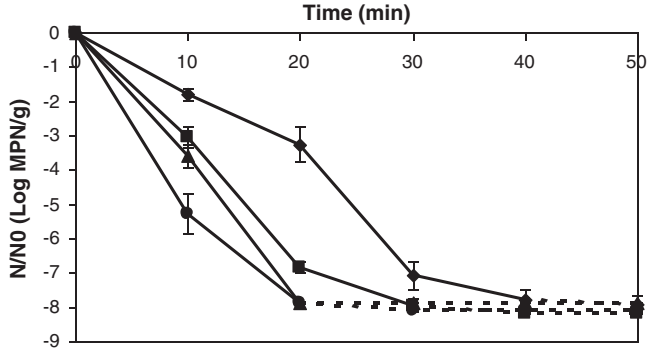

Fig. 1. Inactivation of *Salmonella* in cocoa beans during roasting at different temperatures. (♦) 110 °C, (■) 120 °C, (▲) 130 °C, (•) 140 °C. (—) Count below the detection limit.

Table 1
D and Z values determined for *Salmonella* during roasting of cocoa beans and nibs.

Matrix	Temperature (°C)	Means D-value ± SD (min)[a]	r^2 [b]	Z-value (°C)	r^2 [b]
Cocoa beans	110	4.79 ± 0.22a	0.948	102.60	0.898
	120	3.62 ± 0.07b	0.962		
	130	2.55 ± 0.11c	0.998		
	140	2.55 ± 0.11c	0.962		
Nibs	110	8.86 ± 0.69a	0.977	50.30	0.932
	120	8.02 ± 0.23b	0.972		
	130	3.75 ± 0.19c	0.988		
	140	2.47 ± 0.06d	0.976		

[a] Values obtained from three independent trials with standard deviation. Means with different letters in the same sample are significantly different ($p < 0.05$).
[b] r^2: correlation coefficient.

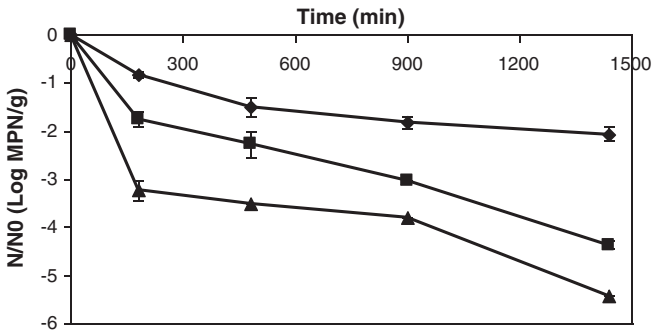

Fig. 3. Inactivation of *Salmonella* in milk chocolate during conching at different temperatures. (◆) 50 °C, (■) 60 °C, (▲) 70 °C.

In nibs, there was significant difference ($p<0.05$) among all D-values calculated. The time required to achieve a reduction of 1-log at 110, 120 and 130 °C was 3.6, 3.2 and 1.5-fold higher than at 140 °C. The z-value was determined by linear regression of D-values log versus the corresponding temperature heating. Roasting temperature must increase 102.60 °C in cocoa beans and 50.30 °C in nibs to reduce the D-value by tenfold (Table 1).

3.2. Conching of milk chocolate

Fig. 3 shows the thermal inactivation curves of *Salmonella* during milk chocolate conching at 50 °C to 70 °C. At 50 °C, reductions of 0.83 and 1.52 log MPN/g were observed after 180 and 480 min, respectively. After that, there was no significant decline in the inactivation rate, with a reduction of only 0.56 log MPN/g throughout the period between 480 min and 1440 min. The curve of thermal inactivation at 60 °C showed a trend closer to linearity, and reductions of 1.76, 2.28, 3.03 and 4.35 log MPN/g at 180, 480, 900 and 1440 min were observed. The treatment at 70 °C showed a sharp decline in the initial count (3.50 log MPN/g) in the first 180 min. However, after increasing the process time to 480 or 900 min no significant addition reduction was observed (0.31 log MPN/g). Only after 1440 min, a considerable decrease in the pathogen count (>5.41 log MPN/g) was obtained. *Salmonella* was recovered by enrichment after analysis of 100 g of all samples that showed a count below the detection limit.

Due to *Salmonella* behavior during chocolate conching, which showed a high inactivation rate in the first 180 min followed by a lower death rate (Fig. 3), two D-values were calculated for each treatment (Table 2). For the first 180 min of the process, the D-values were 216.87, 102.27 and 50.99 min at 50, 60 and 70 °C, respectively. For the period between 180 and 1440 min, the D-values calculated were 1076.76 min at 50 °C, 481.94 min at 60 °C and 702.23 min at 70 °C. Since the *Salmonella* death rate showed distinct behaviors in the two analyzed time intervals, it was not possible to estimate a representative z-value for the chocolate conching process.

Table 2
D and Z values determined for *Salmonella* in two different period of time during milk chocolate conching process.

Time interval (min)	Temperature (°C)	Means D-value ± SD (min)[a]	r^2 [b]
0–180	50	216.87 ± 14.81a	0.999
0–180	60	102.27 ± 8.61b	0.999
0–180	70	50.99 ± 3.07c	0.999
180–1440	50	1076.76 ± 9.33a	0.884
180–1440	60	481.94 ± 26.55c	0.989
180–1440	70	702.23 ± 32.65b	0.821

[a] Values obtained from three independent trials with standard deviation. Means with different letters in the same sample are significantly different ($p<0.05$).
[b] r^2: correlation coefficient.

4. Discussion

The roasting process of cocoa beans results in the reduction of moisture from 8 to around 2% and of water activity from 0.75 to 0.50. This decrease may be the main factor that greatly increases the thermal resistance of *Salmonella*. There are no published data comparing the inactivation of *Salmonella* in the roasting of cocoa beans and nibs. However, in a study carried out by the Almond Board of California's Expert Technical Review Panel (ABC, 2007), *S.* Enteritidis PT30 was reduced by 5-log in almonds after 2 min at 126 °C. In the current research, to achieve the same reduction at 130 °C, 13 and 19 min would be required for cocoa beans and nibs, respectively.

The thermal inactivation of microorganisms in food can be influenced by several factors, such as food composition (fat, NaCl, pH, aw) (Mattick et al., 2001). In this study, thermal resistance of *Salmonella* was slightly 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 between the reductions of the two matrices studied only at 140 °C. The difference observed at lower temperatures may be attributed in part to a larger contact surface and to greater fat content present in nibs. Despite the higher D-values observed in nibs, the z-value was half that calculated for cocoa beans; this may be related to higher speed of heat spread in nibs. Lee et al. (2006) and Izurieta and Komitopoulou (2012) noted a variation in the thermal resistance of *Salmonella* in different nut shells. This oscillation may be due to the difference of the surface microstructures shown by different nuts. Comparing the results obtained here with others that also evaluated samples with low water activity, it is possible to conclude that the sample type significantly influences the thermal resistance of *Salmonella*. The D-values calculated for 110 and 120 °C for cocoa beans and nibs were higher than those reported by Harris (2008) for almonds ($D_{121\,°C}=0.8$ min) and by Izurieta and Komitopoulou (2012) for cocoa bean shells ($D_{105\,°C}=0.72$ to 1.0 min) and hazelnut shells ($D_{105\,°C}=2.0$ to 2.5 min).

Conching is the last step in chocolate manufacturing that uses heat. This step should eliminate any possible microbial contamination present in the raw materials to ensure the safety of the final product. Indeed, according to the present results, conching is not efficient to eliminate a high contamination of *Salmonella*. The initial count of 3–4 log was not completely reduced after 1440 min (24 h) at any tested temperature. Furthermore, increasing the processing time does not necessarily result in the increase of microbial death. Other research on thermal inactivation of *Salmonella* in chocolate was compared to our data. The $D_{70\,°C}$ value calculated for the second part of the inactivation curve was less than the D-value reported by Barrile and Cone (1970) for milk chocolate (1,200 min) and higher than that found by Krapf and Gantenbein-Demarchi (2010) for bitter chocolate (600 min).

The first-order kinetics model assumes that all cells of a microbial population have the same thermal resistance, but in some cases significant deviations were observed from linearity (Shachar and Yaron, 2006, Ma et al., 2009). In the current research, high inactivation rate observed in the first 180 min (3 h) of the chocolate conching indicates heterogeneity in the thermal resistance of the bacteria. It could have been influenced by the different serotypes used and by the characteristics of the chocolate which expose the cells to different environments. Moreover, at the beginning of this process in general the solid particles (sucrose, cocoa solids) are not fully overlaid by the fatty phase and in this condition *Salmonella* cells are less resistant to heat. According to Shachar and Yaron (2006), some bacteria could aggregate into particles within or near the aqueous phase of the product, while others could be located in a drier or more hydrophobic and fatty environment, which would give them greater protection against high temperatures. Behavior similar to that observed in our study was reported by Goepfert and Biggie (1968) in chocolate. In peanut butter Shachar and Yaron (2006)

and Ma et al. (2009) obtained concave curves of thermal inactivation of *Salmonella*, with a sharp initial decline of the population in the first minutes of process (10 to 20 min), after which a slow decrease was observed.

Validation of a thermal process for low water activity food, such as cocoa and its derivatives, involves the determination of the critical limit for the target microorganism. A targeted 2 to 5-log reduction is commonly selected based on a hazard analysis that includes historical association of ingredients with *Salmonella*, prevalence and extent of contamination, and the intended use of the final product. The U.S. Food and Drug Administration issued guidance documents for peanuts and pistachios recommending the application of a thermal inactivation step capable of reducing the *Salmonella* level by a minimum of 5-log (FDA, 2009a, 2009b). For almonds a treatment capable of achieving a minimum of 4-log reduction of *Salmonella* is necessary (USDA, 2007).

For cocoa there is neither legislation in force nor published data on risk assessment of *Salmonella*. Besides, little is known about the contamination level of this pathogen in cocoa beans and nibs. However, the National Confectioners Association Chocolate Council (NCACC, 2011) suggests reductions between 4 and 5-log for the roasting step. Based on this recommendation, according to the D-values calculated in the current study, while for cocoa beans the time–temperature combination of 24 min–110 °C, 18 min–120 °C, 13 min–130 or 140 °C will be needed to achieve 5-log reduction, for nibs, 44 min at 110 °C, 40 min at 120 °C, 19 min at 130 °C or 13 min at 140 °C should be used. For chocolate conching, if we consider an initial count of 1 log MPN/g and the lowest contamination level associated with an outbreak of *Salmonella* in chocolate, that was −1.37 log MPN/g (0.043 MPN/g), a reduction of 3-log will be necessary to result in a final count of −2 log MPN/g (0.01 MPN/g). To achieve that level of reduction, the process would take *ca.* 42 h at 50 °C, 13 h at 60 and 2.5 h at 70 °C.

Therefore, results in the current study have shown that the type of matrix (cocoa beans or nibs) played a significant role on the heat resistance of *Salmonella*. In addition, the results indicate that depending on the initial load and the process parameters time/temperature even when higher temperatures were used the death rates were not sufficient to achieve the detection limit. However, heat resistance data obtained under given experimental conditions cannot be used by cocoa and chocolate manufacturers to validate thermal processes. A risk assessment analysis is necessary to establish a safety level of *Salmonella* contamination in this kind of product and consequently parameters of its thermal processes.

Acknowledgment

This research was supported by the Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), process 11406-2.

References

ABC, Almond Board of California, 2007. Processing authority kit. http://www.almondboard.com/about/content.cfm?ItemNumber=25135andtoken=58482anduserID.

Abd, S.J., McCarthy, K.L., Harris, L.J., 2008. Use of the Weibull model to evaluate the impact of storage time and temperature on oil-roasted almonds. P1-33, IAFP 2008 Abstract Book. IAFP Annual Meeting, Columbus, OH, 3 to 6 August, 2008.

Andrews, W.H., Hammack, T.S., 2005. Salmonella. FDA, Food and Drug Administration, Bacteriological Analytical Manual Online http://www.cfsan.fda.gov/ebam/bam-5.html.

Barrile, J.C., Cone, J.F., 1970. Effect of added moisture on the heat resistance of *Salmonella* Anatum in milk chocolate. Applied Microbiology 19, 177–178.

Barrile, J.C., Ostovar, K., Keeney, P.G., 1971. Microflora of cocoa beans before and after roasting at 150 °C. Journal of Milk and Food Technology 34, 369–371.

Beckett, S.T., 2008. The Science of Chocolate, 2nd ed. Royal Society of Chemistry, Cambridge. 234 pp.

Beuchat, L.R., Mann, D.A., 2011. Inactivation of *Salmonella* on pecan nutmeats by hot air treatment and oil roasting. Journal of Food Protection 74, 1441–1450.

Cordier, J.L., 1994. HACCP in the chocolate industry. Food Control 5, 171–175.

D'Aoust, J.Y., 1977. *Salmonella* and the chocolate industry. A review. Journal of Food Protection 40, 718–727.

Food and Drug Administration, 2009a. Recall of products containing peanut butter: *Salmonella typhimurium*. http://www.fda.gov/oc/opacom/hottopics/salmonellatyph.html. Updated: January 28, 2009.

Food and Drug Administration, 2009b. Peanut Corporation of America (PCA), Blakely, GA:FDA form 483 (inspectional observations) January 9–27, 2008 [2009]. http://www.fda.gov/ora/frequent/default.html.

Goepfert, J.M., Biggie, R.A., 1968. Heat resistance of *Salmonella typhimurium* and *Salmonella senftenberg* 775W in milk chocolate. Applied Microbiology 16, 1939–1940.

Harris, L.J., 2008. Salmonella — factors affecting resistance to heat. IAFP Symposium S8 Validating Process for Reducing *Salmonella* in Low Water Activity Foods: IAFP Annual Meeting, August 3–6, Columbus, Ohio.

Hockin, J.C., D'aoust, J.Y., Bowering, D., Jessop, J.H., Khanna, B., Lior, H., Milling, M.E., 1989. An international outbreak of *Salmonella* nima from imported chocolate. Journal of Food Protection 52, 51–54.

ICMSF, International Commission on Microbiological Specifications for Foods, 2005. Cocoa, chocolate, and confectionery, Microorganisms in Foods 6: Microbiological Ecology of Food Commodities, 2nd ed. Kluwer Academic/Plenum Publishers, New York, pp. 467–479.

Izurieta, W.P., Komitopoulou, E., 2012. Effect of moisture on *Salmonella* spp heat resistance in cocoa and hazelnut shells. Food Research International 45, 1087–1092.

Krapf, T., Gantenbein-Demarchi, C., 2010. Thermal inactivation of *Salmonella* spp. during conching. LWT- Food Science and Technology 43, 720–723.

Lee, S.Y., Oh, S.W., Chung, H.J., Reyes-De-Corcuera, J.I., Powers, J.R., Kang, D.H., 2006. Reduction of *Salmonella enterica* serovars Enteritidis on the surface of raw shelled almonds by exposure to steam. Journal of Food Protection 69, 591–595.

Ma, L., Zhang, G., Gerner-Smidt, P., Mantripragada, V., Ezeoke, I., Doyle, M.P., 2009. Thermal inactivation of *Salmonella* in peanut butter. Journal of Food Protection 72, 1596–1601.

Mattick, K.L., JoRgensen, F., Wang, p, Pound, J., Vandeven, M.H., Ward, L.R., Legan, J.D., Lappin-Scott, H.M., Humphrey, T.J., 2001. Effect of challenge temperature and solute type on heat tolerance of *Salmonella* serovars at low water activity. Applied and Environmental Microbiology 67, 4128–4136.

Melo, L.L.M.M., Bolini, H.M.A., Efraim, P., 2007. Equisweet milk chocolates with intense sweeteners using time-intensity method. Journal of Food Quality 30, 1056–1067.

Nascimento, M.S., Silva, I.F., Silva, J.C., Reolon, E.M., Santos, A.R.B., Silva, N., 2010. Enteropathogens in cocoa pre-processing. Food Control 21, 408–411.

NCACC, National Confectioners Association Chocolate Council, 2011. Raw chocolate: risk and recommendations. The Manufacturing Confectioner 57–59.

Podolak, R., Enache, H., Stone, W., Black, D.G., Elliot, P., 2010. Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low — moisture foods. Journal of Food Protection 73, 1919–1936.

Schwan, R.F., Wheals, A.E., 2004. The microbiology of cocoa fermentation and its role in chocolate quality. Critical Reviews in Food Science and Nutrition 44, 1–17.

Shachar, D., Yaron, S., 2006. Heat tolerance of *Salmonella enterica* serovars Agona, Enteritidis, and Typhimurium in peanut butter. Journal of Food Protection 69, 2687–2691.

Stobinska, H., Krysiak, W., Nebesny, E., Kozanecka, E., 2006. Effects of convective roasting conditions on microbial safety of cocoa beans. Acta Agrophysica 7, 239–248.

Tamminga, S.K., Beumer, R.R., Kampelmacher, E.H., van Leusden, F.M., 1976. Survival of *Salmonella* Eastbourne and *Salmonella* Typhimurium in chocolate. Journal of Hygine 76, 41–47.

Tomlins, R.I., Ordal, Z.J., 1976. Thermal injury and inactivation in vegetative bacteria. In: Skinner, F.A., Hugo, W.B. (Eds.), Inhibition and Inactivation of Vegetative Microbes. Academic Press, London, pp. 153–190.

USDA, United States Department of Agriculture, 2007. Rule 7 CFR Part 981. Almonds grown in California, outgoing quality control requirements. Docket n. FV06-981-1 FR.

Van Asselt, E.D., Zwietering, M.H., 2006. A systematic approach to determine global thermal inactivation parameters for various food pathogens. International Journal of Food Microbiology 107, 73–82.

Werber, D., Dreesman, J., Feil, F., van Treeck, U., Fell, G., Ethelberg, S., Hauri, A.M., Roggentin, P., Prager, R., Fisher, I.S.T., Behnke, S.C., Bartelt, E., Weise, E., Ellis, A., Siitonen, A., Andersson, Y., Tschäpe, H., Kramer, M.H., Ammon, A., 2005. International outbreak of Salmonella Oranienburg due to German chocolate. BMC Infectious Diseases 5, 7–17.