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Research Note

# Thermal inactivation of *Salmonella* spp. during conching

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ABSTRACT

Although chocolate is a microbiologically stable product it has been described as a vehicle for *Salmonella* spp. Because of the low water activity ($a_w$) and the high fat content of chocolate *Salmonella* spp. shows an increased heat resistance, even during the thermal process of chocolate making. The aim of this study was to evaluate the thermal inactivation of *Salmonella* spp. during conching in various masses of chocolate and cocoa butter at different temperatures (50–90 °C). The effect of thermal treatment on *Salmonella* spp. was determined with the MPN (Most-Probable-Number) method. Results of thermal treatment showed approximate *D*-values for cocoa butter from $D_{50°C} = 245$ min to $D_{60°C} = 306$ min, for cocoa liquor from $D_{50°C} = 999$ min to $D_{90°C} = 26$ min and for dark chocolate of $D_{50°C} = 1574$ min. *z*-values were found to be $z = 20$ °C in cocoa liquor and $z = 14$ °C in dark chocolate. This study demonstrates that the conching process alone does not ensure the inactivation of *Salmonella* spp. in different chocolate masses and that an additional decontamination step at the beginning of the process as well as an HACCP concept is necessary during chocolate production to guarantee the absence of *Salmonella* spp. in chocolates and related products.



## 1. Introduction

Food poisoning caused by *Salmonella* spp. is of high relevance in the industrialized world. Although salmonellosis is often associated with the consumption of contaminated raw or undercooked food such as milk, eggs and (poultry) meat, chocolate has also been described as a vehicle for *Salmonella* spp. Since the 1970s several international outbreaks associated with food poisoning following the consumption of contaminated chocolate have been described (D'Aoust, 1977). The most recent international outbreak was reported in 2005 (Werber et al., 2005), in which the serovar *Salmonella* Oranienburg was involved. In 2006, a recall of chocolate contaminated with *Salmonella* spp. was reported in the United Kingdom (Anonym, 2006). As chocolate has a low $a_w$-value of 0.3–0.5 and high sugar content (approx. 60%) as well as high fat content (≥ 18%), microorganisms are not able to proliferate in it. Still, microorganisms may be present in the final product. However, beside low $a_w$-value of chocolate it has been suggested that certain food ingredients such as fat may protect microorganisms against thermal treatment during food processing (D'Aoust & Pivnick, 1976). Considering the high temperatures ranging from 110 to 140 °C – applied during the dry roasting of the cocoa beans, followed by conching the cocoa liquor at 50–80 °C for 12–24 h, one would assume that microorganisms such as *Salmonellae* would be completely inactivated. Nevertheless, studies already performed in the 1960s and 1970s indicated increased heat resistance of *Salmonella* spp. in milk chocolate and dark chocolate and the bacterial cells could neither be inactivated during manufacture nor during storage (Goepfert & Biggie, 1968; Rieschel & Schenkel, 1971; Tamminga, Beumer, & Kampelmacher, 1976, 1977). Although different studies on thermal resistance of *Salmonellae* in different food products have been performed (Doyle & Mazzotta, 2000), not many investigations have been published on the inactivation of *Salmonella* spp. during the conventional conching process of chocolate. Therefore the aim of this study was to assess the effectiveness of thermal treatment on *Salmonella* spp. during the conching process of chocolate. Additionally, experiments were carried out to determine thermal inactivation at 90 °C, although such high temperatures are not applied during long conching of chocolate. Furthermore, the range of chocolate products was extended to related products such as cocoa butter and cocoa liquor.

## 2. Materials and methods

### 2.1. Raw materials and their preparation

Cocoa butter, cocoa liquor and dark chocolate were obtained from a chocolate manufacturer in 10 kg plastic tubs and were tested free of *Salmonella* spp. according to the method described by the FOPH (2001). Further microbial loads of the matrices investigated,

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whether pathogenic or not, have not been tested. Chocolate masses were melted in plastic tubs for 18–24 h at the temperatures to be tested (50 °C, 60 °C, 70 °C, 80 °C, 90 °C) using an oven (type Leventi Holland, Leventi International). Additionally, 500 g chocolate mass was weighed into a sterile 1 L Duran bottle and kept melted at 50 °C in a water bath (Bain Marie Biostat®, Bioblock Scientific).

### 2.2. Bacteria and preparation of the inoculum

A strain of *Salmonella* spp., which had been isolated from chocolate related products and successfully identified using API 20E (BioMérieux, 20 100/20 160), was chosen for inactivation experiments. The frozen strain was reactivated aerobically overnight at 37 °C in 10 mL Brain Heart Infusion Broth (BHI, Biolife) and then stored on Plate Count slope agar (PC, Biolife) at 4 °C for max. 4 weeks. Colonies were harvested from the PC slope agar and reactivated overnight (aerobically, BHI broth, 37 °C). 200 μL of this culture was spread on 4 Brilliant Green-Phenol Red-Lactose-Sucrose agar plates (BPLS, Merck) and incubated aerobically overnight at 37 °C. The cell mass grown on these agar plates was harvested quantitatively, suspended in BHI broth, and homogenized by vortexing. The cell concentration of this suspension was determined by means of a counting chamber (Neubauer, depth: 0.01 mm). Cell suspensions with an approximate concentration of $10^{10}$ cells per mL were used as an inoculum.

### 2.3. Thermal treatment and simulation of conching

Conching of the chocolate masses was simulated in an ESCO mixer (type EL 6 glass, ESCO Labor AG). The double-wall glassware of the mixer was filled with water heated to the test temperatures and covered with tinfoil to avoid heat loss. During the tests the chocolate masses were stirred at U = 20/min for 23 h. Cocoa butter, chocolate liquor and dark chocolate were preheated as described (2.1.). Thermal treatments were performed at temperatures of 50 °C, 60 °C, 70 °C, 80 °C and 90 °C (±1 °C), except for cocoa butter which was only heated up to 80 °C. For the experiments 1.5 kg portions of the preheated chocolate mass were poured into the ESCO mixer, the temperature being controlled using a Pt 100 reading every 10 min. The *Salmonella* inoculum was added to the chocolate mass as soon as the chocolate mass had reached the final testing temperature (2.4.). All inactivation experiments on different masses and inactivation temperatures were carried out once.

### 2.4. Inoculation, sample preparation and determination of *Salmonella* spp.

An inoculum of 2 mL (2.2.) was added to 0.5 kg preheated chocolate in a Duran bottle (2.1.). The bottle was shaken vigorously by hand for approx. 3 min. Then the inoculated chocolate subset was combined with the 1.5 kg chocolate in the ESCO mixer to reach a final cell concentration of approx. $10^7$ cells per g product. The first sample was taken 30 min after inoculation, followed by further samples drawn every 90 min over a period of 8 h. Finally, the last sample was collected 23 h after inoculation. All samples were taken in duplicate.

For microbiological examination 10 g chocolate mass was added to 90 mL of NaCl solution (0.85% NaCl, 0.1% peptone). The suspension was homogenized for approx. 10 min on a heated (50 °C) magnetic stirrer plate until chocolate was dispersed homogeneously and was then diluted decimally (1:10). For the quantitative determination of *Salmonella* spp. the Most-Probable-Number method (MPN; detection limit: 0.30 cfu/g) with three parallels was applied. MPN tables from de Man (1983) were used to calculate the viable counts. This procedure (homogenization of chocolate in preheated NaCl solution, estimation by MPN) was tested before the heat treatment investigations. Comparing the direct plating method on BPLS agar and the MPN-method directly after the homogenization described, the latter showed the best recovery results. From each dilution, 1 mL was transferred to 9 mL Buffered Peptone Water (BPW, Oxoid Ltd.) and incubated (aerobically, 18–24 h, 37 °C). After incubation, 100 μL of turbid tubes was added to 9 mL Rappaport-Vassiliadis-Soya Broth (RVS-B, Oxoid Ltd.) and again incubated (aerobically, 24 h, 42 °C). Afterwards *Salmonella* spp. was confirmed by white colonies and change in colour on BPLS agar plates (aerobic incubation, 18–24 h, 37 °C).

### 2.5. Calculation of D- and z-value

For the determination of the *D*- (1) and *z*-value (2) of the heat resistance of *Salmonella* spp. in different chocolate masses, the following two equations were used. Data were analysed with MS Excel®.

$$D = \frac{t}{\lg \frac{N_0}{N}} \quad (1)$$

$$z = \frac{T_1 - T_2}{\lg \frac{D_2}{D_1}} \quad (2)$$

with: $t$: duration of heat treatment [min]; $N_0$: initial cell concentration; $N$: final cell concentration after heat treatment; $D_1$: $D$-value at temperature $T_1$; $D_2$: $D$-value at temperature $T_2$

## 3. Results

### 3.1. Thermal inactivation of *Salmonella* spp. in different chocolate masses

Trials on thermal inactivation were investigated in cocoa butter, cocoa liquor and dark chocolate at different temperatures (50 °C, 60 °C, 70 °C, 80 °C and 90 °C). Results of the trials performed with **cocoa butter** indicated that at 50 °C and 60 °C *Salmonella* spp. was detectable 5 h after inoculation (Fig. 1). The conching experiments at 50 °C showed an initial concentration of *Salmonella* spp. of $1.4 \times 10^7$ cfu/g at the beginning and led to a mean of 4.3 cfu/g after 23 h of treatment. Trials with thermal treatment at 60 °C showed

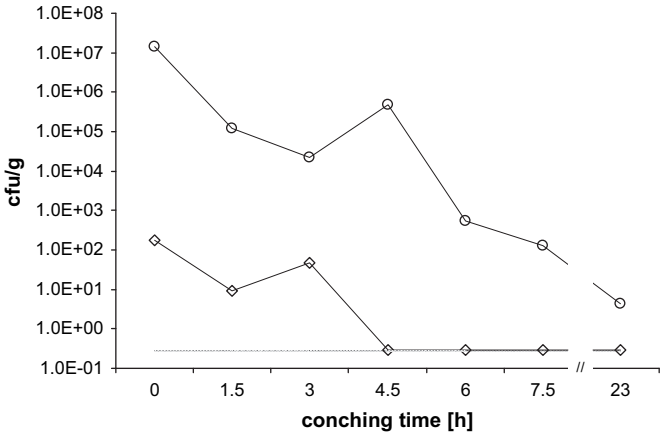


**Fig. 1.** Curves of thermal reduction of *Salmonella* spp. in **cocoa butter** during conching at different temperatures: (○) 50 °C, (◇) 60 °C, (·····) detection limit; mean of duplicate samples (deviation: ±1 $\log_{10}$).