



Contents lists available at ScienceDirect

## LWT - Food Science and Technology

journal homepage: [www.elsevier.com/locate/lwt](http://www.elsevier.com/locate/lwt)

## Research Note

Thermal inactivation of *Salmonella* spp. during conching

T. Krapf\*, C. Gantenbein-Demarchi

ZHAW Zurich University of Applied Sciences, School of Life Sciences and Facility Management, Institute of Food and Beverage Innovation, Gruental, CH-8820 Wädenswil, Switzerland

## ARTICLE INFO

## Article history:

Received 29 July 2008

Received in revised form

9 October 2009

Accepted 20 October 2009

## Keywords:

*Salmonella* spp.

Chocolate

Conching

 $D$ -value

MPN-method

## 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  $D_{50^\circ\text{C}} = 245$  min to  $D_{60^\circ\text{C}} = 306$  min, for cocoa liquor from  $D_{50^\circ\text{C}} = 999$  min to  $D_{90^\circ\text{C}} = 26$  min and for dark chocolate of  $D_{50^\circ\text{C}} = 1574$  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.

© 2009 Elsevier Ltd. All rights reserved.

## 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 ( $\geq 18\%$ ), 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,

\* Corresponding author.

E-mail address: [tamara.krapf@zhaw.ch](mailto:tamara.krapf@zhaw.ch) (T. Krapf).

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<sup>®</sup>, 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<sup>10</sup> 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<sup>7</sup> 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<sup>®</sup>.

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

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

with: *t*: duration of heat treatment [min]; *N*<sub>0</sub>: initial cell concentration; *N*: final cell concentration after heat treatment; *D*<sub>1</sub>: *D*-value at temperature *T*<sub>1</sub>; *D*<sub>2</sub>: *D*-value at temperature *T*<sub>2</sub>

## 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 × 10<sup>7</sup> 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

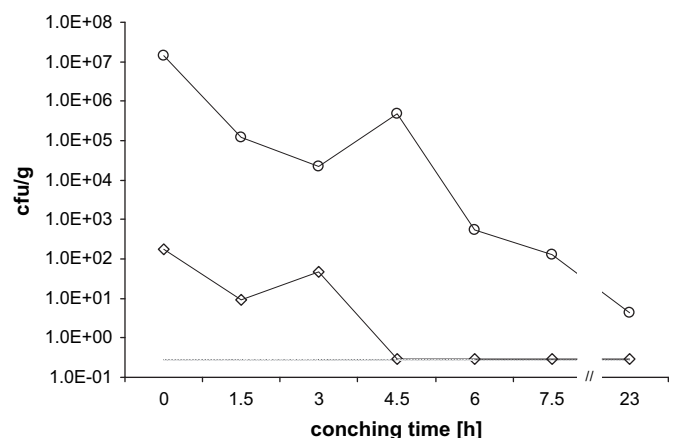


Fig. 1. Curves of thermal reduction of *Salmonella* spp. in **cocoa butter** during conching at different temperatures: (○) 50 °C, (◇) 60 °C, (---) detection limit; mean of duplicate samples (deviation: ±1 log<sub>10</sub>).

an initial load of *Salmonella* spp. of  $1.8 \times 10^2$  cfu/g. The microbial load of the sample taken 4.5 h after inoculation fell below the detection limit of  $<0.30$  cfu/g. Tests performed at 70 °C and 80 °C showed that *Salmonella* spp. was no longer detectable either at the beginning or at the end of the test (data not shown). In **cocoa liquor**, which contains cocoa butter and cocoa powder but no sugar, *Salmonella* spp. was not completely inactivated at 50 °C, 60 °C and 70 °C within 23 h of conching. The viable count reduction ranged from 1 to 6 log<sub>10</sub> starting with an initial concentration of approx.  $10^5$ – $10^7$  cfu/g. At 80 °C the concentration of *Salmonella* spp. fell below the detection limit after 7.5 h (Fig. 2). At 90 °C the same effect was observed after 3 h (data not shown). Inactivation experiments of *Salmonella* spp. during conching of **dark chocolate**, which is mainly composed of cocoa liquor (cocoa butter and cocoa powder) and sugar in varying amounts, showed the lowest reduction of *Salmonella* spp. (Fig. 3).

### 3.2. D- and z-values for *Salmonella* spp. in different chocolate masses

Table 1 shows the **D-values** which were determined for cocoa butter, cocoa liquor and dark chocolate. The values obtained are only of approximate relevance because inactivation experiments were investigated once for each temperature-time-profile and R<sup>2</sup> of the linear regression was not  $>0.9$  in every case. Nevertheless, the data indicate the heat resistance of *Salmonella* spp. subject to the different chocolate matrices. In general, *Salmonella* spp. showed the highest heat resistance in dark chocolate, whereas in cocoa butter D-values were at the lowest level. For cocoa butter, the D-values could not be estimated at 70 °C and 80 °C as only one data point above the detection limit was determined. z-values of 20 °C (cocoa liquor) and of 14 °C (dark chocolate) were calculated, but no z-value was calculated for cocoa butter.

## 4. Discussion

In general, the lowest reduction of *Salmonella* spp. was observed during conching of dark chocolate (3.1). Chocolate masses were inoculated with liquid droplets of cell suspension (2.2.), but the recovered initial cell concentrations were always below the calculated counts. In cocoa butter the initial cell concentration of approx.  $10^7$  cfu/g was reduced by 5 log<sub>10</sub> at 60 °C. In cocoa liquor and dark chocolate initial cell concentration was reduced by 2 log<sub>10</sub> for all temperatures of heat inactivation

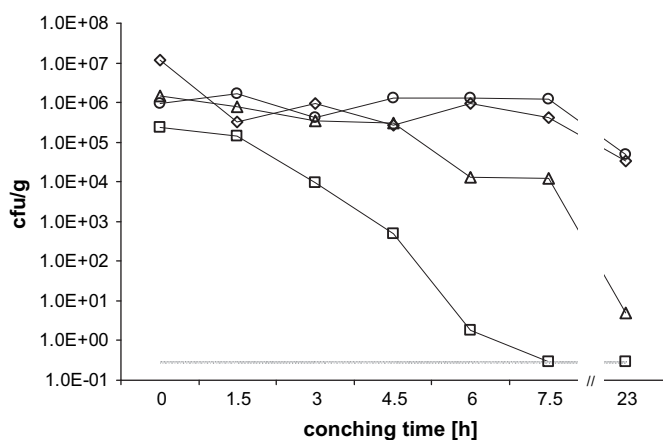


Fig. 2. Curves of thermal reduction of *Salmonella* spp. in **cocoa liquor** during conching at different temperatures: (○) 50 °C, (◇) 60 °C, (△) 70 °C, (□) 80 °C, (----) detection limit; mean of duplicate samples (deviation:  $\pm 1$  log<sub>10</sub>).

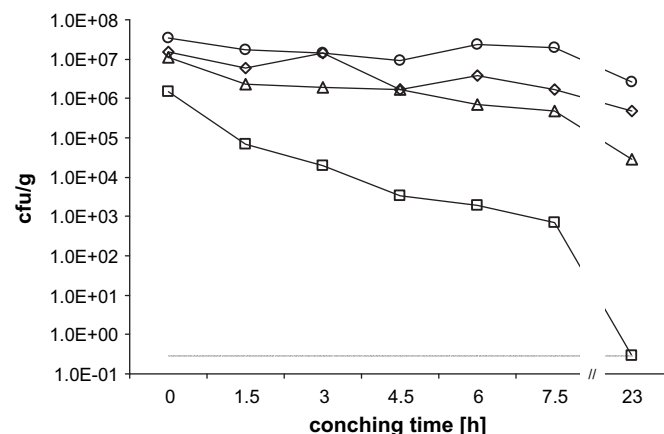


Fig. 3. Curves of thermal reduction of *Salmonella* spp. in **dark chocolate** during conching at different temperatures: (○) 50 °C, (◇) 60 °C, (△) 70 °C, (□) 80 °C, (----) detection limit; mean of duplicate samples (deviation:  $\pm 1$  log<sub>10</sub>).

investigated. A similar effect was reported by Sumner, Sandros, Harmon, Scott, and Bernard (1991) as well as by Juven et al. (1984), where a loss of 1–2 log<sub>10</sub> of the initial concentration was estimated. They suggested that an osmotic shock of wet inoculation might be responsible for their findings. Indeed, as thermal resistance in cocoa liquor was higher than in cocoa butter, it is likely that the liquid droplet of inoculum was hardly dissolved in cocoa butter due to the absence of solutes. Within these droplets the  $a_w$ -value was high and inactivation took place in the case of cocoa butter. This effect might also explain why *Salmonella* spp. were not detected at 70 °C and 80 °C in this matrix from the start of thermal treatments. As cocoa liquor and dark chocolate contain more solutes than cocoa butter, it is assumed that the  $a_w$ -value of the liquid inoculum was reduced due to better dissolution in these matrices. Therefore better survival of *Salmonella* spp. in cocoa liquor and dark chocolate can be understood. Conching of cocoa liquor and dark chocolate showed a lower reduction of *Salmonella* spp. than in cocoa butter. It appears that the high sugar content which lowers the  $a_w$ -value in chocolate contributes to the increase in heat resistance of *Salmonella* spp. For example, Baird-Parker, Boothroyd, and Jones (1970) reported an increase in the heat resistance of *Salmonellae* induced by glucose. Mattick et al. (2001), who investigated a mixture of glucose and fructose to reduce the  $a_w$ -value in broths, found that  $a_w$ -values of 0.65–0.90 exhibit some protection to *Salmonella* spp. at temperatures above 70 °C, whereas at lower temperatures low  $a_w$  increased the effect of thermal inactivation. This fact was also shown in our investigations where *Salmonella* spp. in cocoa butter showed a higher D-value at 60 °C than at 50 °C (Table 1). Davies, Blood, and Gibbs (1990) found z-values of 16.42–26.82 for *Salmonellae* in cocoa liquor, which are in the same range as our findings. We therefore assume that a combination of a low  $a_w$ -value as well as a possible protective effect of fat and other components such as proteins in the matrices may increase the heat resistance of *Salmonella* spp., as already suggested by D'Aoust and Pivnick (1976) and Hiramoto, Matsumoto, Sakae, and Miyazaki (2005).

Table 1

Overview of determined D-values [min] for *Salmonella* spp. in different chocolate masses

Product	D <sub>50°C</sub>	D <sub>60°C</sub>	D <sub>70°C</sub>	D <sub>80°C</sub>	D <sub>90°C</sub>
Cocoa butter	245	306	n.e.	n.e.	n.d.
Cocoa liquor	999	760	248	70	26
Dark chocolate	1570	1008	600	142	25

n.e. = not evaluable, n.d. = not determined.

## 5. Conclusions

Cocoa beans are exposed to microorganisms after harvesting and during both fermentation and the drying process and may be contaminated with pathogens such as *Salmonellae*. As chocolate production includes steps with high temperature exposure such as roasting and conching, one would assume that pathogenic microorganisms are inactivated. However, studies have shown that roasting (Peñaloza-Izurieta, Krapf, Diep, Benz, & Gantenbein-Demarchi, 2008) and conching (Rieschel & Schenkel, 1971) do not guarantee the thermal inactivation of *Salmonellae*. An ultrasonic conching process as described by Lee, Kermasha, and Baker (1989) may lead to a reduction of *Salmonella* spp. in thin films of chocolate. Nevertheless, as part of an integrated HACCP concept steam-based debacterisation of cocoa beans with guaranteed inactivation even of heat-resistant endospores of *Bacillus stearothermophilus* should be integrated at the very beginning of chocolate production (Cordier, 1994; Stehli, Gantenbein-Demarchi, & Benz, 2002). In combination with a defined zoning concept that separates raw and processed material, a debacterisation of cocoa beans might help to ensure the safety of chocolate.

## References

- Anonym. (2006). Press release, [www.food.gov.uk/enforcement/alerts/2006/jun/cadburychoc](http://www.food.gov.uk/enforcement/alerts/2006/jun/cadburychoc) [11.03.2008].
- Baird-Parker, A. C., Boothroyd, M., & Jones, E. (1970). The effect of water activity on the heat resistance of heat sensitive and heat resistant strains of *Salmonellae*. *Journal of Applied Bacteriology*, 33, 515–522.
- Cordier, J.-L. (1994). HACCP in the chocolate industry. *Food Control*, 5(3), 171–175.
- Davies A. R., Blood R. M., & Gibbs P. A. (1990). Effect of moisture level on the heat resistance of *Salmonellae* in cocoa liquor. Research Reports. Leatherhead Food R.A. (contact for this report: Dr. Evangelia Komitopoulou; email: [EKomitopoulou@LeatherheadFood.com](mailto:EKomitopoulou@LeatherheadFood.com))
- D'Aoust, J.-Y. (1977). *Salmonella* and the chocolate industry – a review. *Journal of Food Protection*, 40(10), 718–727.
- D'Aoust, J. Y., & Pivnick, H. (1976). Small infectious doses of *Salmonella*. *The Lancet*, 1, 866.
- Doyle, M. E., & Mazzotta, A. S. (2000). Review of studies on the thermal resistance of *Salmonellae*. *Journal of Food Protection*, 63(6), 779–795.
- FOPH – Swiss Federal Office of Public and Health (2001). Schweizerisches Lebensmittelbuch (SLMB), Kapitel 56: Mikrobiologie
- Goepfert, J. M., & Biggie, R. A. (1968). Heat resistance of *Salmonella typhimurium* and *Salmonella senftenberg 775W* in milk chocolate. *Applied Microbiology*, 16(12), 1939–1940.
- Hiramatsu, R., Matsumoto, M., Sakae, K., & Miyazaki, Y. (2005). Ability of shiga toxin-producing *Escherichia coli* and *Salmonella* spp. to survive in a desiccation model system and in dry Foods. *Applied and Environmental Microbiology*, 71(11), 6657–6663.
- Juven, B. J., Cox, N. A., Bailey, J. S., Thomson, J. E., Charles, O. W., & Shutze, J. V. (1984). Survival of *Salmonella* in dry food and feed. *Journal of Food Protection*, 47(6), 445–448.
- Lee, B. H., Kermasha, S., & Baker, B. E. (1989). Thermal, ultrasonic and ultraviolet inactivation of *Salmonella* in thin films of aqueous media and chocolate. *Food Microbiology*, 6, 143–152.
- de Man, J. C. (1983). MPN tables, corrected. *European Journal of Applied Microbiology and Biotechnology*, 17, 301–305.
- Mattick, K. L., Jørgensen, F., Wang, P., Pound, J., Vandeven, M. H., Ward, L. R., et al. (2001). Effect of challenge temperature and solute type on heat tolerance of *Salmonella* serovars at low water activity. *Journal of Applied and Environmental Microbiology*, 67(9), 4128–4136.
- Peñaloza-Izurieta, W., Krapf, T., Diep, B., Benz, K., & Gantenbein-Demarchi, C. (2008). Reduktion von Salmonellen bei unterschiedlichen Röstparametern. *Lebensmittel-Technologie*, 6, 10–12.
- Rieschel, H., & Schenkel, J. (1971). Das Verhalten von Mikroorganismen, speziell Salmonellen, in Schokoladewaren. *Alimenta*, 2, 57–66.
- Stehli, Ch., Gantenbein-Demarchi, C., & Benz, K. (2002). Sporeinaktivierung auf Kakaobohnen. *Lebensmittel-Technologie*, 35(7–8), 258–263.
- Sumner, S. S., Sandros, T. M., Harmon, M. C., Scott, V. N., & Bernard, D. T. (1991). Heat resistance of *Salmonella typhimurium* and *Listeria monocytogenes* in sucrose solutions of various water activities. *Journal of Food Science*, 56(6), 1741–1743.
- Tamminga, S. K., Beumer, R. R., & Kampelmacher, E. H. (1976). Survival of *Salmonella eastbourne* and *Salmonella typhimurium* in chocolate. *Journal of Hygiene*, 76, 41–47.
- Tamminga, S. K., Beumer, R. R., & Kampelmacher, E. H. (1977). Survival of *Salmonella eastbourne* and *Salmonella typhimurium* in milk chocolate prepared with artificially contaminated milk powder. *Journal of Hygiene*, 79, 333–337.
- Werber, D., Dreesmann, J., Feil, F., van Treeck, U., Gell, G., Ethelberg, S., et al. (2005). International outbreak of *Salmonella* Oranienburg due to German chocolate. *BMC Infectious Diseases*, 5(7), 1472–2334.