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# Predictable and unpredictable survival of foodborne pathogens during non-isothermal heating

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ABSTRACT

In previous work, extreme survival of various bacterial species during cooking was reported when attached to chicken meat. In this paper the effects of an extremely high challenge temperature on survival of *Salmonella* Typhimurium and *Campylobacter jejuni*, attached to chicken breast fillets or pork to test for matrix effects are reported. Survival was predicted, using standard *D*- and *z*-values from the literature, and compared to experimentally obtained data. Attached to meat, both *S*. Typhimurium and *C. jejuni* survived longer than predicted, longer when attached to chicken meat than when attached to pork.

Additionally, the effect of non-isothermal heating on survival of *almonella* in buffer is described. In buffer, when slowly heated, *Salmonella* died off as predicted. When *Salmonella* was heated in buffer according to a heating profile mimicking that of the surface of meat in boiling water, it appeared that cells died off much slower than predicted. It is shown that the thermal characteristics of *Salmonella* surviving the first 35 s of fast heating had changed. After these 35 s, remaining *Salmonella* survived for minutes, even at a challenge temperature of 90 °C.

During heating, cell size decline was observed. A loss of intracellular water during cooking might have resulted in smaller, dehydrated cells, in cells with altered thermal resistance characteristics. This could explain why the use of standard *D*-and *z*-values did not allow the correct prediction of survival of *Salmonella* during fast heating in buffer, or during cooking, being attached to the surface of meat.

Many factors affect the level of heat resistance of bacteria. The results of this and a former study show that attachment to meat contributes to an increased level of heat resistance of bacteria. A fast heating process further contributes to the increased level of heat resistance possibly as the result of changed thermal characteristics due to a loss of water.

## 1. Introduction

Adequate thermal processing or cooking is the most important as well as one of the simplest methods to eliminate pathogenic parasites, bacteria and viruses from food. However, inadequate reheating was identified as a high risk factor for Salmonellosis (Mataragas et al., 2008). Cross-contaminated undercooked meals, ready-to-eat poultry-meat products with extended shelf life and partially cooked products intended to be reheated are high risk products (Mataragas et al., 2008). *Salmonella* infections are also often associated with the consumption of raw or undercooked poultry (EFSA, 2007).

According to Dutch legislation, *Salmonella* should be absent in products intended to be consumed without any further hazard reducing treatment. The general European food law (EC) No 178/2002 “shall pursue one or more of the general objectives of a high level of protection of human life” (Anonymous, 2002). In the EC commission regulation (EC) No 2073/2005 (Anonymous, 2005), food safety criteria for *Salmonella* are described which vary between absent in 5 samples of 10 or 25 g product, depending on the type of food category. In 2011, the (EC) No 2073/2005 was amended ((EU) No 1086/2011; Anonymous, 2011) and a food safety criterium (1.28) for *Salmonella* Enteritidis and *S*. Typhimurium in raw poultry meat (absent in 5 samples of 25 g) was added. In the United States, regulations are provided to ensure destruction of pathogenic bacteria and viruses that may be present in raw meat and poultry products. For meat products *Salmonella* is the microorganism of concern. This organism is associated with a high number of outbreaks, it is more heat resistant than *E. coli* O157 and expected contamination levels are higher than of the more heat resistant *Listeria monocytogenes*, which is usually present as the result of recontamination. Any time/temperature protocol should result in a 6.5 log reduction of *Salmonella* (USDA-FSIS, 1999).

The heat resistance of bacteria can be described by *D*- and *z*-values.

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Reviews on the heat resistance of *Salmonellae* were written by Doyle and Mazzotta (2000), who focused on factors affecting the heat resistance of *Salmonella*, Sörqvist (2003) collected and summarized data of heat resistance in liquids of various species, among which *Salmonella*, and Van Asselt and Zwietering (2006) collected *D*- and *z*-values for *Salmonella* and other food pathogens and determined average *D*- and *z*-values. Based on reported *D*-values, Sörqvist (2003) determined that at a certain temperature, the *D*-value for *Salmonella* in liquids can be described by:

$$\log D(\mathrm{s}) = 12.9511 - 0.19282 \times \mathrm{T}\,(^{\circ}\mathrm{C}).$$

According to Doyle and Mazzotta (2000), *D* is best described by:

$$\log D(\mathrm{min}) = 11.7 - 0.188 \times \mathrm{T}\,(^{\circ}\mathrm{C}).$$

From both formula's it follows that at 70 °C, a tenfold reduction is achieved within 2.1 (Doyle and Mazzotta) or 0.3 (Sörqvist) seconds. Based on 1141 *D*-values collected from the literature, Van Asselt and Zwietering (2006) calculated an average *D*-value at 70 °C of 0.147 min (8.8 s) for *Salmonella*, based on:

$$\log D(\mathrm{min}) = 6.86 - 0.110 \times \mathrm{T}\,(^{\circ}\mathrm{C}).$$

The reported *D*-values (measured in various products) showed a large variation, with an upper limit of the 95% prediction interval of 3.9 min at 70 °C ($\log D(\mathrm{min}) = 8.28 - 0.110 \times \mathrm{T}\,(^{\circ}\mathrm{C})$).

*Salmonella* heat resistance generally increases with reducing moisture (Podolak et al., 2010). High resistance to high temperatures is found in food products with a low water and high fat content, like chocolate and peanut butter (Goepfert and Biggie, 1968; Ma et al., 2009; Shachar and Yaron, 2006). The level of protection to high temperatures offered by a low moisture environment varies with type of solute. At 55 °C and an $a_w$ of 0.9, sucrose is giving a better protection than a mixture of glucose/fructose, a mixture of glucose/fructose giving a better protection than NaCl (Mattick et al., 2001). A similar difference in protective effect between glucose and NaCl was reported by Aljarallah and Adams (2006). It was suggested that (accumulated) solutes protected specific targets for heat inactivation (Aljarallah and Adams, 2006; Mattick et al., 2001). Additionally, residence in a low $a_w$ environment might have resulted in dehydrated cells, with improved heat resistance (Kirby and Davies, 1990).

The effect of attachment to meat on the heat resistance of bacteria was already described by Humphrey et al. (2003). Morild et al. (2011) mentioned that there are no significant differences in surface attachment, between *Salmonella*, *Yersinia* and *Listeria*, which indicates that surface and environmental factors may influence attachment more than bacterial properties. A more profound location of attached bacteria at muscle compared to skin was indicated. In muscle tissue, bacteria tended to "hide" between the muscle fibres and may be entrapped at those sites.

Other factors, among which type of strain, physiological status of the cell, the presence of food additives and experimental setup further affect the heat resistance of bacterial species (Doyle and Mazzotta, 2000).

When determining *D*-values, a small amount of a bacterial culture is transferred to a relatively large volume of preheated medium of which the temperature is kept constant. To test for the heat resistance of bacteria in food or (ground) meat products, bacteria are usually mixed with a small amount of such products. Samples are compressed in a bag into a thin layer and submerged in a temperature-controlled water bath. Experiments are usually performed within a temperature range of 50 to 65 °C, as the inactivation of bacterial cells starts at approximately 50 °C, and inactivation rates are too high at temperatures above 65 °C to allow for accurate measurements. In poultry products, $D_{55}$ values of 43.3 to 6.9 min have been reported (Bucher et al., 2008; Murphy et al., 1999, 2004a, 2004b; Tuntivanich et al., 2008).

In a recent paper (De Jong et al., 2012), survival at high temperature of *Salmonella* on a chicken breast fillet was studied using a consumer-style cooking technique. Unexpectedly, under such non-isothermal conditions, *Salmonella* survived for a long period of time. Submerged in boiling water, a tenfold reduction of the number of bacteria applied to the surface of fillets required 2 min, whereas the surface temperature reached 70 °C within 30 s, a temperature at which the *D*-value is at most in the order of a few seconds. This extreme heat resistance of *Salmonella* on chicken meat was also observed in other species, like *Escherichia coli*, *Campylobacter jejuni* and *Lactobacillus casei* (De Jong et al., 2012). The fact that extreme heat resistance was observed in a variety of micro-organisms and that cells became extreme heat resistant within a very short period of only 30 s, too short to allow for microbial adaptation (Guisbert et al., 2008), suggest that the mechanism behind the extreme level of heat resistance might have a physical rather than a physiological, adaptive character.

The cooking of biological tissue, like muscle tissue, results in the loss of water caused by shortening of intracellular proteins (Wu et al., 2007). Such cooking losses are greater in fast cooked (high temperature) tissue than in slow cooked (low temperature) tissue (King et al., 2003; Wu et al., 2007). Like muscle tissue, sudden exposure of bacteria to an extremely high temperature, as in the study by De Jong et al. (2012) might have resulted in a loss of water and consequently in smaller cells with an increased level of heat resistance as in a low $a_w$ environment (Kirby and Davies, 1990).

In this paper, the matrix effects of chicken breast fillet, pork and buffer on survival of *S*. Typhimurium and *C. jejuni* at an extremely high challenge temperature are reported. To test for the effect of the heating rate, survival of *Salmonella* was measured in a small (fast heating) or large (slow heating) volume of buffer. Observed and predicted survival rates were compared. Cell size changes during heating, as a possible explanation for increased heat resistance, were monitored by optical density measurements.

## 2. Materials and methods

### 2.1. Bacterial strain and growth media

*Salmonella* Typhimurium 7945, a DT104 serovar isolated in our laboratory and a cocktail of five *Campylobacter jejuni* strains (NCTC 11168, NCTC 11828, B258, LB99hu and 82/69) were stored and cultured as described by De Jong et al. (2012).

### 2.2. Preparation of inoculated meat products

Chicken breast (140–175 g) and pork fillets (100–175 g) were bought at local stores and stored at −20 °C. Prior to inoculation, meat products were thawed overnight at 5 °C. After thawing, meat products were placed on a paper tissue and dried for 1 h in a laminar flow cabinet, each side 30 min. After drying, 1 ml of an o/n culture of bacteria (approximately $10^9$ CFU/ml) was spread over the surface of a piece of meat, 0.5 ml on each side. Inoculated meat products were stored overnight at 5 °C in a plastic bag.

### 2.3. Cooking, sampling and microbiological enumeration procedures

Inoculated, refrigerated fillets (chicken, pork) were transferred to 4 l of boiling water (one item at a time) at time zero and heated for 0 to 18 min. After each heat treatment, the fillet was immediately transferred into a sterile blender (a Waring HGB2WT, Torrington, Connecticut, US), weighed, and together with 200 ml phosphate buffered saline (PBS, 4 °C; Biotrading, the Netherlands) blended for 1 min. The pan was washed up after cooking for every single food item and clean tap water was brought to the boil again before adding another inoculated fillet.

For experiments in which PBS was used as heating menstruum, a test tube containing 10 ml of PBS, or a 300 ml glass bottle filled with 160 ml of PBS were inoculated with 0.1, respectively 1.6 ml of an o/n

culture of *Salmonella*. The tube or bottle was placed in 4 l of boiling water and heated for 0 to 10 min. After heat treatment, 10 ml tubes were directly cooled in ice-water. To monitor survival in 160 ml PBS, samples of 1 ml were taken at various time intervals and transferred to 9 ml of a peptone (1 g/l) physiological saline (9 g/l NaCl solution (PPS) that was kept on melting ice. Experiments were conducted at least in duplicate, on different days.

Chicken breast fillets and pork meat were sampled and analyzed as described by De Jong et al. (2012). Culturability of the inoculated bacteria on the food items after heat-treatment was determined by use of the Most Probable Number method (MPN, see DeMan (1975)) in combination with spread-plating suitable dilutions on agar plates. The lower level of detection used was 1.4 organism/food item. Media used for the MPN method and plate counts, respectively, were as described by De Jong et al. (2012): Preston broth and Karmali agar for *C. jejuni*, for *S.* Typhimurium Buffered Peptone Water (NVI) and Brilliant Green Agar (Oxoid) were used. MPN samples were checked for growth of the respective organisms after incubation by streak plating on above mentioned agar plates. Suspected colonies of *C. jejuni* were confirmed by phase contrast microscopy. Media for *S.* Typhimurium were aerobically incubated overnight at 37 °C. *C. jejuni* media were microaerobically incubated (broth: 48 h, agar: 72 h; 37 °C) either in a three-gas incubator (5% $CO_2$, 10% $O_2$, 85% $N_2$) or in jars with BBL Campypak (Becton Dickinson, Sparks, USA). The contamination levels of the food items after heat treatment were calculated, taking into account the exact weights used for enumeration, using an Excel spreadsheet based on the MPN method described by DeMan (1975).

When testing for the effect of the heating rate in PBS, *Salmonella* was counted by spread-plating on BGA only.

### 2.4. Optical density measurements

Changes in optical density of bacterial suspensions were monitored at 660 nm, using a Pharmacia Biotech Ultrospec 1000 spectrophotometer.

### 2.5. Calculations

#### 2.5.1. Temperature of chicken fillets

The temperature at the surface of a chicken fillet in boiling water was both calculated and measured as described by De Jong et al. (2012).

#### 2.5.2. Temperature profiles in PBS

To describe the temperature profile during fast and slow heating, test tubes containing 10 ml or bottles containing 160 ml PBS were places in a pan with 4 l of boiling water. Using an ASL precision thermometer F200 (ASL LTD, UK), the temperature was measured at fixed, regular time intervals. Experiments were repeated at least three times. Average temperature measurements at specific time points were used to build temperature profiles. As a temperature control during survival experiments in 10 or 160 ml PBS we measured the temperature in a parallel test tube or bottle.

### 2.6. Heat resistance parameters

The *D*-value is the time of heat treatment required at a certain temperature to destroy 90% of the bacterial cells, the *z*-value is the number of degrees of temperature change needed to change the *D*-value by a factor of 10. *D*- and *z*-values are related according to:

$$z = (T_1 - T_2)/(\log D_2 - \log D_1). \quad (1)$$

The *z*-value is used to calculate the lethality (F) of any time/temperature combination:

$$F = 10\text{^}((T_{x,t} - T_{ref})/z). \quad (2)$$

We used a $T_{ref}$ of 55 °C, a $D_{ref}$ -value of 222 s and a *z*-value of 5.2 °C (Sörqvist, 2003). In combination with the calculated or measured temperature profiles described above, the overall lethality during heating can be calculated. For example, heating for 1 s at 60.2 °C has the same lethal effect of $10^{((60.2-55)/5.2)} = 10$ s at 55 °C and results in a 10/222 = 0.045 log reduction. For a non-isothermal process of 600 s, the overall thermal pathogen lethality is calculated by accumulation of F:

$$\sum_{s=0}^{s=600} F, \quad (3)$$

using time intervals of 1 s during which the temperature was considered to be constant. After each interval the log number of bacteria equals the log number of bacteria before the interval minus F.

## 3. Results

Refrigerated chicken breast fillets with *Salmonella* spread over the surface were put in a pan with boiling water for various periods. The pan was constantly heated and the water temperature did not drop below 99 °C (not shown). *Salmonella* could still be detected on chicken breast fillet after 9 min (Fig. 1A). The cell log number declined following a straight line that was best described by: $\log N_{(t)} = 9.4 - 0.65 \times t$ ($R^2 = 0.96$) from which a *D*-value of 1.54 min was calculated. With pork instead of chicken breast fillets (Fig. 1B) we measured a *D*-value of approximately 1 min: $\log N_{(t)} = 8.9 - t$ ($R^2 = 0.81$). Again, unexpectedly high, though smaller than when using chicken meat.

Experiments with *Campylobacter jejuni* gave similar results (Fig. 2). They survived unexpectedly long when inoculated on the surface of chicken meat (tenfold reduction time: 2.9 min) and survival on pork (tenfold reduction time: 1.46 min) was similar, though less longer than when using chicken meat (Fig. 2).

### 3.1. Temperature profiles

We measured the surface temperature ($T_M$) of chicken fillets (140–175 g) in boiling water by pressing a sensor to the surface. We also calculated the temperature profile 1 mm under the surface of a chicken breast fillet ($T_C$; see Materials and methods section). The measured and calculated heating profiles are shown in Fig. 3. According to both methods, the surface temperature reached at least 70 °C after 30 s.

### 3.2. Heating profiles and survival predictions

Based on *D*- and *z*-values from the literature (Sörqvist, 2003) in combination with the heating profile based on $T_C$, we calculated the process lethality F for *Salmonella* (see Materials and methods section). Results of this calculation and of calculations based on the 95% upper prediction level for *Salmonella* in 'various products' (Van Asselt and Zwietering, 2006) were compared with the experimentally obtained data see Fig. 4.

The number of surviving *Salmonella* strongly differed from our predictions. To check the validity of our calculations, we predicted and measured survival in 160 ml PBS of which the temperature increased with 0.2 °C per second, following a profile that was best described by:

$T_{160ml} = -1.20 \times t^2 + 19.98 \times t + 15.96$ ($r^2 = 0.99$; n = 6; t in seconds). As shown in Fig. 5A, under non-isothermal conditions, *Salmonella* died-off as predicted.

The surface temperature of chicken breast fillets ($T_C$) reached 70 °C within 30 s. whereas 160 ml buffer reached 70 °C only after 4 min. In order to mimick the heating rate of chicken breast fillets, we used a smaller amount of buffer, 10 ml instead of 160 ml. The temperature profile of 10 ml of buffer in 4 l of boiling water fitted to

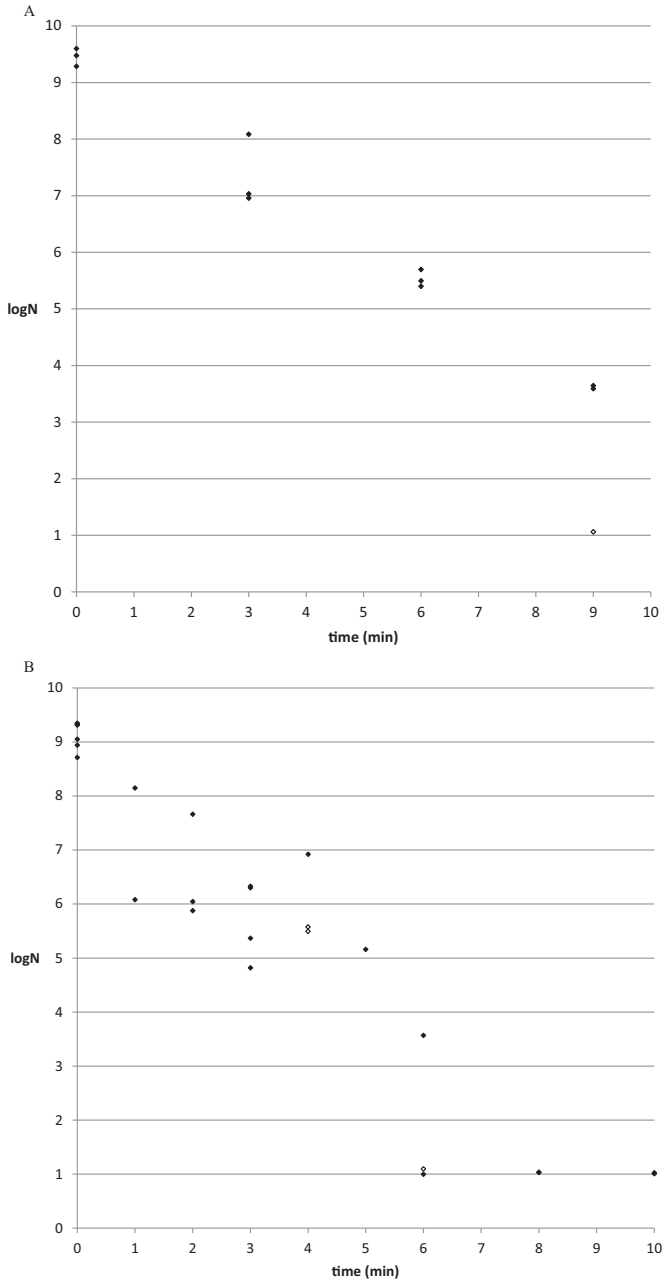


**Fig. 1.** Survival of *Salmonella* on the surface of chicken meat (1A) and pork (1B).
Open symbols: counts below detection limit. LogN: log number of bacteria per ite (of about 150 g).

$T_{10ml} = -66.34 \times t^2 + 136.22 \times t + 20.79$ ($r^2 = 0.99$; n = 6; t in seconds) and mimicked the $T_C$-based heating profile of chicken breast fillets: a temperature of 70 °C was reached within 30 s (Fig. 5B). In 10 ml of buffer we also predicted and measured survival of *Salmonella* and now observed that *Salmonella* survived for a longer period of time than predicted. We could still detect *Salmonella* after 60 s when the temperature reached 90 °C.

These results suggested that the heat resistance characteristics of *Salmonella* had changed during the first 35 s of fast heating. This was confirmed by the results of experiments in which we tested survival at a constant temperature after first being rapidly or slowly heated. Bacteria slowly heated to 60 °C behaved as predicted when subsequently kept at a constant temperature of 60 °C (Fig. 6A), but rapidly heated bacteria were unexpectedly resistant when transferred to a constant temperature of 60 °C or even 90 °C (Fig. 6B).

Rapid heating of bacteria might have resulted in a loss of water and consequently in smaller cells with an increased level of heat resistance as in a low $a_w$-environment (Kirby and Davies, 1990). The optical density of a bacterial suspension reflects the cell size (effective surface area: $\pi r^2$) and number of cells (Stevenson et al., 2016). As *Salmonella* does not grow during cooking and cooking of biological tissue might result in loss of water, any change in the optical density of a culture during cooking might reflect a change in cell size. We tested for cell size changes during cooking of *Salmonella* using a spectrophotometer and observed that the OD660 declined during heating. Fast heating of 10 ml resulted in a fast decline in OD660, but the optical density also declined during slow heating of 160 ml. When plotted against the temperature, it turned out that in the temperature range from 20 to 99 °C the OD660 declined with increasing temperatures independently from the heating rate (Fig. 7). From the observed changes in optical density in response to the temperature raise, we calculated (not shown) that the volume ($4/3\pi r^3$) declined 0.22% per degree Celsius raise. This means that at 70 °C the volume had dropped by approximately 10%.

## 4. Discussion

In a previous paper (De Jong et al., 2012), we reported on unexpected survival of various bacteria, among which *Salmonella*, *E. coli*, *Lactobacillus* and *Campylobacter*, on chicken breast fillets in boiling water. In this contribution, using *Salmonella* and *Campylobacter*, this phenomenon was repeatedly observed. We also showed that both *Salmonella* and *Campylobacter* survived for an unexpectedly long period when attached to pork. In buffer, using standard *D*- and *z*-values, survival of *Salmonella* under non-isothermal conditions was as predicted, but only if the heating rate was relatively slow. At a relatively fast heating rate, *Salmonella* survived longer than predicted, still being detectable after 1 min when the temperature had reached 90 °C. During rapid heating, the thermal resistance characteristics of *Salmonella* changed. Cells that survived after 35 s of rapid heating survived for minutes when subsequently challenged at 60 or 90 °C.

Many factors contribute to the heat resistance of bacteria like the type of matrix and species. We used three different matrices, chicken meat, pork and a liquid buffer and two types of bacteria. Using comparable heating rates (approximately 1.4 °C/s), both *Salmonella* and *Campylobacter* survived longer when attached to chicken meat than to pork. The thermal conductivity of chicken breast meat (0.412 W/(m·K) is lower than of pork (0.505 W/(m·K) (http://pcfarina.eng.unipr.it/Public/Termofluidodinamica/Utility/Tabelle%20Alimenti.pdf). This means that the heat transfer rate of pork is higher and that the temperature of pork increases more quickly, leading to faster heating of attached bacterial cells. A difference in thermal conductivity between chicken breast meat and pork thus could explain the observed difference between survival on chicken meat and pork.

In buffer, at a fast heating rate of approximately 1.6 °C/s, *Salmonella* also survived for a longer period than predicted, but not as long as when attached to chicken meat or pork. This might be explained by the fact that bacteria are fully surrounded by heating medium in buffer unlike when being attached to meat. The difference might also have resulted from a difference in physiological status, either being attached to meat or suspended in buffer or, again, from a difference in matrix composition. This was not further tested.

The experimental set-up is known to affect the heat resistance of bacteria. We varied in experimental set-up by using different matrices (see above) and we varied, using buffer as a matrix, in heating rate. Survival was as predicted if the temperature of buffer increased slowly from 20 to 90 °C (approximately 0.2 °C/s). Within the same temperature range however, *Salmonella* numbers declined slower than predicted if the temperature increased with 1.6 °C per second.

*D*- and *z*-values were taken from the literature. Such parameters are determined and can be used under isothermal conditions. Inactivation

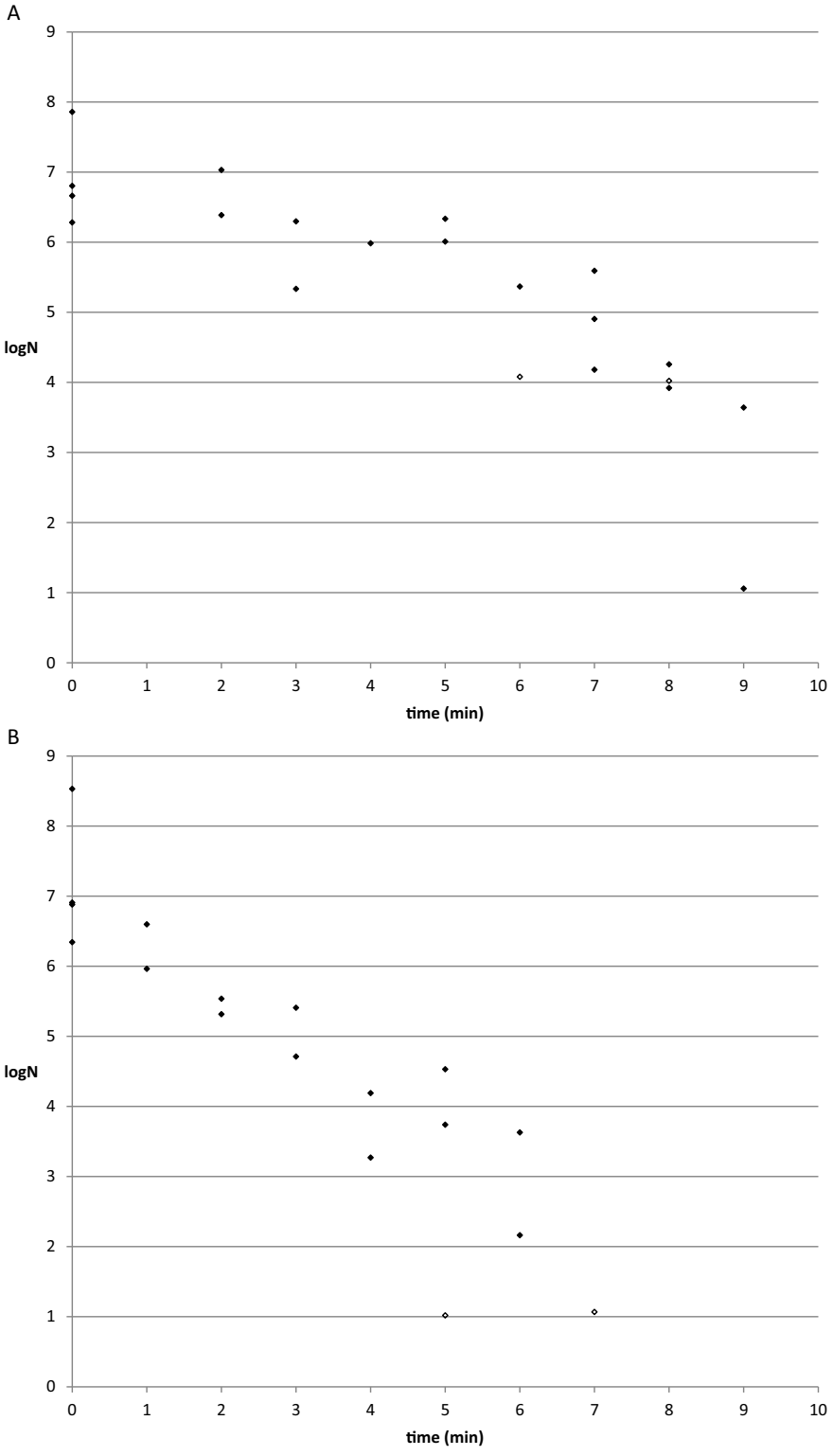


**Fig. 2.** Survival of *Campylobacter* on the surface of chicken meat (2A) and pork (2B).
Open symbols: counts below detection limit. Data points are from different experiments. For each experiment, different time intervals were used. LogN: log number of bacteria per item (of about 150 g).

under non-isothermal conditions cannot always be estimated from such *D*- and *z*-values, as microbial adaptation or sensibilization can occur during the heating up. Guisbert et al. (2008) showed that in *Escherichia coli* the activation of the heat shock response during a temperature increase from 30 to 42 °C requires more than 1 min and that *E. coli* is fully adapted after 5 min. This heat shock response therefore seems not relevant as in our experiments a temperature of 70 °C was reached within half a minute. Hassani et al. (2007) showed that predictions on survival of both *S*. Typhimurium and *S*. Senftenberg under non-isothermal conditions, using standard *D*- and *z*-values, were close to the experimental data. The same observation was done by Mackey et al. (2006). And we were able to predict survival of *Salmonella* using a slow

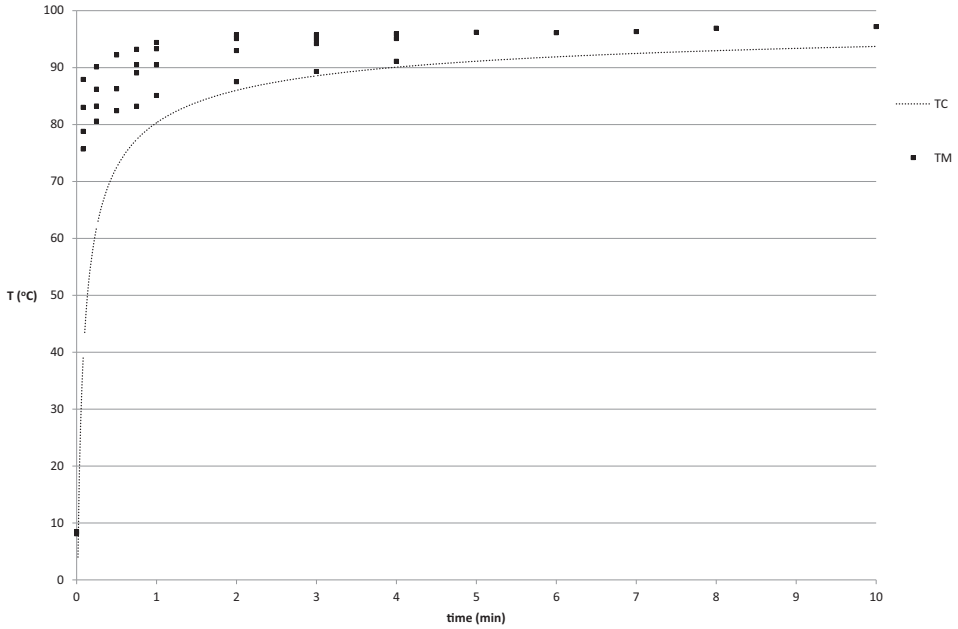


**Fig. 3.** Heating profiles of chicken refrigerated chicken breast fillets in boiling water. $T_C$: calculated temperature, 1 mm under the surface; $T_M$: measured temperature.

non-isothermal cooking procedure. Apparently, when determining the lethality of a non-isothermal process for *Salmonella*, standard *D*- and *z*-values can be used, but not under all non-isothermal conditions.

An intriguing observation was the constant die off rate of both *Salmonella* and *Campylobacter* under non-isothermal conditions. We calculated that the heat resistance of the *Salmonella* attached to chicken meat equaled that of *Salmonella* isothermally challenged at 56 °C (not shown). The fact that they were challenged at a much higher temperature indicated that the thermophysical characteristics had changed before the temperature reached a lethal value and that the characteristics kept changing as the temperature increased while the die off rate remained constant. That the thermophysical characteristics had changed was confirmed by the results shown in Fig. 6 where *Salmonella* did not rapidly die at a constant temperature of 60 °C or even 90 °C after being first rapidly heated for 35 s.

The heat transfer across the bacterial membrane occurs via conduction and is described by Fourier's law: $Q = -\lambda \cdot dT/dx$, in which Q is the amount of heat transferred per unit time, λ the conductivity and dT/dx the temperature difference over a cell membrane with thickness x. Intracellular heat transfer occurs via convection, which is described by: $Q = h \cdot A \cdot (T_{cm} - T_c)$ where Q is the heat transferred per unit time, h is the heat transfer coefficient, A is the area of the cell membrane, $T_{cm}$ is the cell membrane's temperature and $T_c$ is the temperature of the cytoplasm. The heat transfer coefficient h depends on the physical properties of the cytoplasm.

If the assumption that cells stay intact (no cell disruption) during the heating process is correct, then the observed decline in optical density during heating reflects a decline in surface area ($(\pi r^2)$ and thus in volume of cells ($4/3\pi r^3$) which can be explained by a loss of water. This loss of water could have resulted in a lower heat transfer coefficient (h) of the cytoplasm. A lower water content is also known to protect bacterial spores from heat stress (Setlow and Johnson, 2001). The observation that *Salmonella* survived for more than 3 min during slow heating, while the optical density of this suspension declined

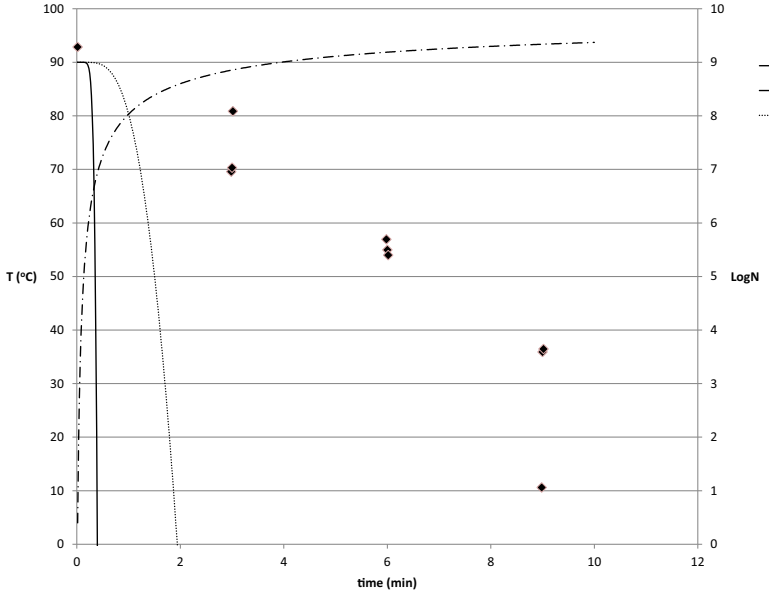


**Fig. 4.** Observed and predicted number of *Salmonella* on a chicken breast fillet in boiling water. $T_C$: calculated temperature profile (see Fig. 3). LogN: log number of bacteria per item (see Fig. 1A). For the predictions, the following *D*- and *z*-values were used:
Predicted logN: $D_{55}$: 222 s; $z$ = 5,2 °C (Sörqvist, 2003); predicted logN upper 95%: $D_{70}$ = 233 s; $z$ = 9.1 °C (Van Asselt and Zwietering, 2006).

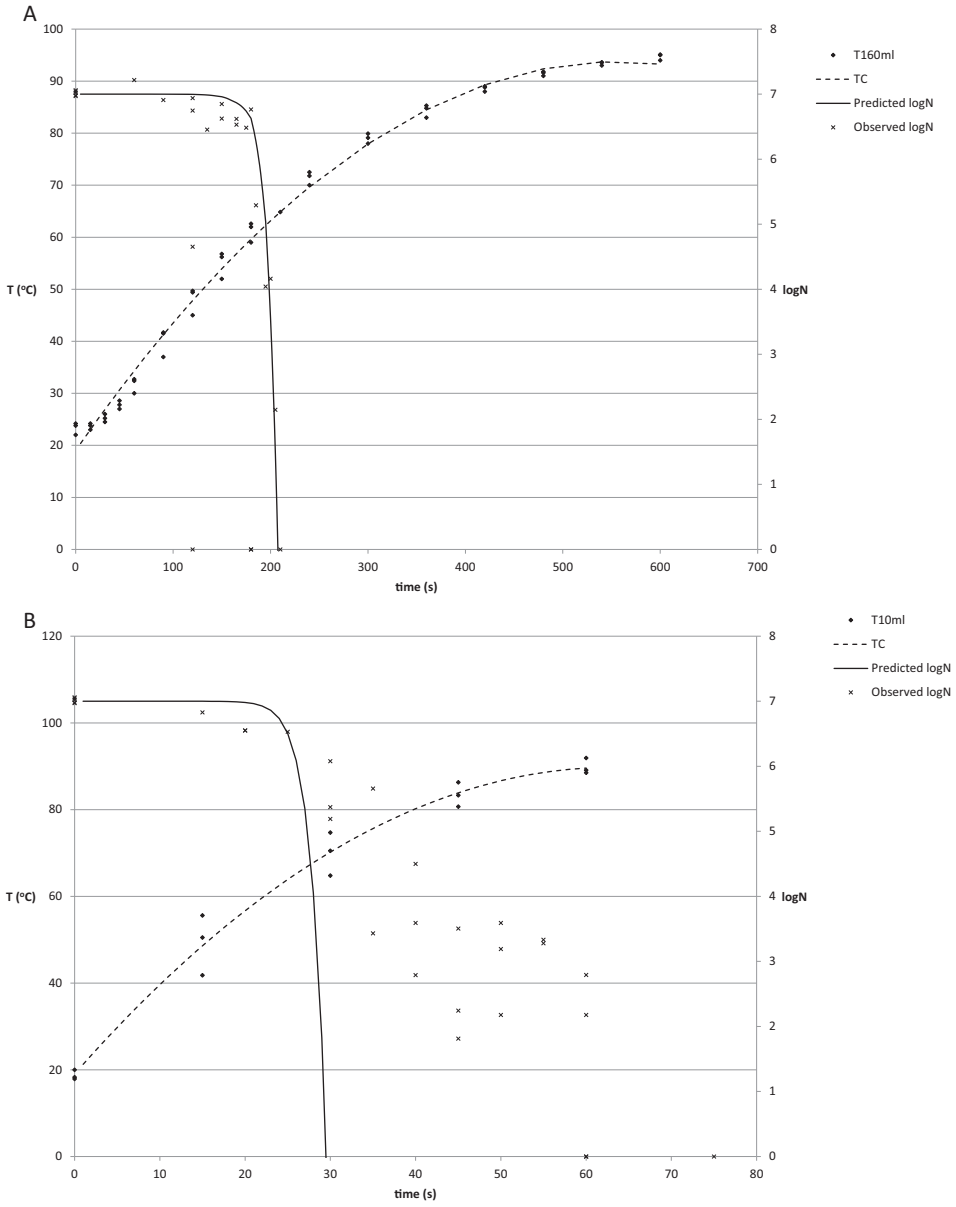


**Fig. 5.** Predicted and observed non-isothermal survival of *Salmonella* in different volumes of buffer, following a slow (5A) or fast (5B) heating rate. T160 ml: measured temperature in a bottle containing 160 ml buffer, in boiling water; T10 ml: measured temperature in a test tube containing 10 ml buffer, in boiling water; $T_C$: calculated temperature, taken from Fig. 3.

constantly during 10 min suggested that cells stayed intact and gradually shrunk.

However, similar cell size changes in response to the temperature were observed during slow and fast heating. Apparently, a loss of water in heated cells alone cannot explain the difference in predictability of cell survival during fast and slow heating.

To reach a certain intracellular temperature, a certain amount of heat needs to be transferred over the cell membrane. The amount of heat (Q) transferred depends on ΔT. During cooking, this ΔT changes in time. When exposed to a large ΔT (as in our meat cooking experiments) or if the external temperature changes too fast (as in 10 ml of buffer), the intracellular temperature of bacteria might (temporarily) stay behind. In combination with reduced convection due to a constant loss of cytoplasmic water, this could explain extremely high heat resistant cells that survived at normally lethal temperatures. The combination of limited heat transfer and the gradual loss of water can also explain why cells die at a constant rate during non-isothermal heat resistance testing.

## 5. Conclusion

Many factors affect the level of heat resistance of bacteria. The results of this and a former study show that attachment to meat contributes to an increased level of heat resistance of bacteria. In *Salmonella*, a fast heating process further contributes to an increased level of heat resistance, possibly as a result of a combination of reduced convection and limited conduction of heat. If the suggested explanation appears to be true, fast heating not only contributes to an increased level of heat resistance in *Salmonella*, but also in other species.

### 5.1. Implications

In heat resistance testing, a small amount of a bacterial culture is transferred to a relatively large volume of preheated medium of which the temperature is kept constant. Although all bacteria are instantaneously exposed to the same challenge temperature, survival curves are described by log linear inactivation kinetics. Why does 90%

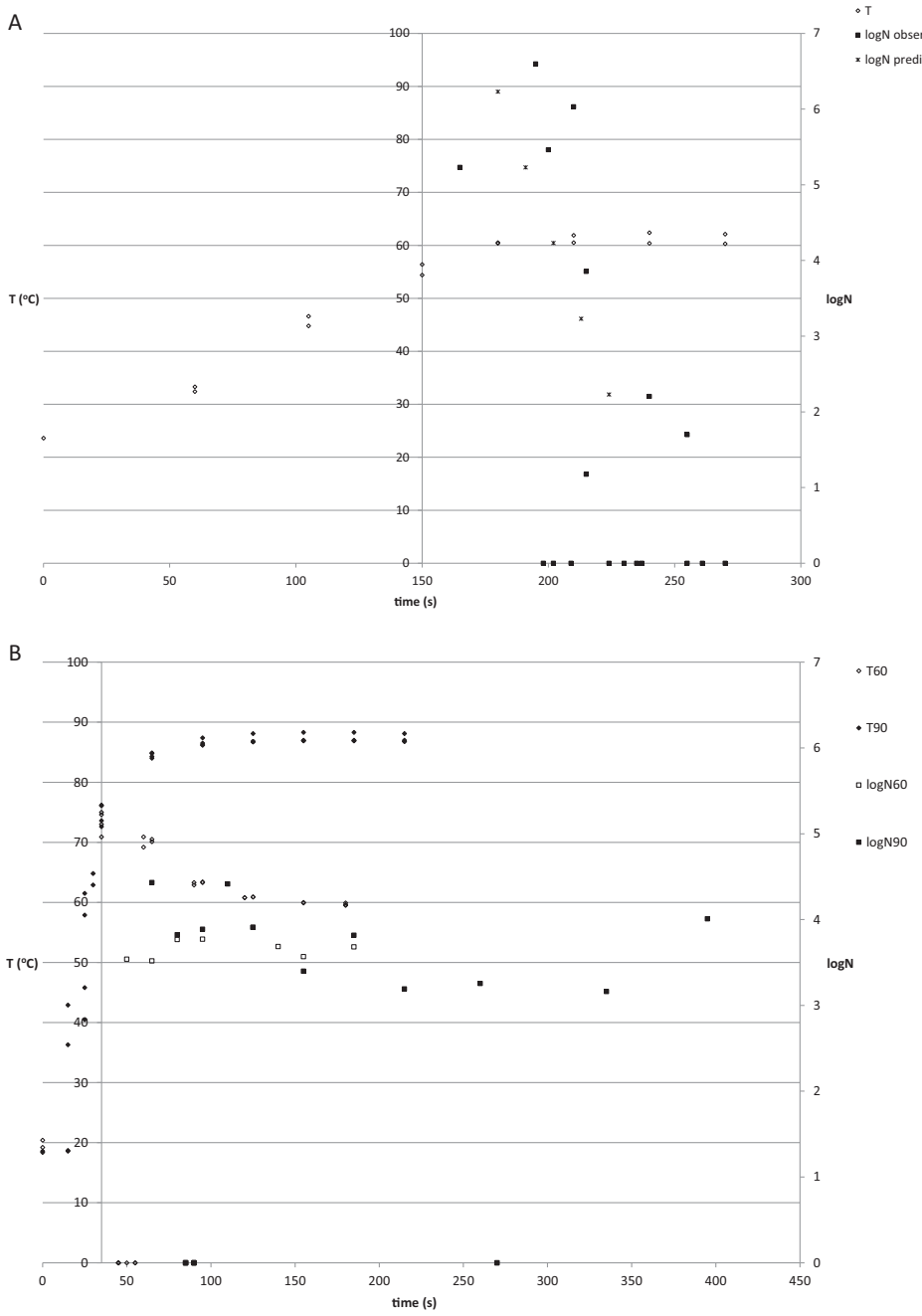


**Fig. 6.** A. Heat resistance of slowly heated *S.* Typhimurium at 60 °C.
A bottle containing 160 ml of a *Salmonella* suspension in PBS at room temperature was heated in boiling water. After 150 s, having reached 55 °C, the bottle was transferred to a waterbath preheated at 60 °C. After transfer, at regular time intervals, samples (1 ml) were taken and transferred to test tubes kept on melting ice, containing 9 ml of PBS. Observed and predicted numbers of *Salmonella* is also shown.
B. Heat resistance of rapidly heated *S.* Typhimurium at 60 or 90 °C. Test tubes containing 10 ml of a *Salmonella* suspension in PBS at room temperature were heated in boiling water. After 35 s, having reached approximately 75 °C, test tubes were transferred to a waterbath, preheated at either 60 or 90 °C. After regular time intervals, individual test tubes were removed from the waterbath and put on melting ice.

of all cells die off within one unit of time, why does 9% of all cells die off at a two times lower rate? And why does 0.9% die off at a three times lower rate? The heat resistance of different isolates of a single species (Juneja and Marks, 2005) or culture might vary. Or cells might have adapted during testing (expression of heat shock proteins; Humpheson et al., 1998; Guisbert et al., 2008). The latter explanation only helps to understand differences in survival within a temperature range in which adaptation can occur. The results and suggested explanation presented in this paper help to understand the gradual die off of bacteria, also at heating rates that do not allow for adaptation and at time/temperature combinations at which no bacterial cell is predicted to survive.

Isothermal microbial survival curves are not always linear (Corradini et al., 2007). When challenged, cells initially die off at a relative high rate, followed by a second phase (tail) in which bacteria die off at a lower rate. De novo synthesis of heat shock proteins could only partly explain tailing (Humpheson et al., 1998). Tailing is more obvious at higher challenge temperatures (e.g. 60 °C) than at a lower challenge temperature (49 °C; Humpheson et al., 1998). This is exactly what is expected when water loss (larger at higher temperatures) induces a higher level of heat resistance. Possibly, a loss of cytoplasmatic water during heating also contributes to tailing, to non-linear, bi-phasic survival.

Time/temperature combinations where extreme survival of bacterial cells is possible might be limited to a small number of food-related, practical situations. For example, butchers with a small business who prepare large sausages or hams, cut these sausages or hams after cooking into two parts. One part is for direct sales, the other half is vacuum packed and shortly heat treated in boiling water to kill any microbiological contamination from the knife or cutting board to extend the shelf life (Houben and Eckhausen, 2006). Another example from the literature: heating profiles of unfrozen beef patties show that

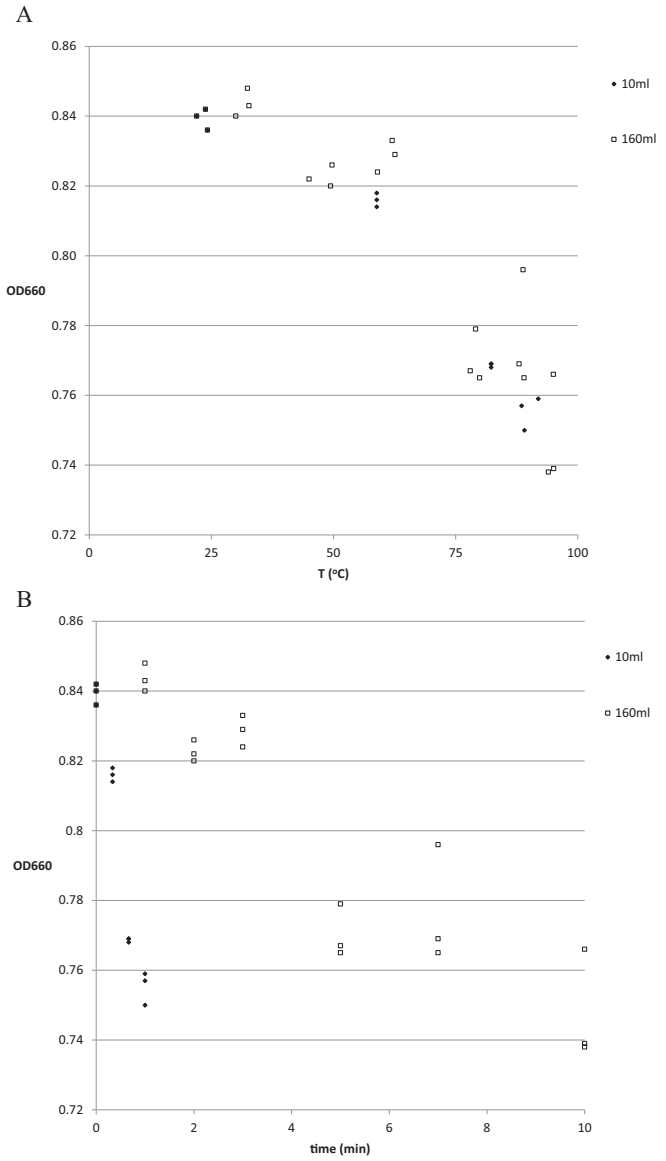


**Fig. 7.** Effect of temperature (7A) and heating rate (7B) on the OD660 of a *Salmonella* suspension. 10 ml: fast heating; 160 ml: slow heating.

during double-sided pan-frying at 160 °C, the surface temperature reaches 70 °C (starting at 5 °C) in approximately 30 s (Ou and Mittal, 2006). This is very similar to our fast heating profile. When the geometric centre of the patty gradually reached 71 °C (in 90 s), the surface temperature was 100 °C. This suggests that bacteria might have survived at the surface. If so, then the risk of double-sided fried beef patties is not only in the centre of patties, but also at their surface. Unfortunately, authors did not validate predicted survival of *E. coli*, *Listeria innocua* or *Salmonella*.

Finally, the results from this study show that the heat resistance characteristics of bacterial cells can change in an unpredictable way, depending on the way a heat treatment is delivered. It is important to realize that this may affect safety margins. Therefore, when designing industrial heat treatments, it is important to validate its design before being applied in full scale processing.

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