International Journal of Food Microbiology 300 (2019) 53–63

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Home style frying of steak and meat products: Survival of *Escherichia coli* related to dynamic temperature profiles

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ARTICLE INFO

Keywords:
D-z model
Home-preparation
Inactivation
Consumer
E. coli
Meat

ABSTRACT

Microbial survival of heating and cross-contamination are the two transmission routes during food preparation in the consumers' kitchen that are relevant for QMRA (Quantitative Microbial Risk Assessment). The aim of the present study was to extend the limited amount of data on microbial survival during real-life preparation of meat and meat products and to obtain accessory temperature data that allow for a more general (product unspecific) approach. Therefore survival data were combined with extensive measurements of time- and location dependent temperature using an infrared camera for the surface and buttons for the inside of the product, supplemented with interpolation modelling. We investigated the survival of heating of *Escherichia coli* O111:H2 in beefsteak, hamburgers (beef and 50% beef 50% pork (HH)), meatballs (beef and HH) and crumbs (HH).

For beefsteak, survival as a whole is dominated by the sides, giving a log reduction of 1–2 (rare), 3–4 (medium) and 6–7 (done). Limited measurements indicated that done preparation gave 5–6 log reduction for crumbs and at least 8–9 log for the other products. Medium preparation gave a higher reduction in hamburgers (2–4 log) than in meatballs (1–2 log) and in beef (3–4) than in HH (2–3) hamburgers. In general, our 'done' results give larger inactivation than found in literature, whereas 'rare' and 'medium' results are similar.

The experiments resulted in two types of curves of D_{70}/z-values, dependent on product, doneness and for beefsteaks sides vs. top/bottom. One type of curve agrees reasonably with literature D_{70}/z estimates from isothermal temperature experiments, which supports using these estimates for home style cooking QMRA calculations. In case of the other type of curve, which is mainly found for (near) surface contamination in close contact with the pan, these literature estimates cannot be applied.

We also applied a simplified approach, assuming thermal inactivation is dominated by the highest temperatures reached. The time duration of this highest temperature gives accessory D-values which prove to fit with isothermal temperature literature data, thus suggesting application of such data for QMRA is possible by this approach also, which is less labor intensive both in terms of measurements and modelling.

In real life, variability in product properties and preparation styles is large. Further studies are needed to analyze the effect on survival, preferably focusing on determining the essential variables. More variation in heating time will allow for estimating D_{70}/z point estimates rather than curves representing possible sets of D_{70}/z-values.

1. Introduction

The exposure assessment is that part of the Quantitative Microbiological Risk Assessment (QMRA) whereby the exposure of a human population to a microbiological hazard is estimated. This part relies on modelling the sequential steps of the pathway that bring the microbiological hazard into food at the point of consumption. The fate of a pathogen in food and, in turn, the level of human exposure, is associated with the consumer behaviour in the kitchen. According to the European Food Safety Authority (EFSA), the largest proportion of food borne outbreaks occurring in the European Union originates in the household with meat and meat products as type of food most frequently implicated (EFSA, 2016). Notably, there is a paucity of information on the extent of microbiological survival in meat products after heating in the consumer kitchen. The extent of the thermal inactivation of bacteria has been assessed through experiments at isothermal temperature carried out in part, with bacteria embedded in meat, but mostly in other food matrices or liquid media (Lahou et al., 2015; Sörqvist, 2003; Stringer et al., 2000). Nevertheless, home-style preparations are not static thermal process. The hypotheses behind our study, is that the

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https://doi.org/10.1016/j.ijfoodmicro.2019.03.020
Received 31 August 2018; Received in revised form 15 February 2019; Accepted 29 March 2019
Available online 09 April 2019

inactivation of bacteria in consumer kitchen might be better represented by dynamic thermal experiments. The aim of the study is on the one hand, to extend the availability of survival ratios in meat for the real world situation to be used in QMRA, and, on the other hand, apply generalized thermal inactivation models explaining bacterial heat survival in the dynamic situation of meat prepared at home so that survival calculations can be done for other meat products also. Model parameters will be compared with parameters from literature on inactivation at a isothermal temperature to see whether these are of use for home style frying. We used a strain of *Escherichia coli* as model organism, while appreciating strain variability will account for the main part of the about the two orders of magnitude range in experimental temperature-specific heat resistance (den Besten et al., 2018), which must be taken into account when using our results and literature data for QMRA calculations. Beef steaks, plain beef and mixed (50% beef and 50% pork) meat preparations (meatball, hamburger and meat crumble) were spiked with a known amount of *E. coli* and then fried simulating home conditions until meeting different levels of readiness preferred by the consumers whereupon survival is measured. Temperatures on and in the food were measured by an infrared camera and by button loggers embedded in the food matrix.

2. Materials and methods

2.1. Bacterial culture

A Shiga toxin-producing *Escherichia coli* (STEC) serotype O111:H2, cured from its natural stx2-gene, (original name: ED191 CURE, kindly provided by Istituto Superiore di Sanità, Italy) was used for all experiments. *E. coli* were grown overnight at 37 °C in 20 ml Brain Heart Infusion broth (BHI, Biotrading, The Netherlands). The culture was centrifuged (3500 $g \times 15$ min,), and the pellet was suspended in 2 ml peptone physiological salt solution (PPS) (Biotrading, Mijdrecht, The Netherlands) to obtain a 10-fold concentrated overnight culture of about 6 to 9 $\times 10^9$ CFU/ml.

2.2. Inoculation and frying beefsteak, hamburger, meatball and crumbs

The experiments with beefsteaks were performed using only rump steaks ranging from 111 to 143 g in weight, all bought from the same butcher. For a part of the steaks, the surface of the side was estimated by multiplying the average of four thickness measurements per steak (measured with a caliper and the circumference, which were measured with a string. The circumference was also used to obtain surface area estimates of and top-bottom surfaces, which were assumed to be flat.

The bacterial inactivation occurring in the top-bottom surfaces and in the side were carried out in two distinct sets of experiments. For the top-bottom surfaces, one aliquot of 50 µl was inoculated on the top and another one on the bottom of the beefsteak

and evenly distributed over the surfaces with a spatula. For the side, five aliquots of 20 µl were applied in 5 different point of the steak side in order to evenly distribute the bacteria along the all side surface and to prevent the inoculum from dripping off the steak. The single aliquot was spread around the adjacent meat area with a spatula. Three types of readiness, namely “rare”, “medium” and “done” were investigated, while realizing these are subjective qualifications. The frying time and procedures were adapted from a previously study simulating home-frying (Lahou et al., 2015).

A tablespoon of fluid concentrated butter (AH culinair, Albert Heijn, The Netherlands) was added in the 20 cm diameter steel frying pan (de Buyer, France), which was heated on an electric cooking plate (SLK-2, Schott AG, Germany) for 2 min (Table 1). The electric cook marks ranged from 1 (low) to 7 (high. Power: 1800 W. Maximum temperature: +600 °C). Afterwards, the steak was fried for 2, 4 or 6.5 min on each side (Table 1), being the frying times for rare, medium or done steak, respectively. These appear as ‘almost raw, just fried on the outside’, ‘only the core red’ and ‘cooked all through’, respectively.

Four meatballs, 4 hamburgers and the crumbs were prepared by using 500 g, 400 g and 300 g of minced meat (Albert Heijn, The Netherlands), respectively. The meat was either plain beef or a mixture of 50% beef and 50% pork, called “Half and Half” (HH). One ml of bacterial culture was inoculated in the minced meat, which was then thoroughly mixed manually. After inoculation, each food type was stored in the fridge overnight and shifted to room temperature 15 min before frying. Preparation details are given in Table 1. Medium preparation for hamburgers and meat balls is defined as ‘red on the inside, but no blood dripping from it when cut open’. Done preparation for hamburgers, meat balls and crumbs is defined as ‘cooked all through’.

After frying, they were left for 3 min on a plate, before further investigations, as to include the additional effect of heat that will also occur in a realistic home or restaurant situation. In addition, for every experiment, one contaminated portion (1 meatball; 1 hamburger; a crumbs portion size of 100 g) was not fried and instead used to estimate the initial bacterial contamination, another portion, which was not inoculated and only PPSD was added, was not fried and used to estimate natural contamination of the meat (*E. coli* already present when purchasing the meat).

The hamburger shape was given through a mould that had 10 cm diameter and 1.9 cm thickness. The spherical shape of the meatball was given manually (diameter: 6 ± 1 cm). Butter was added before placement of the food in the pan. The pan was preheated before the placement of the food Medium and done were investigated for both the hamburger and the meatball. The meatball was initially turned every minute and then with 2 min intervals after the placement of a lid on the pan after 6 min of frying. The hamburger was turned over at intervals of 1 min. The crumbs consisted of 300 g of mixed minced meat composed by 50% beef and 50% pork (Albert Heijn, The Netherlands) which were placed in the pan and continuously stirred during frying.

The experiments were carried out in duplicate or triplicate for beefsteak and singular measurements for the other products. For single measurements we used a so-called mathematical matrix approach, testing all combinations of product shape and meat type.

Table 1
Details of the time, readiness, and electrical heater of the cooking experiments.

Food type	Beefsteak	Beefsteak	Beefsteak	Crumbs	Hamburger	Hamburger	Meatball	Meatball
Readiness	Raw	Medium	Done	Done	Medium	Done	Medium	Done
Tablespoon butter (n°)	1	1	1	2	1	1	3	3
Preheating pan and plate mark	2 min–mark 7	2 min–mark 7	2 min–mark 7	3 min–mark 5	3 min–mark 5	3 min–mark 5	3 min–mark 5	3 min–mark 5
Frying time and plate mark	4 min–mark 5	8 min–mark 5	13 min–mark 5	1.5 min–mark 5 3.5 min–mark 7	6 or 7 min–mark 5	10 min–mark 5	6 min–mark 5 9 min–mark 3	6 min–mark 5 14 min–mark 3
Cooling down on plate (min)	3							
Laboratory analysis (min)	2	2	2	1	10	10	6	6

2.3. Detection and enumeration of E. coli

After frying, the beefsteak and the other meat preparations, except for the crumbs for which only 100 g was analysed, were weighed and an equal volume of PPS was put in a stomacher-bag and homogenized in a stomacher (Type 400 circulator, Seward, UK). From this primary dilution, ten-fold serial dilutions were prepared in PPS, and pour-plated (1 ml) in duplicate on TBX-agar (Biorad, CA, USA), while storing the remainder in the refrigerator. The numbers of *E. coli* colonies (blue-green appearance) on the plates were determined after overnight incubation at 37 °C. In case of no colonies, a 25 ml aliquot of homogenate (the remainder left in the fridge) was added to 225 ml buffered peptone water (BPW) (BioTrading, Mijdrecht, The Netherlands). After overnight incubation at 37 °C, a loop-full of this broth was plated on TBX plates in duplicate. The plates were checked for the presence of suspected *E. coli* colonies after overnight incubation.

2.4. Calculation of bacterial survival

The number of microorganisms present in the food portion before and after the heating was used to calculate a survival ratio (s);

$$s = \frac{\text{No. of CFU after frying}}{\text{No. of CFU raw}} \quad (1)$$

which served as measure of the inactivating effect of the cooking style applied.

The number of microorganisms was assumed to be Poisson (λt) distributed in the samples, where λ is the concentration (CFU/g) and t is the analysed sample size (g). In case of availability of direct counts, uncertainty of λ was described by a Gamma distribution (Vose, 2008). The survival ratio, including its uncertainty, is then equal to the ratios of Gamma distributions, each multiplied by w, the total portion size (g).

$$s = \frac{\text{Gamma}\left(\alpha_f, \frac{1}{t_f}\right) \times w_f}{\text{Gamma}\left(\alpha_r, \frac{1}{t_r}\right) \times w_r} \quad (2)$$

Here α is the total number of CFU counted in the serial dilutions and t is the total analysed number of g of the food matrix in the dilutions. Where the subscripts f and r stand for after frying and raw, respectively.

Direct counts of micro-organisms before and after frying already give an estimate of survival, but we carried this further using Bayesian statistics which also incorporates enrichment results in case of negative direct counts and provides uncertainty estimates. In case of no colonies observed in the dilution series, enrichment results were used. Then an exponential distribution was used as a prior for λ (Eqs. (3)–(5)):

$$\pi(\lambda) = e^{-\lambda} \quad (3)$$

This prior distribution multiplied with the likelihood function gives the posterior distribution for λ, f(λ).

In case of negative enrichment:

$$f(\lambda) = e^{-\lambda} \times e^{-\lambda(t_1+t_2)} \quad (4)$$

In case of positive enrichment:

$$f(\lambda) = e^{-\lambda} \times e^{-\lambda t_1} \times (1 - e^{-\lambda t_2}) \quad (5)$$

where t_1 and t_2 are the total number of g of the food matrix analysed in the dilutions and in the enrichment, respectively.

For a parameter p that can take any positive value, often $1/p$ is recommended as uninformed prior (Vose, 2008) However, taking $1/\lambda$ as prior gave severe numerical problems in estimating with @Risk simulations the distribution of λ, which is located very near the Y-axis. Therefore Exp $(-\lambda)$, which behaves less extreme near the Y-axis, was taken as a prior. This prior gave similar results as another alternative, a constant value as a prior. This indicates that the choice of the prior has limited influence on the end result, which gives some confidence in the estimated uncertainty distributions.

Survival ratios were calculated analogously to Eq. (2) where the numerator equals the distribution described by Eq. (4) or (5), multiplied by w_f.

Uncertainty distributions were obtained by simulations of 10,000 iterations from the respective distributions. Simulations were performed in @Risk (Palisade Corporation, Ithaca, USA), an add-in to Microsoft Excel. For beefsteak, for replicated experiments, s was calculated as the geometric mean of survival ratios of the replicates. The separate measurements for s for side and top-bottom were used to calculate s for the whole beefsteak. To this aim, we explored two approaches, which stemmed from two different assumptions. One approach assumed the bacteria to be uniformly distributed all over the surface of the beefsteak (uniform approach). In this case, the s of the whole beefsteak was obtained by adding the s measurements of the top-bottom and the side adjusted for the fractions of the top-bottom and the side of the total surface of the beefsteak, 0.76 (T/B) and 0.24 (side), respectively. The other, more realistic approach, considers that the top-bottom surfaces of the beefsteak only become contaminated during cutting by the butcher (cutting approach). The beefsteak derives from a cylinder-shaped original piece of meat from which a slice (the beefsteak) is cut by the butcher who cleans the knife after every cut. This frequency of cleaning was assumed to simplify the model approach and limit considering the cross-contamination through the sequential slices by means of the knife. One cut will contaminate a top and a bottom of a beefsteak. We assumed the number of bacteria transmitted from the original meat surface to the knife by a cut to be proportional to the spine thickness of the knife (0.175 cm) (https://www.knivesandtools.com/) and a fraction 0.5 of bacteria transmitted. Subsequently, a fraction 0.5 of the bacteria on the knife is transmitted to the top-bottom. The thickness of the slice (the side) is on average 1.88 cm (12 beefsteaks measured). It can be derived that for a beefsteak, the fraction of bacteria on the top + bottom (f_{t+b}) and the side f_s equal:

$$f_{t+b} = \frac{0.175 * 0.5^2}{1.88 - 0.175 * 0.5^2} = 0.024 \quad (6)$$

$$f_s = \frac{1.88 - 0.175 * 0.5}{1.88 - 0.175 * 0.5^2} = 0.976 \quad (7)$$

The scenarios have different uses: the cutting scenario describes survival for a contaminated beefsteak in retail, whereas the uniform scenario describes survival for a homogeneously contaminated beefsteak in a laboratory experiment.

2.5. Temperature measurements

The infrared camera (Ti450, Fluke, Eindhoven, The Netherlands) was used to take pictures at regular intervals (of about 20 s) providing temperature at pixel level (Table 2). Nevertheless, some pictures were taken at shorter interval during the flipping of the food. When the food was simply laying on the pan, picture were taken at larger intervals. In the case of the meatball and the hamburger, infrared data were integrated with the measurements of three temperature buttons (iButton, Maxim's, Son José, USA) located in the center and midway between the center and the surface (meat ball) or side (hamburger). We assumed that the presence of the buttons would not affect the temperature distribution in the product. At the end of the experiments, the location of the buttons was measured.

2.6. Modelling temperature profile

Data processing and modelling of temperature is described in Table 2.

The measured temperatures by infrared camera or buttons were taken as the midpoint of an interval of about 5–30 s in which temperature was taken to be constant, as a numerical approximation. The

Table 2
Modelling of temperature and survival ratio of the beefsteak and meat products. (T = temperature; s = survival ratio). Temperature at the bottom of product is assumed 100 °C.

Food type	Measurements	Temperature model and survival calculation
Beefsteak	– Infrared camera picture of top surface and side	During Frying +3 min: – top surface: average pixel T. – side: layers based on 10 equally spaced pixel T from top to bottom. During laboratory analysis: average top surface T; side layers T scaled to top surface T Survival: *s* is calculated separately per layer and top/bottom and these values are averaged (weighed for surface or contamination)
Crumbs	– Infrared camera pictures of top surface	During frying: – top surface: 10 percentiles of a PERT distribution for T. – inside: from top to bottom 10 layers of exponential course of T; separate per surface PERT percentile T value. During 3 min and laboratory analysis: 10 percentiles of a PERT distribution for T. Survival: average *s* of 100 values is calculated per time interval and these average *s* values are multiplied.
Hamburger	– Infrared camera top surface and side – Temperature buttons in the matrix	During frying +3 min: Top surface: average pixel T. – inside horizontal: exponential course of nine T values from side to center to side interpolating between and including buttons. – side and inside vertical: 20 layers of exponential course of T from top to middle layer (buttons) to bottom. During laboratory analysis: average of button values. Survival: *s* is calculated separately for 180 parts and these values are volume-weighted averaged.
Meatball	– Infrared camera surface temperature – Temperature buttons in the matrix	During frying +3 min: – Surface: 10 percentiles of a PERT distribution for T; for fraction of surface covered with fat $T = 100$ °C. – inside: exponential course of 19 T values from side to centre to side interpolating between and including buttons. During laboratory analysis: average of button values. Survival: *s* is calculated separately per layer by calculating the average per time interval and multiplying these averages; then the volume-weighted average is taken.

infrared camera provides the mean (m), highest (a) and lowest (b) temperature of an indicated region. The required mode value when applying the Pert distribution equals:

$$mode: 6 \times a \times b \quad (8)$$

The temperature between two measured points in the meat (temperature as a function of location) was assumed to follows an exponentially shaped curve. The assumption that the temperature between two measured points in the meat (temperature as a function of location) follows an exponentially shaped curve is supported by previous studies. Tran et al. (2002) modelled temperature profiles based on a series of experiments with a finite elements model using heat flow equations and physical properties of meat. Both the experimental results and the model show exponentially shaped curves when temperature profiles for the respective time points are plotted. A similar heat flow model was developed by EFSA (Hill et al., 2010) in a hamburger. In fact, after extracting data from a run of this model, an exponentially shaped curve for the temperature profiles was also confirmed. Our own measurements (camera pictures and button measurements) of the meatballs and hamburger also followed an exponential curve. Based on these three sources, it seems reasonable to describe the decrease of temperature from the surface to the center during frying and from the center to the surface after transferring the product to the plate with an exponential function.

The relationship between temperature and bacterial survival is not linear (Eq. (13)–(14)). On that account, the interpolation of additional thermal values within two adjacent points for which the temperature was recorded is important to more accurately describe the temperature course from surface to inside and therefore having a better estimate of the bacterial inactivation occurring. During the frying phase, the exponential course of Temperature T (°C) as a function of location × (cm) (distance from surface) levels off towards the lower temperature and was described as

$$T = T_1 \times e^{a(x-x_1)} \quad (9)$$

with

$$a = \frac{LN\left(\frac{T_2}{T_1}\right)}{x_2 - x_1} \quad (10)$$

where (x_1, T_1) and (x_2, T_2) are the values of the measured distance - temperature points flanking the point for which temperature was interpolated.

During cooling down, the exponential course levels off towards the higher temperature according to:

$T = T_1 + (100 - T_1)(1 - e^{-a(x-x_1)})$ (11).

with

$$a = \frac{-LN\left(\frac{100-T_2}{100-T_1}\right)}{x_2 - x_1} \quad (12)$$

For each food type, the temperature of the bottom surface was not measurable through the infrared camera pictures. During the experiments, the maximal temperature reached by the oil in the surroundings of the food had a range of 130–175 °C. On that account we used a cautious approach and the surface in contact with pan was assumed to be constant at 100 °C. For meatball, this was extended to the fraction of the surface covered with fat due to the turning of the ball, which was set at 0.175 based on the infrared pictures. When the food was transferred on the plate, the pixel nearest to the bottom was used to determine the temperature of the bottom. In case of the crumbs, the mean temperature of the minced meat was used since crumbs underwent a mixing during the transfer on the plate.

2.7. The D, z model and survival ratio s

The thermal inactivation of microorganism is determined by the time-temperature profile. The effect of temperature is often described by the so-called "D/z-model" (Bigelow, 1921; Van Asselt and Zwietering, 2006):

$$D = D_{ref} \times 10^{-\frac{(T-T_{ref})}{z}} \quad (13)$$

where

The D-value is the time in minutes required to inactivate 90% of a defined microorganism at a specific temperature,
T_{ref} is the chosen reference temperature (°C),
D_{ref} is the value of D at the reference temperature (T_{ref}. was set at 70 °C, being the temperature often used in D/z-modelling. Therefore T_{ref} and D_{ref} are termed T_{70} and D_{70}).
z is the temperature increase (°C) needed to reduce the D-value with a factor of 10.

The survival ratio (*s*) (the fraction of microorganisms that survives) in a chosen time period *t* (min) can be calculated as follows:

$$s = 10^{-\frac{t}{D}} \quad (14)$$

assuming a log-linear decrease of survival with time.

The Microsoft Excel Solver add-in was used to determine which set of (D_{70}, z) pairs results in the survival ratio *s* we measured in our experiment. D_{70} values were determined for the z value interval between 2 and 5 and 15.

Thermal inactivation is dominated by the highest temperatures reached. In order to compare the D-values present in literature, which were obtained at certain constant temperature, we also applied a simplified calculation. We considered as relevant for the D-value calculation, the time interval during which the temperature was less then 10 °C lower than the second or third highest measured temperature (to get rid of outliers). This value of 10 °C was chosen as a cut-off value as van Asselt and Zwietering (2006) reported a z-value of about 10.6 °C for *E. coli*, thus a 10 °C lower temperature will already have a 10 times higher D-value. Together with the measured survival ratio *s*, the D-value based on this approach was termed D-high temperature D_{hT} (the inactivation rate most compatible with measured survival ratio *s*), was then calculated using Eq. (14). In the D_{hT} calculation for the whole beefsteak the survival on top/bottom was neglected (the temperature being much higher there), only the initial estimated fraction of *E. coli* on the side was taken into account.

3. Results

3.1. Survival ratio (s) of E. coli after frying

No *E. coli* was detected after plating ten-fold serial dilutions of the raw uncontaminated beefsteaks. Except for one of the beef hamburgers, which showed no contamination, all the remnant food types had a baseline contamination ranging from 1×10^2 to 2.1×10^3/portion (data not shown). The limit of detection of the microbiological culture protocol applied was 10 CFU/portion (beefsteak or meatball) or 8 CFU/portion (hamburger and crumbs). The level of contamination of the spiked beefsteaks was similar across the experiments (Table 3). Rare and medium gave countable numbers, but done frying led to positive direct plating results only in one case (Exp#S2). In the other done repeats, positive and negative results after enrichment for the side and the top/bottom were observed, respectively.

Table 3
Microbiological results of the beefsteaks experiments. Duplicate or triplicate measurements results are expressed as CFU/portion. S = side, T/B = top-bottom. N = negative result after direct plating and after enrichment. P = positive after enrichment. Detection limit of the enrichment 10 CFU/portion.

Exp #	Raw contaminated (CFU/portion)	Rare (CFU/portion)	Medium (CFU/portion)	Done (CFU/portion)
S1	1.4×10^9	1.7×10^7	1.2×10^6	P
S2	1.4×10^9	2.6×10^7	1.1×10^6	5.1×10^3
S3	2.1×10^8	1.1×10^7	2.2×10^5	–
T/B1	1.4×10^9	2.1×10^5	–	–
T/B2	1.4×10^9	1.3×10^6	5.1×10^3	–
T/B3	1.4×10^9	–	1.8×10^2	–
T/B4	1.4×10^9	–	–	N
T/B5	1.4×10^9	–	–	N

The survival of bacteria on the beefsteak decreased with the length of thermal treatment and was lower on the T/B for all levels of readiness compared with the side (Tables 3 and Table S1, Fig. 1). In case of the cutting approach, both the sides and the whole beefsteak have a log

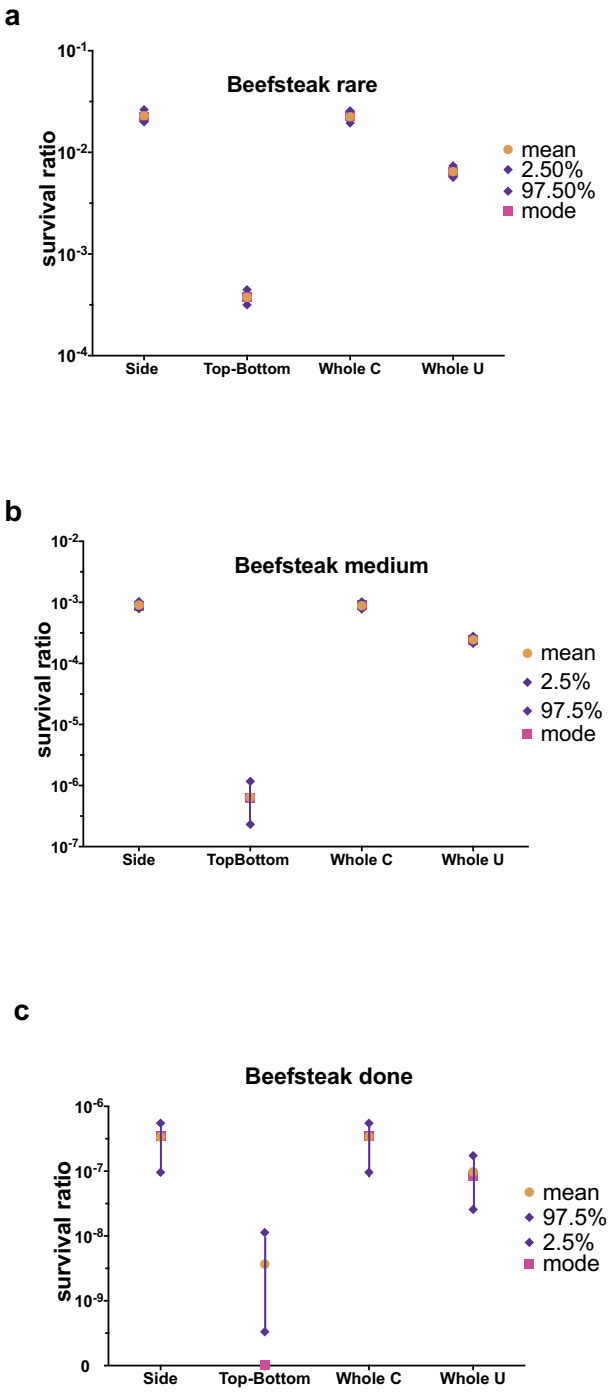

Fig. 1. Survival ratio of the side surface, top-bottom surfaces and the whole beefsteak for the raw rare (a), medium (b) and done readiness(c). The survival ratio of the whole beefsteak (duplicate or triplicate measurements) is the sum of the survival ratios of the top-bottom and side multiplied by (a) 2.4% and 97.6% (cutting approach 'whole C') or (b) 73% and 27% (uniform approach 'whole U'), the estimated fraction of bacteria residing on the top-bottom and side, respectively (see Section 2.4). Results were illustrated as mean (orange circle), 2.5–97.5% percentiles (blue diamonds) and mode (purple square). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Microbiological results of the meatball, hamburger and crumbs experiments. Singular measurements results are expressed as CFU/portion. N = negative after direct plating and after enrichment. (Detection limit of the enrichment 10 CFU/portion (meatball), 8 CFU portion (hamburger and crumbs).

Exp#	Raw contaminated (CFU/portion)	Medium (CFU/portion)	Done (CFU/portion)
Beef ball	3.0×10^9	4.6×10^7	–
Beef ball	7.8×10^9		N
HH ball	3.0×10^9	9.9×10^7	N
Beef hamburger	1.8×10^9	9.2×10^5	–
Beef hamburger	2.4×10^9	–	N
HH burger	3.0×10^9	1.7×10^7	N
Crumbs	1.2×10^9	–	1×10^4

reduction of 1–2 (rare), 3–4 (medium) and 6–7 (done).

The bacterial load after spiking was similar across the raw portions of the different food types and ranged from 1.2×10^9 to 3.01×10^9/portion (Table 4).

Regardless of either the shape or the composition, the amount of *E. coli* was reduced dramatically for the done readiness for all types of meat. In most cases, no survival of bacteria was found. Meatballs and hamburgers showed a reduction of at least 8–9 logs, looking at the upper 95% CI. Survival was only detected for beefsteak (7–8 log reduction) and crumbs (5–6 log reduction) (Fig. 1, Fig. 2, Table 4 and Table S2).

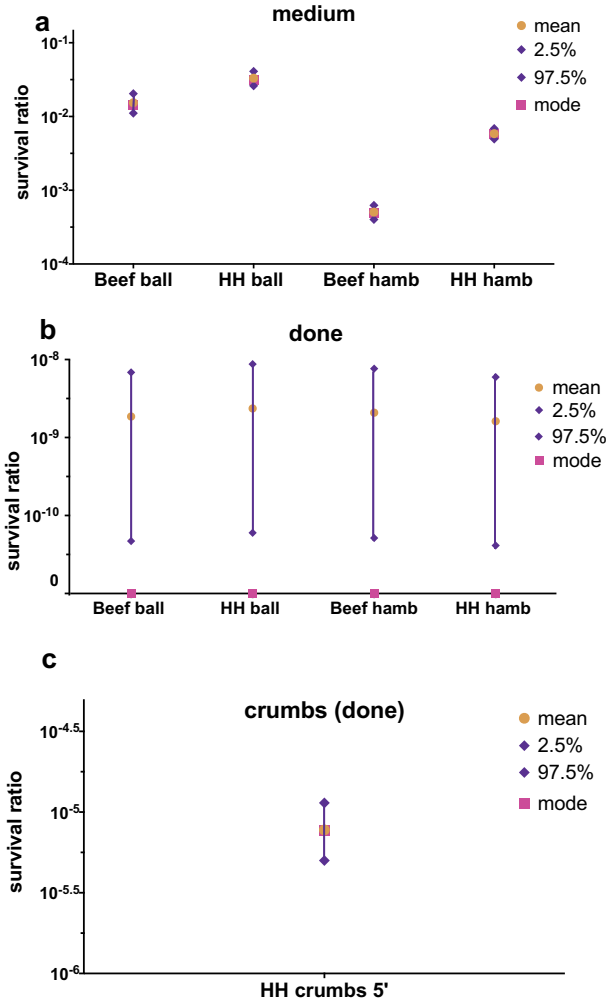

Fig. 2. Survival ratio of the medium (a), done (b) meatball and hamburger (b) and crumbs (c). Results were illustrated as mean (orange circle), 2.5–97.5% percentiles (blue diamonds) and mode (purple square). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

When considering frying to medium readiness, the shape had an impact on the microbial survival in minced meat preparations. In fact, bacterial inactivation in hamburgers (2–4 log) was higher than in meatballs (1–2 log). Lower bacterial inactivation was observed in HH hamburger (2–3 log) than in beef hamburger (3–4 log). Note that the uncertainty of the results for hamburger, meatball and crumbs is larger than for beefsteak, being based on singular and duplicate/triplicate measurements, respectively.

One beef ball done experiment exhibited much higher bacterial survival (*s* mean 2.9×10^{-5}, 95% CI: 2.1×10^{-5}–3.8×10^{-5}) than the other repeats. This finding was associated with a change into an ovoid shape of the meatball during the frying process, which caused part of the ball to remain relatively raw as was apparent visually. The medium beef hamburger experiment was repeated, extending the cooking time from 6 to 7 min since the inner part was too raw after 6 min to be acceptable for consumption. Correspondingly, the reduction increased from 2 to 3 log (*s* mean 5.8×10^{-3}; 95%CI: 4.9×10^{-3}-6.9×10^{-3}) to 3–4 log.

Overall, the crumbs, which were fried for only 5 min, showed greater bacterial inactivation than all the compressed medium meat preparations. The lowest level of bacterial reduction was (1–2 log) was found in rare beefsteak and medium beef and HH balls.

3.2. D_{70} and z value curve for the heating process

The curves of paired D_{70-} and z-values estimated from the inactivation observed in our experiments are plotted in Fig. 3–5. Two types of curves occur, (1) where z increases with D_{70} with increasing slope and (2) where z decreases with D_{70} with decreasing slope. The whole beefsteak curves are dominated by the sides related to higher loads of *E. coli* and lower temperatures. Type 1 curves are found for beefsteak sides rare and medium and for hamburgers and meatballs, beef and HH. The differences between these curves are limited. Type 2 curves are found for beefsteak T/B rare, medium and done, side done and for crumbs. These curves differ in location, shifting more towards the literature values in the order mentioned.

3.3. D_{hT}-value of beefsteak, hamburger, meatball and crumbs for the heating treatment

The thermal inactivation of microorganism is determined by the time-temperature profile of the heating treatment. In Fig. 6 we reported the time-temperature profile registered to achieve a done readiness of the beefsteak, hamburger, meatball. The effectiveness of the cooking process in inactivating bacteria can be described by the D_{hT}-value (see Section 2.8). The higher the D_{hT}-value, the less efficient is the inactivation process. In Table 5 and Fig. 7 are reported the D_{hT}-values of real life frying conditions executed in our experiments.

D_{hT}-values were lowest for beefsteak and crumbs, being products where the bacteria are at or just below the surface. The steak values show no clear relation with readiness. The differences in D_{hT} and *s* between side and T/B fit with each other.

The heating process was more effective in hamburger then in meatballs and in beef then in HH. The hamburger/meatball difference can be explained by the higher temperatures reached in hamburgers, however temperatures are similar in beef and HH. So, the product composition appears to influence the inactivation rate.

4. Discussion

A better estimate of the bacterial inactivation resulting from the home-frying of meat and meat products could help in refining the exposure assessment in QMRA. Our model, built on the results of realistic

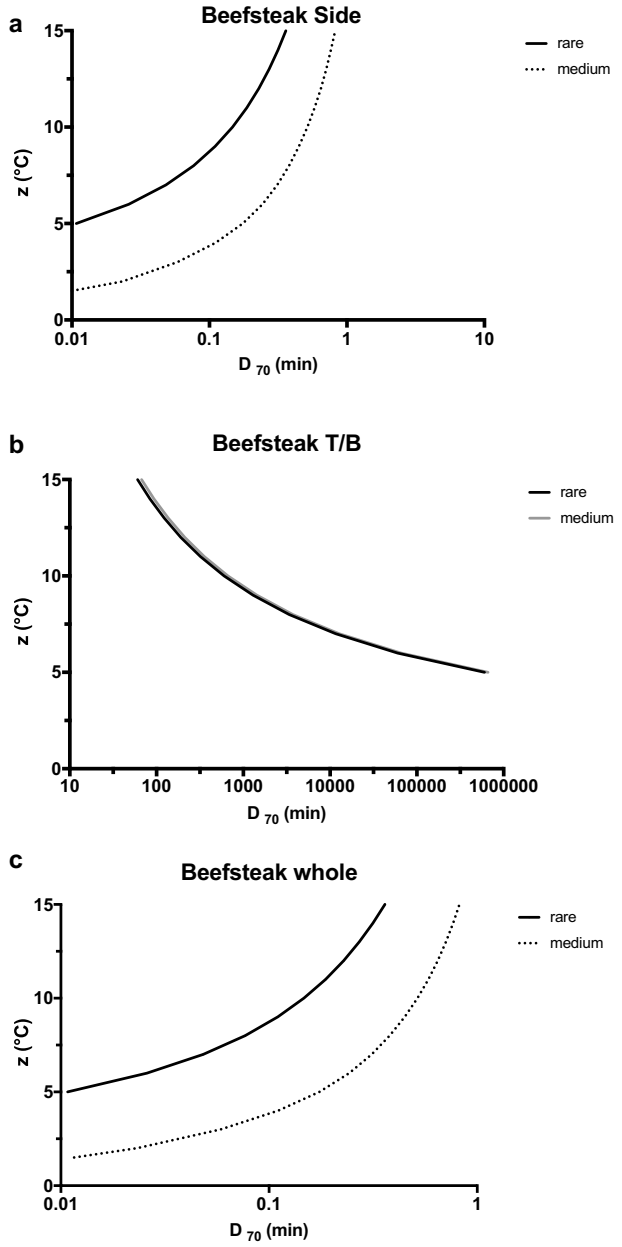

Fig. 3. Curves of D_{70}/z values compatible with the inactivation observed for the side (a), top-bottom surfaces (b) and whole (c) beefsteak rare and medium. Curves of the T/B surfaces rare and medium overlap.

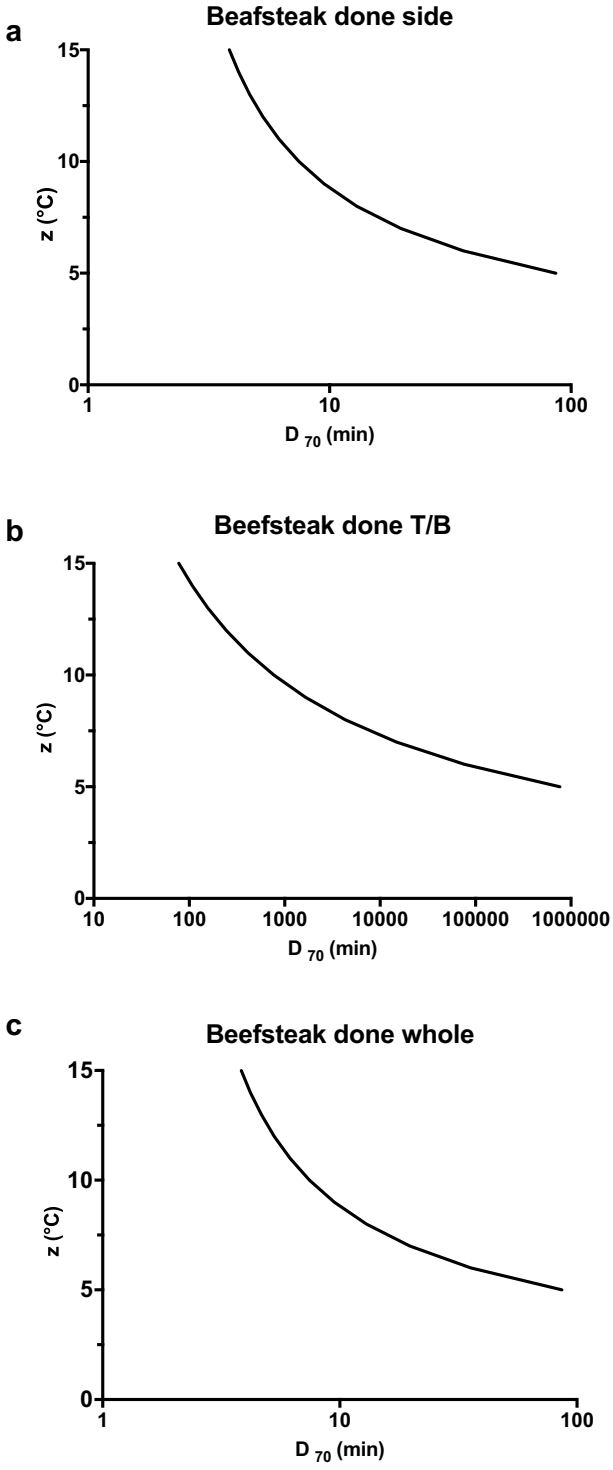

Fig. 4. Curves of D_{70}/z values compatible with the inactivation observed for the side (a), top-bottom surfaces (b) and whole beefsteak done.

experiments, provides *s*, D and D_{70}/z-values of beefsteak, hamburger, meatball and crumbs fried to achieve distinct degrees of readiness.

The temperature of food bottom surface is not measurable with infrared camera pictures. In our calculations, we use a maximum temperature of 100 °C, although we appreciate that the temperature of the oil and maybe the crust will exceed this value. This approach is supported by literature data (Lahou et al., 2015; Tran et al., 2002), which showed that the temperature inside of minced meat never exceeds 100 °C supposedly due to the effect of the water in the meat reaching the boiling point. Furthermore, for values above 100 °C, the exact temperature hardly affects the calculation results. Based on these observations, in our model the maximum temperature for meat was set at 100 °C.

The two beefsteak scenarios ("uniform" or "cutting") on the distribution of the bacteria over the meat surface give quite different bacterial survival results. When bacteria were assumed to reside almost totally on the side surface ("cutting"), bacterial survival ratios were always greater than when "uniform" distribution was assumed.

The bacterial inactivation observed in our experiments was confronted with the published studies in which domestic heating were simulated (Table 6). A large variability in methods and results can be observed, related to *E. coli* (strain, type and antibiotic resistance), product size and structure, storage method, inoculation method,

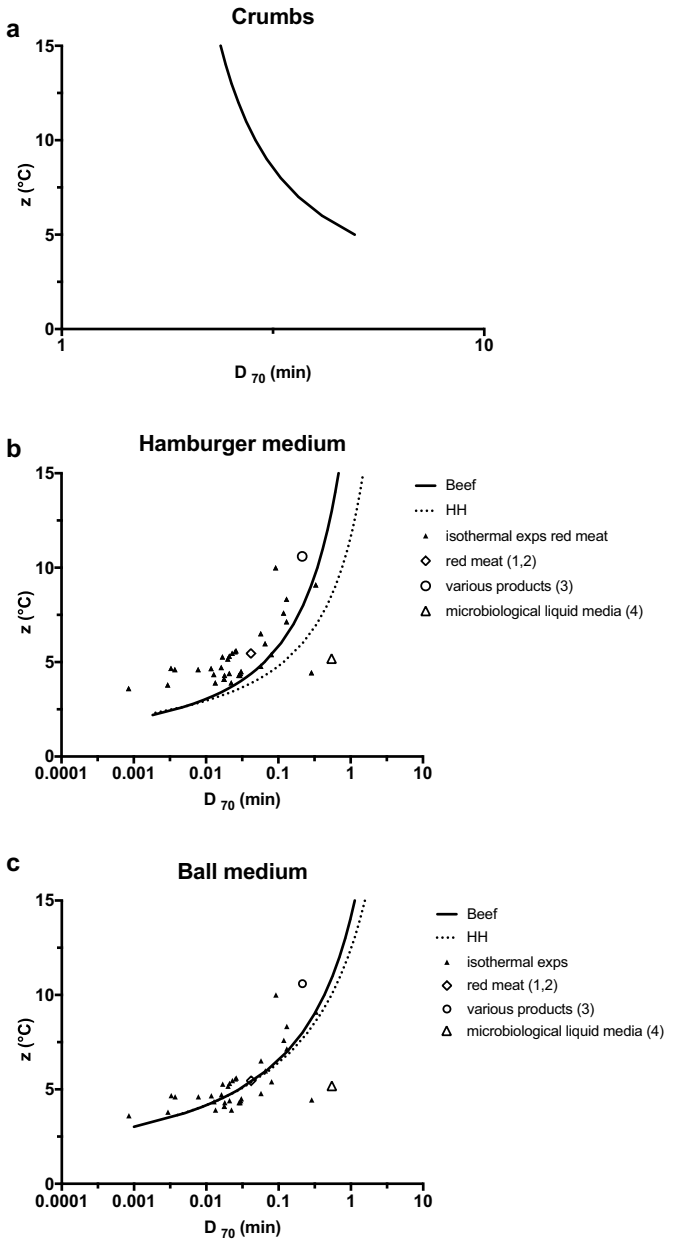

Fig. 5. Curves of D_{70}/z values compatible with the inactivation observed for the crumbs (a). Beef and HH medium hamburger (b), Beef and HH medium ball (c) and D70/z-point estimates from 1. Halder et al., 2010; 2. Stringer et al., 2000; 3. Van Asselt and Zwietering, 2006; 4. Sörqvist, 2003.

heating time, heating method and, last but not least, the evaluation of the degree of doneness of the meat, which widely varies between consumers. The inactivation we found for the done readiness is generally larger than the values of literature (Table 6). The microbiological negative results after enrichment set the inactivation for hamburgers and meatballs up to at least 8–9 log and in the case of the beefsteak, we found 6–7 log. The medium heated beefsteaks and hamburgers experienced an inactivation value of 3–4 and 2–4 logs, respectively. These values are compatible with those found in literature. Interestingly, we found no clear difference between inactivation in medium heated beefsteaks and hamburgers. The same is found in literature, comparing surface contaminated products and internally contaminated products (Lahou et al., 2015; Adler et al., 2012) vs. the other references in

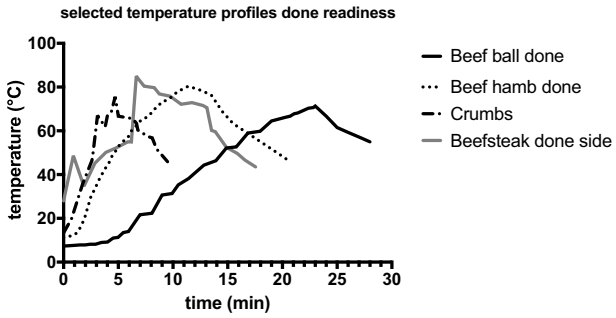

Fig. 6. Selected temperature profiles done readiness. Crumbs, top surface: average infrared pixel temperature; Beef hamburger done, internal: average of three buttons; Beefsteak done, side surface (halfway): average of the 2 middle infrared pixels (pixel 5 and 6); Beef meatball done, internal: average of three buttons.

Table 6). We found the inactivation of medium readiness to be less in meatballs than in hamburgers and one might postulate this is due to the thickness of the product. However, considering previous results for ground products (Boqvist et al., 2015; Røssvoll et al., 2014; Shen et al., 2010, 2011) only gives a hint of decreasing inactivation with product thickness and moreover, Shen et al. (2010) shows a clear increase of inactivation with product thickness (1.5, 2.5 and 4.0 cm) which is explained by a longer cooking time for thicker products to reach the same internal temperature. This could well explain the contradiction with our results, where observed internal redness of the product is the criterion for medium heating instead of a fixed internal temperature. The inactivation values found in literature for ground products rare to medium (Table 6) have a large range (1–8 log), which comprises the range we obtained for medium prepared hamburgers and meatballs (1–4 log).

One important question is how isothermal D_{70}/z values relate to our 'dynamic' D_{70}/z values for prediction purposes. Comparing the type 1 curves with literature 'isothermal temperature' - data (see Section 3.3 and Fig. 3a–c) shows a reasonable agreement. This is not the case of microbiological liquid media, providing evidence for their cautious application to in real food products. The type 2 curves do not agree with previously published data with the beefsteak T/B values being the most shifted to the right of all type 2 curves and thus is the most distant from the literature values in Fig. 5b–c. One explanation for this divergence is that when all the *E. coli* are at or very near the surface of the meat and come in close contact with the butter or the metal of the pan (beefsteak T/B, crumbs) this might disturb the 'normal' D/z-relationship. This could be related to a high or very fast rise of temperature for these bacteria, which, as speculated by de Jonge (2019), lead to a high resistance to heat inactivation- see e.g. *Campylobacter* survival when boiling chicken filet (de Jong et al., 2012). This however does not explain the type 2 curve found for beefsteak done side, which corresponds with the deviating behaviour of beefsteak done in Fig. 7. This product-heating time reached the highest temperature between those given in Fig. 3 (with the exception of beefsteak T/B) and the D/z-model is possibly not valid.

We determined (D_{hT}, T_{hT}) values for the products in our experiments assuming that the highest temperatures achieved determine inactivation. These values are compared with static temperature (D, T) results in Fig. 7. It appears that there is no or a limited difference between the results obtained in a dynamic and a static situation, except for beefsteak done. This suggests that the large set of static temperature (D, T) results from literature can be used to estimate survival in a real life dynamic situation. This would only necessitate measurement of the temperature in the product part where maximum temperature is expected to be lowest, together with the time period of maximum

Table 5
D_{hT}-value for the heating treatment applied to the side, T/B top-bottom and whole beefsteak, hamburger, meatball and crumbs.

Food	D_{hT}-value (min) rare	*t* (min) rare	T_{hT} (°C) rare	D_{hT}-value (min) medium	*t* (min) medium	T_{hT}- (°C) medium	D_{hT}-value (min) done	*t* (min) done	T_{hT} (°C) done
Beefsteak T/B	0.6	2.0	95	0.7	4.0	95	0.8	6.5	95
Beefsteak Side	1.3	2.1	54.7	1.7	5.3	59.5	1.1	6.8	75
Beefsteak whole[a]	1.3			1.7			1.1		
Beefsteak whole[b]	0.8			1.3			0.9		
Beef hamburger	–	–	–	2.12	7	61	–	–	–
HH hamburger	–	–	–	2.78	6.2	59	–	–	–
Beef meatball	–	–	–	4.58	8.3	53	–	–	–
HH meatball	–	–	–	8.98	13.3	54.5	–	–	–
Crumbs	–	–	–	–	–	–	0.89	4.4	63

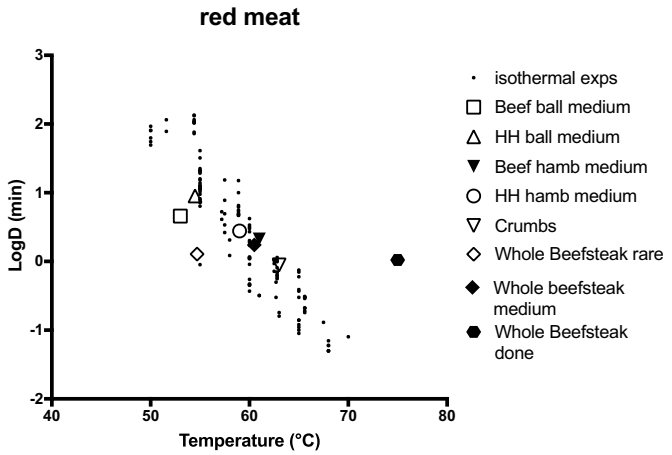

Fig. 7. (LogD, T) values for isothermal experiments from red meat found in literature and ($LogD_{hT}$, T_{hT}) values from the present experiments. The beefsteak value displayed is related to the "cutting" scenario. Isothermal values retrieved from (Brar et al., 2018; Byrne et al., 2002; Clavero et al., 1998; Duffy et al., 2006; Huang and Juneja, 2003; Juneja, 2003; Juneja et al., 1997, 1998; Juneja and Marmer, 1999; Juneja and Novak, 2003; Line et al., 1991; Liu et al., 2015; Osaili et al., 2007; Smith et al., 2008). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

temperature minus e.g. *z* °C, and a simple calculation. With this rough approach, time and money could be saved.

The results of heating experiments, even those isothermal, are subjected to large variability that can be ascribed to method of heating, the composition of the product and the experimental design of the study (Stringer et al., 2000). Previous exposure of bacterial cells to sub-lethal (46 °C) or refrigerating temperature (4 °C) (de Jong et al., 2012; Duffy et al., 2006) increases their ability to counteract the process of heating. High fat, high mineral content or pH (Huang and Juneja, 2003) in the 5–6 range, decrease the inactivation of bacterial cells. There is evidence that microbiological media have lower ability to support colony formation of heat-stressed *E. coli* cells leading to the overestimation of the inactivating effect of the thermal treatment (Juneja and Novak, 2003). Furthermore, heat resistance varies strongly within the *E. coli* species, with some strains possessing a markedly improved ability to cope with heat and thus requiring longer treatment to be inactivated (Liu et al., 2015).

The concentration of *E. coli* O157:H7 is estimated to rarely exceed the value of 10^3 CFU/g in ground beef at retail (Cassin et al., 1998; Duffy et al., 2006, b). Generally a reduction is targeted in thermal treatments to limit the exposure of the consumers to the pathogen (Stringer et al., 2000). The medium readiness applied in our study was not able to achieve such degree of inactivation. This result becomes even more important in the light of the large variability we observed in some other set experiments that were not shown here. For example the loss of the spherical shape (changed to ovoid) of the meatball during the frying process was coupled with a reduced bacterial inactivation compared to a repeat where the spherical shape was conserved. The ball preferably took only certain orientations in the pan. This in turn caused part of the ball to remain relatively raw as was apparent visually. Another example is that a difference of as little as one minute frying time was found to be able to make the difference between a rare (i.e. bloody inside) and a medium hamburger. These findings suggest that consumers might be exposed to doses which are not negligible and associated with a high probability of infection (Gill and Huszczynski, 2016; Teunis et al., 2008). The evaluation of the inactivating effect of real-life thermal treatment must also take into account consumer's preferences, habits and the method of conservation of minced meat. Survey on consumer's preferences carried out in Norway and USA revealed that a 20% to 43% of participants had a taste for undercooked hamburgers that show a pink appearance in the interior part (Altekruse et al., 1999; Røssvoll et al., 2014; Taylor et al., 2012). Also, a rapid change in colour of minced meat conserved in modified atmosphere packages (MAP) or stored at freezing temperature upon thermal treatment can be observed (Boqvist et al., 2015; Byrne et al., 2002; Røssvoll et al., 2014), called the "premature browning" phenomenon (PBM). Surveys on consumer's habits in the kitchen have shown that visual appearance is frequently the preferred method to assess meat doneness. As a consequence, the temperature experienced by food during frying can be insufficient to ensure a safe level of inactivation due to PBM (Boqvist et al., 2015).

Our work provides *s*- and D-values which can be directly used to improve the exposure assessment steps of QMRA for the meat products studied and which can also be generalized to other meat products. Nevertheless, further research including more replicates, variation in heating time, variation in shape must be carried out in order to assess the importance of the variability in real life meat preparations and ultimately provides D_{70}/z point estimates (rather than curves) to predict *E. coli* survival.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Table 6
E. coli inactivation in studies in which domestic heating were simulated.

−Log s	Bacterium	product	Heating time	Heating method	Reference
2.5–5.7	O157:H7	Beef hamburger (MAP or minced at retail). Thickness 1 cm. Inoculum mixed with product.	4–10 min (slightly to very pink) 8– > 13 min (no evidence of pink)	Skillet on induction stove	(Boqvist et al., 2015)
4.8–5.7					
2.8	13 'O'-types	Beef hamburger (MAP or vacuum). Thickness 1.5 cm. Inoculum mixed with product	6 min(rare)	Grill plate to 60 °C	(Røssvoll et al., 2014)
3.7			7 min (medium)	To 66 °C	
5.9			8 min (done)	To 72 °C	
1.1–4.2	O157:H7	Coarsely ground beef, frozen and cut to steaks. Thickness 1.5–4 cm. Inoculum mixed with product	4–65 min, depending on heating method and steak thickness (rare to medium)	Pan/roast/grill oven, to 65 °C	(Shen et al., 2010)
1.5–5.5	O157:H7	Coarsely ground beef, frozen and cut to steaks. Thickness 2.5 cm. Inoculum mixed with product	25–60 min (rare-medium)	Pan/roast/grill oven, to 65 °C, variable heating temperature	(Shen et al., 2011)
2.3–2.6	O157 and generic	Steak from several animals, inoculation of surfaces and edges	Simulated home pan frying. 2 min (rare)	Frying pan	(Lahou et al., 2015)[a]
2.9–3.4			4 min (medium)		
3.5–4.3			6.5 min (done)		
0.8–5.0	O157:H7, O26, O111 and nonpathogenic, non- and nalidixic resistant, 19 strains	Blade tenderized subprimals, cut and sliced to 185 g steaks; inoculation on and inside muscle.	14-17 min (realistic industrial processing, rare to medium)	Vacuum pack in water in RF oven, increase to 60, 63, 65 °C, 5 min room temperature	(Rincon and Singh, 2016)
0.4–2.9	O157:H7, 8 strains, rifampicin-resistant	Sliced meat slices stacked to steaks of 1.2 or 2.4 cm thickness and blade-tenderized, variation in storage and thawing, inoculation at 0.3–1.2 cm depth	About 3–43 min	Pan-broiling or roasting in oven, increase to 60 °C	(Adler et al., 2012)
0.4–3.4		Inoculation at surface			
4.7–6.9	O157:H7, 8 strains	Beef burger patties 100 g (20% fat-80%) lean) Inoculum mixed with product	4–10.9 min (done)	Single or double sided grill turned once until 71 °C	(Rhee et al., 2003)
1.3–5.7	O157:H7, 5 strains	Beef burgers (110 g) Inoculum mixed with product	4.5–9.9 min (medium)	Single or double sided grill until core 60 °C	(D'Sa et al., 2000)
2.9–6.1			4.6–15.8 (done)	Single or double sided grill until core 68 °C	
1.4–8	O157:H7: 7 strains	Beef burger (200 g) Inoculum mixed with product	2.5 min medium	Grill until core 63 °C	(Liu et al., 2015)
3.4–8			3 min done	Grill until core 71 °C	

[a] Lahou et al. (2015) *s* values were calculated by the authors from the given data.

Acknowledgements

The authors thank John Tonen (Poonawalla Science Park B.V.) for assistance with the TI200 InfraRed-camera. The authors also thank Dr. Stefano Morabito (ISS, Rome) for providing the cured *E. coli* O111 strain. The authors want to thank Mieke Uyttendaele and her group from Ghent University for hospitality and important inspiration at a visit during the initial stage of the study. This work was supported by The Netherlands Food and Consumer Product Safety Authority (grant number 9.2.04.B.10). The research by Pesciaroli Michele was supported by the EU-FORA programme of the European Food Safety Authority (Parma, Italy).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2019.03.020.

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