Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Surface decontamination of beef inoculated with *Salmonella* Typhimurium DT104 or *Escherichia coli* O157:H7 using dry air in a novel heat treatment apparatus

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Keywords
beef, decontamination, dry air, *Escherichia coli* O157:H, *Salmonella* Typhimurium DT104.

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2005/1013: received 7 September 2005, revised 15 February 2006 and accepted 16 February 2006

doi:10.1111/j.1365-2672.2006.02988.x

Abstract

Aims: To determine the effectiveness of a novel dry air decontamination apparatus in the deactivation of *Salmonella* serotype Typhimurium DT104 or *Escherichia coli* O157:H7 on beef surfaces.

Methods and Results: A laboratory scale dry air decontamination apparatus, capable of producing repeatable and known heating time–temperature cycles on food surfaces was used in decontamination trials. Beef samples were surface inoculated with 7–8 $\log_{10}$CFU cm^{-2} of *S.* Typhimurium DT104 or *E. coli* O157:H7 and heated at 60, 75, 90 and 100°C using fast and slow heating rates and subsequently held at these temperatures for up to 600 s. A substantial reduction in pathogen numbers was achieved at higher temperatures (90 and 100°C, 4·18–6·06 $\log_{10}$CFU cm^{-2}) using both heating rates, but cell survival at these temperatures was also observed. At the lower temperatures, deactivation was small at 60°C in particular it was less than one log unit after 3 min heating. No significant differences were observed when total reductions in pathogen counts were compared for all the temperature/heat up time combinations tested. During slow heating at 90°C, and both heating rates at 100°C, the pattern of deactivation of *S.* Typhimurium DT104 or *E. coli* O157:H7 was triphasic.

Conclusions: This study has shown that heating meat surfaces with dry air can achieve substantial reductions in *S.* Typhimurium DT104 or *E. coli* O157:H7. As surface decontamination of beef surfaces with dry air had a negative effect on beef colour and appearance, such a decontamination apparatus would be unsuitable for producing meat for retail sale but it could be used to produce safer meat for use in the catering trade.

Significance and Impact of the Study: This study provides researchers and food processors with data on the dynamic changes in *S.* Typhimurium DT104 and *E. coli* O157:H7 counts on intact beef surfaces during heating with dry air under realistic (time-varying) temperature conditions.

Introduction

Systems for the decontamination of meat have been in operation since the early 1960s. In general, the agents used to decontaminate meat, primarily carcasses can broadly be categorized as chemical (organic acids) and physical interventions, such as heat or cold (Sheridan 2004). Heat is generally regarded as being the most effective in particular steam and is now used extensively in the meat industry to decontaminate beef carcasses (Nutsch *et al.* 1997). Hot water pasteurization is also used, primarily for the decontamination of pig carcasses (Gill and

Jones 1997). The use of hot air however, has largely been confined to the cooking of meat in convection ovens and not as a surface-pasteurizing agent (Murphy *et al.* 2004). This type of heating is unlikely to be applicable to carcass pasteurization because of operational difficulties relating to equipment size and efficiencies in delivering sufficiently hot air to carcass surfaces. This approach could however, have potential in relation to the rapid and effective decontamination of the surface of [smaller] meat pieces, such as beef primals, which become contaminated with pathogens and other undesirable bacteria during carcass boning operations (Sheridan and Lynch 1992). In considering any meat decontamination system, in particular a novel one such as hot air, deactivation of surface pathogens must consider the effect of temperature and the time of application on survival (Kirby and Davies 1990). Of particular importance are the properties of the meat itself, which differ between animal species and meat type and can have a major influence on pathogen survival due to protective effects related to surface topography (McMeekin and Thomas 1979; Schwach and Zottola 1982; McCann *et al.* 2006). Account must also be taken of the pathogens to be investigated and their behaviour when stressed from heat or other inimical processes (Dodd *et al.* 1997). The heat resistance of pathogens will differ depending on whether the cells are in an exponential or stationary phase of growth. Stationary phase cells are known to be more heat resistant and are therefore better able to withstand potentially lethal heat treatments (Doyle and Mazzotta 2000).

This study investigated the effects of dry air applied using a novel decontamination apparatus in reducing the numbers of stationary phase of *Salmonella* serotype Typhimurium DT104 and *Escherichia coli* O157:H7 recovered from surface inoculated beef, using a range of temperatures, at two different heating rates.

Materials and methods

Inocula

Salmonella Typhimurium DT104, a bovine isolate from the Ashtown Food Research Centre culture collection and *E. coli* O157:H7 ATCC 43895, an isolate from a raw hamburger in Michigan and Oregon in 1982 (Strockbine *et al.* 1986), which was obtained from LGC Promochem, London, UK, were stored on cryoprotective beads (Technical Service Consultants Ltd., Heywood, UK) in a freezer at −20°C. One bead of each organism was aseptically placed in 30 ml of brain heart infusion broth (BHI, Oxoid, Basingstoke, UK) in a 250-ml Duran bottle and incubated at 37°C for 24 h. From these cultures, 1 ml was transferred to 99 ml of BHI broth and *S.* Typhimurium DT104 was incubated at 37°C for 16 h and *E. coli* O157:H7 at 37°C for 18 h. After these times the cultures were in late stationary phase of growth, which had been established from previous experiments. Cells from these cultures were centrifuged at 4500 ***g*** for 10 min, washed three times in 9 ml of maximum recovery diluent (MRD; Oxoid) and re-suspended in 1 ml of MRD. Cell numbers in these suspensions were determined by spiral plating (Whitley Automatic Spiral Plater, West Yorkshire, England) with appropriate dilutions on tryptone soya agar (TSA; Oxoid) plates and found to contain approximately 10 $\log_{10}$ CFU ml^{-1}.

Meat sample preparation and inoculation

Musculus tensor fascia latae (knuckle from hind quarter beef) was collected from an Irish beef export plant, transported to the laboratory under refrigerated conditions (0–4°C) and stored in a chill condition at 4°C until use. The beef was sliced to a thickness of approximately 20 mm using a Cookworks slicer (Argos, UK) and a stainless steel circular template was used to cut the slices into approximately 50-mm diameter circles. Samples were placed in heat-resistant glass dishes (55-mm diameter, 22-mm high; Schott Duran, Mainz, Germany), covered with a glass lid to prevent surface drying and refrigerated at 4°C before inoculation. Samples were point inoculated in the centre, as opposed to inoculation and spreading over the entire surface, as under natural conditions beef carcasses are contaminated mainly from the hide in the form of faecal spots. A 'point inoculum' of 5 μl was pipetted on the surface of samples in dishes while in the refrigerator at 4°C, giving a surface inoculum of 7–8 $\log_{10}$ CFU cm^{-2}. After inoculation, the glass lids were replaced and the samples stored for 15 min to allow attachment to the beef surface.

Heat treatment apparatus

A decontamination apparatus was designed and built by the Food Refrigeration and Process Engineering Research Centre (FRPREC) at the University of Bristol, UK. The apparatus is fully computer-controlled and designed to produce repeatable and known heating time–temperature cycles on food surfaces. The design criteria were that the apparatus should be capable of heating the surface of a sample from 5°C to a maximum of 100°C in a time selected by the user. All samples were heated in an air stream at 20·0 m s^{-1} (range 15·0–25·0 m s^{-1}). The sample size used is a compromise between being as small as possible, which allows it to be easily and more uniformly heated, and large enough for microbiological analysis. Heating and holding times were entered into a graphical user interface (GUI), written in Microsoft Visual Basic. A test

involves placing an inoculated sample in a heat-resistant glass dish described previously that fits on top of a metal sample holder in the apparatus. By pressing a button connected to the GUI, a pneumatic actuator moves the sample holder into a heating chamber. In the chamber, a regenerative blower is used to blow air over the sample, through a 3·3-kW heater, using a nozzle adjacent and perpendicular to the surface of the sample. The sample is rotated in the chamber using a motor, to give even surface heating. Surface temperatures in the chamber are monitored throughout a test period, using an infrared (IR) sensor and the heating profile for each sample is automatically recorded and stored on a PC. This apparatus has been described in detail elsewhere Foster *et al.* (2006).

Heat treatments

Inoculated beef samples were subjected to either a fast or slow heating up phase to a set holding temperatures of 60, 75, 90 or 100°C and subsequently held at these temperatures for predetermined time intervals up to 600, 190, 20 and 5 s, respectively (see Table 1). The fast heating times were selected on the basis that they were the maximum heating rates that could be achieved by the experimental apparatus. During fast and slow heating, including the holding phase, 13–19 samples were heated at each target temperature, after which they were cooled in chilled MRD.

At each time point, a single inoculated beef sample was taken from the refrigerator at 4°C, the glass lid removed, weighed on a Sartorius BP2100s balance (Geottingen, Germany) in a Microflow Class III biological safety cabinet (Bioquell UK Ltd., UK), and placed in the heat treatment apparatus. Samples were processed at different times and temperatures, and a survival profile was developed. For example, during slow heating to 100°C, single samples were examined at intervals of 50, 100, 150, 200, 225, 250, 260, 270, 280 and 311 s during the heat up phase. In order to determine survival during the holding phase, samples which had completed the heat up phase to 100°C, were held and examined at intervals for a further 5 s. A temperature profile was recorded for each sample tested using the IR sensor in the heat treatment apparatus. After heating, samples were removed from the apparatus, weighed and placed in stomacher bags containing 100 ml of MRD, prechilled to 4°C. The bags were placed in ice water at approximately 0–1°C and held for further processing. At each sampling point, inoculated samples were subjected to the same processing, omitting the heat treatment step, to provide control data on the initial bacterial numbers on inoculated samples. Uninoculated samples were also processed without heating to verify there was no contamination from *Salmonella* or *E. coli* O157:H7 present. Heat treatment experiments were repeated on three separate occasions.

Prediction of water activity (a_w) from weight loss measurements

Weight loss measurements were taken from beef samples during fast and slow heating and holding when target temperatures were reached (60, 75, 90 and 100°C). The weight loss and the beef surface temperature data were used to derive beef surface a_w values during heating. This was done using the coupled heat–water model developed by Kondjoyan *et al.* (2006) for beef samples subjected to rapidly changing surface temperatures.

Enumeration of surviving bacteria

Uninoculated, inoculated control and heat-treated beef samples in cooled MRD were homogenized for 1 min in a Colworth stomacher (Model BA 6024; A.J. Steward& Company Ltd., London, UK) and serially diluted using 9-ml volumes of MRD. A 50-μl sample of appropriate dilutions was surface plated in duplicate using a spiral plater onto TSA plates. Where samples were thought to have low numbers of survivors, a 1·0 ml of undiluted sample was surface plated. After 2 h of resuscitation at 25°C, to allow for recovery of heat-damaged cells, the TSA was overlaid with 10 ml of xylose-lysine-desoxycholate agar (XLD; Oxoid) for *S.* Typhimurium DT104 or 10 ml of sorbitol MacConkey agar with cefixime tellurite supplement (CT-SMAC; Oxoid) for *E. coli* O157:H7. After overlaying, plates were allowed to solidify and incubated at 37°C for 24 h. Colonies were counted automatically using an Acolyte colony counter (Synbiosis Cambridge, UK).

Statistical analyses

After heat treatment of beef surfaces, surviving populations of *S.* Typhimurium DT104 and *E. coli* O157:H7 were converted to $\log_{10}$ CFU cm^{-2}. Surviving pathogen numbers ($\log_{10}$ CFU cm^{-2}) were plotted against time (s) to produce thermal inactivation curves. Pathogen reductions ($\log_{10}$ CFU cm^{-2}) during heat up (HUR), holding time (HTR) and the total reductions (TR) for each temperature and the heating rates were calculated. These reductions were analysed for the effects of heat up time (fast, slow) at each temperature (60, 75, 90 and 100°C). In addition, the HUR, HTR and TR of the two pathogens were compared at each of the eight temperatures/heat up time combinations. In each case, the data were structured as a 2×2 factorial with three replicates and an analysis of variance (ANOVA) was carried out using Genstat 5 (Sta-

tistics Department, Rothamsted Experimental Station, Hertfordshire, UK).

Results

The uninoculated beef samples showed that no contamination from *Salmonella* or *E. coli* O157:H7 was present. The *S.* Typhimurium DT104 and *E. coli* O157:H7 on the surface of unheated controls showed a consistent inoculum concentration of approximately 7–8 $\log_{10}$ CFU cm^{-2} with low levels of variation between samples. For example, with fast heating at 90°C, the mean untreated control counts were $7{\cdot}65 \pm 0{\cdot}16$ $\log_{10}$ CFU cm^{-2} for *E. coli* O157:H7 and $7{\cdot}45 \pm 0{\cdot}13$ $\log_{10}$ CFU cm^{-2} for *S.* Typhimurium DT104. The data in Table 1 shows the different heating regimes used, the rates of change in beef surface temperatures during heating and the bacterial reductions achieved. The rate of change in beef surface temperatures during fast (1·97°C s^{-1}) and slow (0·33°C s^{-1}) heating were similar for each temperature tested.

At each temperature and heating rate, HUR in counts was observed before the target temperature was reached for both organisms. During the heat up phase, the largest reductions in cell numbers occurred at the higher temperatures, for example at 100°C, during slow heating for 311 s (5·52 $\log_{10}$ CFU cm^{-2} for *S.* Typhimurium DT104 and 5·91 $\log_{10}$ CFU cm^{-2} for *E. coli* O157:H7). With the exception of 60°C, reductions in counts were higher, during slow heating, compared to fast, for both pathogens but the differences were significant only at 90 and 100°C ($P < 0{\cdot}05$). Holding time reductions during fast and slow heating for both organisms were generally small, not significant and did not exceed 3·07 $\log_{10}$ CFU cm^{-2}. The TR for the different heat treatments showed that the higher temperatures (90 and 100°C) were the most effective in reducing pathogen numbers and differences between fast and slow heating at all temperatures were not significant.

When pathogen reductions were compared during heat up, significant differences were observed for fast heating at 75°C and slow heating at 90°C only ($P < 0{\cdot}05$). During holding significant differences in pathogen reductions were only observed for fast heating at 100°C. No significant differences were observed when the TR in pathogen counts were compared for all the temperature/heat up time combinations tested. It was noted that there was a large amount of variation among replicates resulting in low levels of significant differences between treatments.

The heating profiles and subsequent changes in pathogen counts of a representative selection of these treatments are shown in Figs 1–4. At 75°C, after the fast or slow heating of *S.* Typhimurium DT104 (Fig. 1), a linear decline in numbers was observed during the holding phase, and this was also noted for *E. coli* O157:H7. After fast heating *E. coli* O157:H7 cells at 90°C, reductions in counts were linear during the holding phase (Fig. 2a). During slow heating however the change in counts was triphasic. This consisted of an initial period during heat up in which no decline occurred (shoulder), followed by a logarithmic decline. In the final phase, during the holding time, tailing was observed in which reductions in cell numbers were variable (Fig. 2b). Similar types of curves during fast and slow heating to 90°C were observed for *S.* Typhimurium DT104. Figures 3 and 4 illustrate the decline in cell numbers during fast and slow heating to 100°C for both pathogens. These show the same triphasic pattern of cell deactivation as that described for slow heating at 90°C.

Using the coupled heat–water model, the predicted meat surface a_w values at all fast heating temperatures (60–100°C) declined rapidly (within 25–30 s) to very low values (0·1–0·2). Slow heating also achieved a_w values in this range, but took much longer, i.e. 150–200 s. These a_w reductions commenced when the meat surface temperature was approximately 20–30°C, during fast and slow heating.

Table 1 Mean reductions ($\log_{10}$ CFU cm^{-2}) in *Salmonella* Typhimurium DT104 and *Escherichia coli* O157:H7 counts on beef after heat treatment

Heating temp (°C)	Heat up time (s)	Holding time (s)	Rates of change (°C s^{-1})	HUR	HTR	TR
S. Typhimurium DT104						
60	30	600	2·00	0·56	1·20	1·76
	180	600	0·33	0·52	0·94	1·46
75	38	190	1·97	0·22*	3·02	3·24
	229	190	0·33	0·48	2·34	2·82
90	46	20	1·96	1·72	2·46	4·18
	278	20	0·32	2·97†	1·85	4·82
100	51	5	1·96	2·97 (0·93)	0·89‡	3·86
	311	5	0·32	5·52	0·29	5·81
E. coli O157:H7						
60	30	600	2·00	0·55 (0·60)	0·98	1·53
	180	600	0·33	0·31	1·01	1·31
75	38	190	1·97	0·76*	2·85	3·61
	229	190	0·33	1·09	3·07	4·16
90	46	20	1·96	2·03 (0·35)	2·67	4·70
	278	20	0·33	5·05†	1·02	6·06
100	51	5	1·96	2·33 (0·22)	3·63‡ (0·90)	5·95
	311	5	0·32	5·91	0·00	5·91

HUR, heat up reduction; HTR,
holding time reduction; TR, total reduction.
Significant differences between fast and slow treatments are shown by SED values in parentheses.
Significant differences between pathogens are shown by SED values 0·20*, 0·32† and 1·05‡.

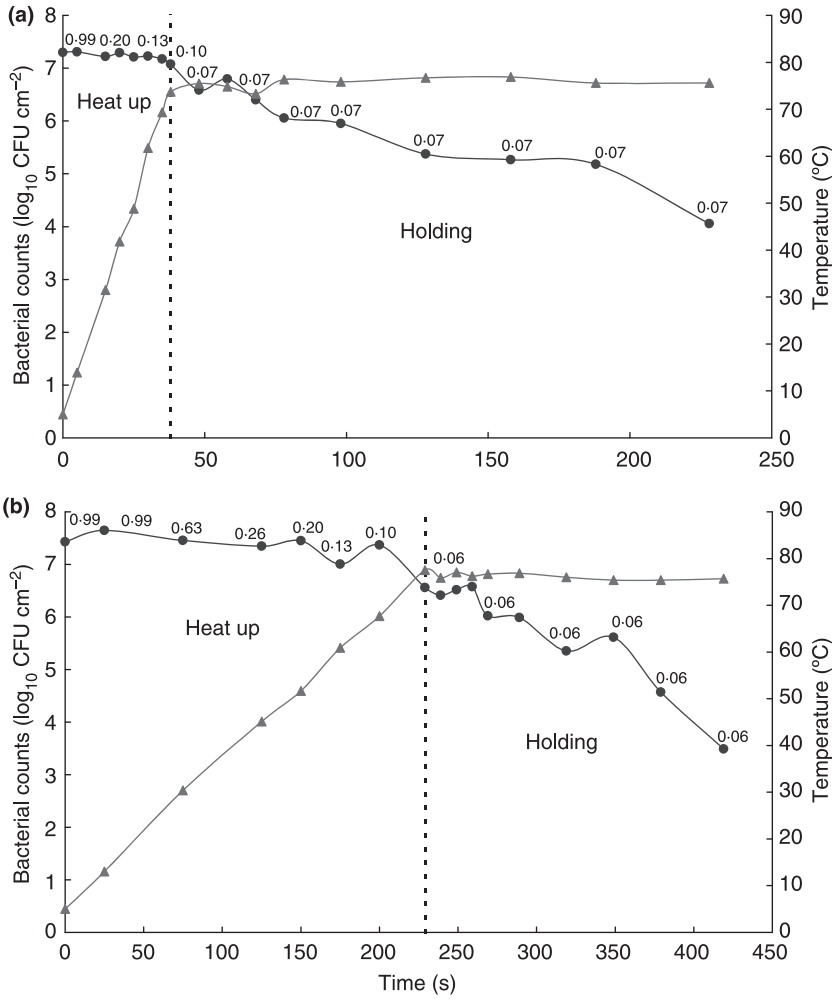

Figure 1 Deactivation of *Salmonella* Typhimurium DT104 on beef surfaces after heating for (a) 38 s and (b) 229 s, from 5 to 75°C, and holding for 190 s: (▲) beef surface temperature; (●) *S.* Typhimurium DT104 counts. Numbers on survival plot indicate beef surface a_w values.

Discussion

This study provides information on the dynamic changes in *S.* Typhimurium DT104 and *E. coli* O157:H7 counts on beef surfaces during heating with dry air, at a range of temperatures (60–100°C) using fast and slow heating rates. In this study, it was observed that heating temperature and the rate of heating influenced pathogen survival.

Dry heating of both pathogens on beef surfaces, at different temperatures, with fast or slow heating, revealed significant levels of cell death, particularly at higher temperatures (90 and 100°C, 4·18–6·06 $\log_{10}$ CFU cm^{-2}), but cell survival at these temperatures was also observed. Pathogen survival was higher at the lower temperatures of 60 and 75°C. For example, at 60°C there was approximately a 6 $\log_{10}$ CFU cm^{-2} survival after 10 min heating for both pathogens.

In the present study, variations among replicate experiments were observed. These variations could have been attributed to a number of factors, including the inability to uniformly heat samples, differences in sample thickness and variations in temperature within the test apparatus. A similar study carried out by Gaze *et al.* (2006) using the same apparatus, noted similar variations in replicate experiments on potatoes inoculated with *Listeria monocytogenes*.

Thermal resistance studies previously carried out in this laboratory (Doherty *et al.* 1998; Bolton *et al.* 2000; Walsh *et al.* 2001) and elsewhere, using meat substrates have generally used inoculated vacuum or heat-sealed packaged minced meat, which is subsequently pressed into a thin layer and heat treated in a water bath (Quintavalla and Campanini 1991; Juneja *et al.* 1997, 1998; Mazzotta 2000; Quintavalla *et al.* 2001; Juneja and Marks 2003). In these studies, which were mainly carried out at 50 to 65°C and may have involved a heating step before the test temperature was reached, *D* values (the time to inactivate 90% of micro-organisms) at different temperatures were calcula-

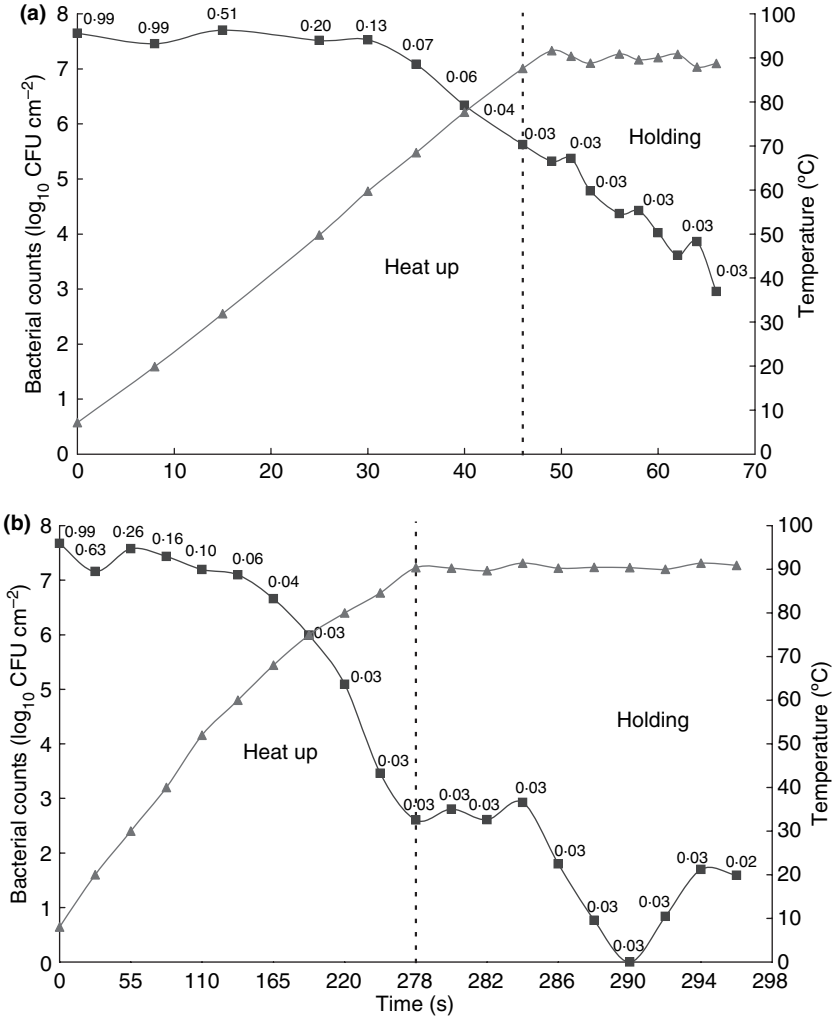

Figure 2 Deactivation of *Escherichia coli* O157:H7 on beef surfaces after heating for (a) 46 s and (b) 278 s, from 5 to 90°C, and holding for 20 s: (▲) beef surface temperature; (■) *E. coli* O157:H7 counts. Numbers on survival plot indicate beef surface a_w values.

ted as a measure of the thermal resistance of different organisms. In the present study, *D* values were not calculated for the different heating regimes used. *D* values could have been calculated at 60 and 75°C, but at the higher temperatures of 90 and 100°C this was not possible. In consequence, deactivation data is presented in the same format for all temperatures, so that comparisons of the different phases of heating could be made.

Other studies, similar to those described here were carried out in the Ashtown Food Research Centre. Circular beef samples (2-mm thick, 50-mm diameter) were point inoculated with stationary phase cells of the pathogens used in the present study, vacuum packaged and immersed in a water bath at 60°C. In the water bath, beef surfaces took 30 s to reach the holding temperature similar to that for fast heating in the heat treatment apparatus. In the heat treatment apparatus, reductions of approximately 1 $\log_{10}$ CFU cm^{-2} were achieved after 10-min holding at 60°C, for both pathogens using fast and slow heating. However, for samples immersed in a water bath a reduction of 5 $\log_{10}$ CFU cm^{-2} in pathogen counts occurred after 70 s for *S.* Typhimurium DT104 and after 7 min for *E. coli* O157:H7 (McCann *et al.* 2004; M. S. McCann *et al.*, unpublished data). The more effective inactivation in the water bath may be related to differences in beef surface a_w values. In the vacuum packs, it is assumed that the beef surface a_w would be constant (0·99) at all times, while in the heat-treatment apparatus the beef surfaces dried rapidly during heating as indicated by the surface a_w values at all the temperatures tested. Even at 60°C, the beef surface a_w reduced rapidly to 0·26 in 20 s and 0·32 in 100 s for fast and slow heating, respectively. It was noted that this reduction in surface a_w commenced when the beef surface temperature was approximately 20–30°C, at all temperatures, for both fast and slow heating. This indicated that surface drying was related to the very fast air speed (20 m s^{-1}) produced in the heat-treatment apparatus and not the heating temperature.

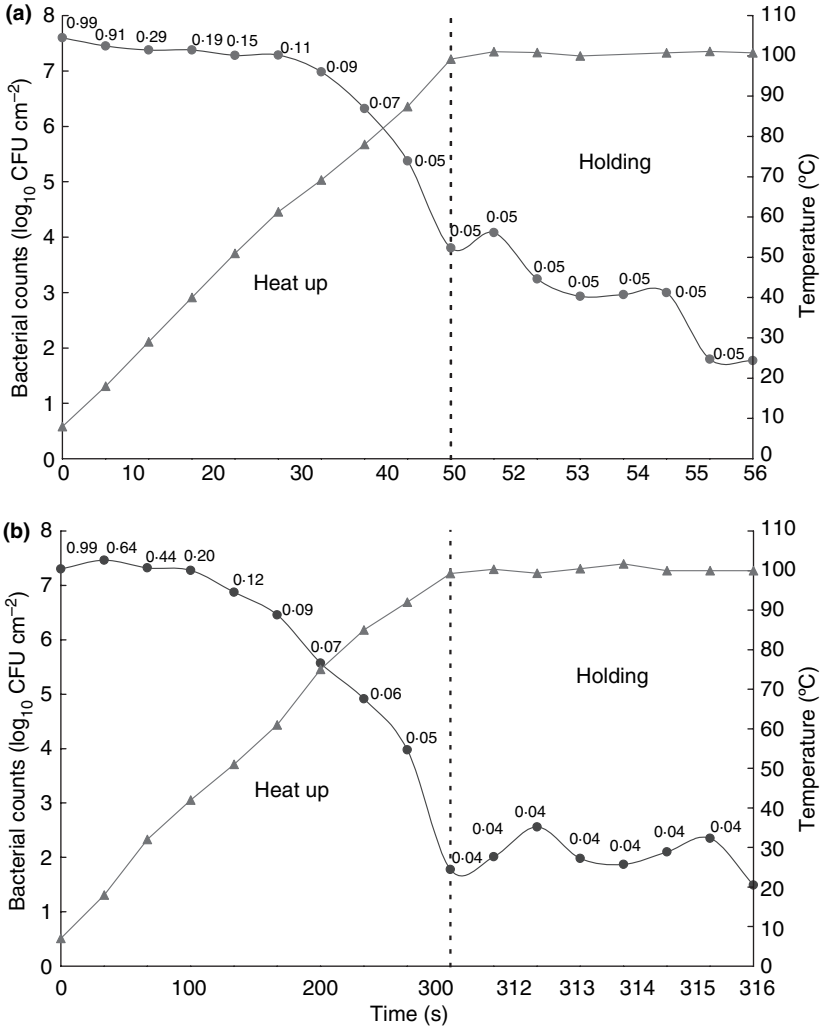

Figure 3 Deactivation of *Salmonella* Typhimurium DT104 on beef surfaces after heating for (a) 51 s and (b) 311 s, from 5 to 100°C, and holding for 5 s: (▲) beef surface temperature; (●) *S.* Typhimurium DT104 counts. Numbers on survival plot indicate beef surface a_w values.

While meat surface a_w values were measured during drying, this effect could not be determined for the pathogen cells. It is reasonable to assume however, that cell drying did occur thereby increasing the pathogens' heat resistance as a result of osmotic stress. Bacterial cells will respond in different ways during drying on meat surfaces, due to physiological variations in the cells, the protective effects of the meat surface structure and the presence of moisture in crevices (Thomas and McMeekin 1982; Schwach and Zottola 1982; Potts 1994). Such a rapid response of cells to a decrease in a_w has been observed in water/glycerol solutions, due to hyperosmotic stress, where a decrease in the total cell volume of about 90% occurred in 9 s (Marechal and Gervais 1994; Berner and Gervais 1994). When dry air is used to deactivate bacterial cells, much higher temperatures are required for bacterial inactivation, compared with heating with moist air (Russell 2003).

During the heating up phase at 75, 90 and 100°C, it was observed that reductions in counts for both pathogens were higher after slow heating compared with the fast. Previous studies have shown that heating rates have a profound effect on the resistance of cells (Mackey and Derrick 1987; Martinez de Maranon *et al.* 1999; Marechal *et al.* 1999). While slow heating to a target temperature is generally associated with an increased ability to survive, a subsequent heat shock possibly due to the production of heat shock proteins (Thompson *et al.* 1979; Mackey and Derrick 1987; Bolton *et al.* 2000; Juneja and Marks 2003) was not evident in the present study and was not reflected in the subsequent survival during the holding time. In experiments where this effect has been demonstrated, the temperatures used were generally below 60°C, where survival during heat up was possible. The effect of heating rates on *S.* Typhimurium has been demonstrated previously in ground beef and it has been shown that the

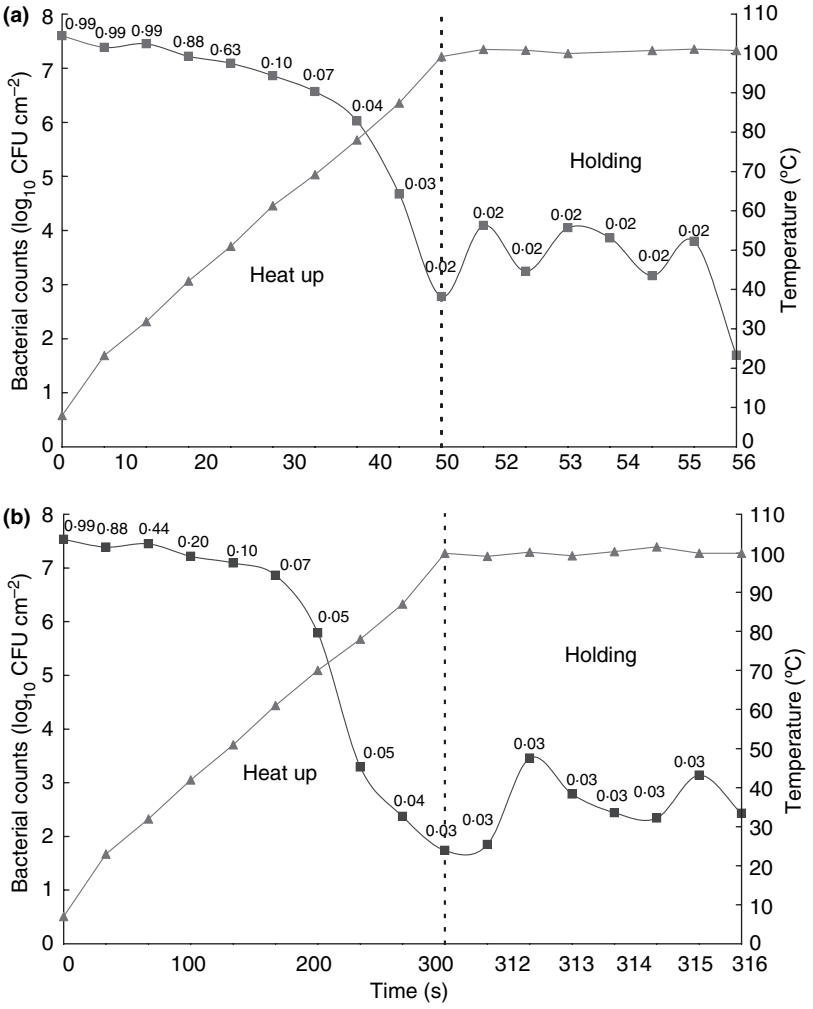

Figure 4 Deactivation of *Escherichia coli* O157:H7 on beef surfaces after heating for (a) 51 s and (b) 311 s, from 5 to 100°C, and holding for 5 s: (▲) beef surface temperature; (■) *E. coli* O157:H7 counts. Numbers on survival plot indicate beef surface a_w values.

slower the temperature rise the greater is the increase in resistance (Thompson *et al.* 1979; Juneja and Marks 2003). With temperatures above 60°C used in the present work, heat shock protein formation may have been masked due to cell death at high temperatures and long heat up times.

The deactivation of *S.* Typhimurium DT104 and *E. coli* O157:H7 during heating depended on the rate of heating and the temperature. During holding at 60°C, inoculated beef samples showed a very slow linear decline in cell numbers over time, which was not related to the heating rate. At 75°C, for *E. coli* O157:H7, the heat up phases were both represented by a shoulder, followed by a linear decline. A similar pattern of deactivation occurred for *S.* Typhimurium DT104. Fast heating at 90°C also resulted in the presence of a shoulder during heat up, and a linear decline for both pathogens. At this temperature for both pathogens, slow heating resulted in triphasic death curves, which were also evident during fast and slow heating at 100°C. The triphasic deactivation curves were composed of an initial phase during heat up, where no decline in bacterial numbers occurred (shoulder), followed by a logarithmic decline in numbers. The final phase during the holding time, tailing, was observed in which reductions in cell numbers were variable. This final phase was considered to represent the presence of a heat-resistant subpopulation, which occurred at high temperatures. Triphasic deactivation has been reported in other studies for *Salmonella* (Moats *et al.* 1971; Mackey and Derrick 1987; Kirby and Davies 1990). In the study of Kirby and Davies, *S.* Typhimurium LT2 cells were placed in a controlled atmosphere with the relative humidity at 57%. The resulting cells were subjected to a heat challenge at 135°C for 30 min and the surviving population was triphasic. The results also showed that after heating at 100°C for 1 h, little or no cell death occurred. While the mechanism for such survival is not known, it almost certainly involves the production of heat shock proteins, which allow the cells to survive such high temperatures.

Heat shock proteins capable of protecting cell protein denaturation at very high temperatures have been described and been shown to be capable of protecting cell lysates of *E. coli* from denaturation at 100°C (Kim *et al.* 1998). Functional diversity amongst heat shock proteins is well known and such a mechanism may be involved in the production of the heat-resistant subpopulation observed at high temperatures, as in the present study (Hengge-Aronis 1993; Haslbeck *et al.* 2004).

The reductions in a_w of beef samples that occurred during dry heating may have had a major influence on the thermal resistance of the pathogens. However, a number of other factors may well have contributed to their heat resistance, including the use of stationary phase cells. The present study used stationary phase cells, which for *E. coli* have been shown to have an enhanced resistance to air-drying (Siegele and Kolter 1992; Potts 1994). The most important factors in relation to *E. coli* surviving heating or drying are the improved adhesiveness of cells, a decreased metabolic rate, an increase in trehalose synthesis and the induction of starvation-specific thermo tolerance and osmoprotective proteins (Notermans and Kampelmacher 1975; Siegele and Kolter 1992; Rees *et al.* 1995; Humphrey *et al.* 1997).

While some of these changes in resistance can be ascribed to heat and osmotic stress responses, some aspects of the observed triphasic curves are more difficult to explain. Thus, the tailing observed may be due to the presence of a heat-resistant subpopulation. As already stated, the occurrence of resistance in heated cells is generally associated with the production of heat shock proteins.

Technologies, such as steam pasteurization of beef surfaces have been shown to give similar reductions, i.e. 3–4 log units using the present test apparatus and other experimental systems (Retzlaff *et al.* 2004; McCann *et al.* 2006). However, under commercial operating conditions steam pasteurization has been shown to be much less effective in reducing bacterial counts on beef carcasses, i.e. *c.* 1 log (McCann *et al.* 2006). This ineffective response is due primarily to the steam/air mixtures in commercial pasteurization units not being sufficiently high to effect a higher reduction in counts. In commercial operations, steam/air mixtures are frequently kept at about 90°C in order to preserve carcass colour, which is insufficiently high enough to obtain large reductions in bacterial counts.

In terms of the practical implications of using dry air to decontaminate beef surfaces, this study has shown that substantial reductions in pathogens can be achieved. A difficulty arises however, in relation to the survival of significant numbers of organisms at high temperatures. In particular, the presence of resistant subpopulations related to osmotic and thermal protective affects needs to be addressed, before this form of surface heating could be recommended as a decontamination process. A further difficulty with the use of air-drying is that after treatment the meat has a poor appearance and colour. This would make the process unsuitable for producing meat for retail sale, but it could be used to produce safer meat for use in the catering trade.

Acknowledgements

The authors would like to thank Dr Alain Kondjoyan for his prediction of beef surface a_w values used in this paper. This study has been carried out with financial support from the Commission of the European Communities specific RTD programme 'Quality of Life and Management of Living Resources', QLK1-2001-01415 'Bugdeath'.

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