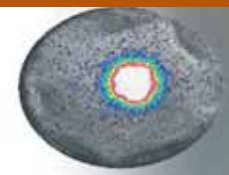
Agriculture and Food Development Authority

FINAL REPORT

Project RMIS No. 5048

Surface decontamination of meat using thermal processes



Ashtown Food Research Centre

RESEARCH & TRAINING FOR THE FOOD INDUSTRY

RESEARCH REPORT NO 87

SURFACE DECONTAMINATION OF MEAT USING THERMAL PROCESSES

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SUMMARY

This study investigated the effectiveness of a novel heat apparatus for decontamination of meat surfaces inoculated with important foodborne pathogens using either steam or dry air.

Steam pasteurisation: Beef, chicken (skin on and off) and pork samples were surface inoculated with 7-8 $\log_{10}$cfu/cm^2 of stationary phase cells of *Salmonella* Typhimurium DT104 or *Escherichia coli* O157:H7 and treated with steam for up to 60 seconds in an experimental test apparatus. Treated discs were examined by a selective overlay resuscitation method to determine surviving pathogen numbers. After treatment for 60 s, numbers of surviving *E. coli* O157:H7 were generally higher than *S.* Typhimurium DT104, though significantly so only for chicken skin, indicating a possible difference in the heat resistance of the two organisms. The numbers of surviving cells were also influenced by meat type, treatment temperature and time. The overall pattern of *E.coli* O157:H7 deactivation was clearly biphasic on all meat types, displaying an initial pattern of rapid decline within the first 10 s followed by much slower decline during the remaining 50 s. This indicated the persistence of a heat resistant sub-population of this pathogen.

Dry air decontamination: Beef samples were surface inoculated with 7-8 $\log_{10}$cfu/cm^2 of *Salmonella* Typhimurium DT104 or *Escherichia coli* O157:H7 and heated at 60, 75, 90 and 100º 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 100º 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; in particular, at 60º C it was less than one log unit after three minutes 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 90º C and both heating rates at 100º C, the pattern of deactivation of *Salmonella* Typhimurium DT104 or *Escherichia coli* O157:H7 was triphasic. This study has shown that heating meat surfaces with dry air can achieve substantial reductions in *Salmonella* Typhimurium DT104 or *Escherichia coli* O157:H7.

INTRODUCTION

Most of the contamination by pathogenic and spoilage organisms is present on food surfaces at the time of harvesting or is transferred to the surfaces during slaughter and processing. Studies (Gill, 1979; 1980) have shown that at the time of slaughter, muscle tissue of a healthy animal is essentially sterile. Meat carcasses and meat cuts are easily contaminated during processing and, if not properly handled, processed and preserved, they may support growth and serve as sources of various spoilage and pathogenic micro-organisms. A variety of sources contribute microbial contamination during slaughtering, dressing, chilling and cutting processes when the muscles of animals are exposed to the environment. Sources of contamination include air, water, soil, faeces, feed, hides, intestines, lymph nodes, processing equipment, utensils and humans (Koutsoumanis and Sofos, 2004).

Centralised processing and preparation of meat products is growing, increasing the distance and time between initial preparation and consumer consumption, thus increasing the risk of pathogen growth (James et al., 2006). If micro-organisms on raw meat surfaces could be eliminated or substantially reduced immediately after slaughter or harvest, the risk of cross-contamination during processing would be substantially reduced. An efficient method of surface decontamination therefore offers substantial advantages in terms of food safety, spoilage and economics.

Currently in the EU there are few decontamination systems for meat; in the USA, decontamination systems for red meat, mainly using steam, are becoming commonplace. Often a hurdle system is used which may require the application of a chemical stage in addition to heating and washing; however, consumers tend to view the use of chemicals with suspicion so surface pasteurisation using rapid thermal heating technologies is an attractive option. Consequently, there is growing interest in the use of rapid heating systems to decontaminate the surface of raw materials and food products.

This project investigated the potential of novel (dry air) and more traditional (steam) techniques for the thermal decontamination of meat inoculated with two foodborne pathogens (*Salmonella* Typhimurium DT104 and *E.coli* O157:H7). *Salmonella* is one of the most important foodborne pathogens of public health significance and continues to present major concerns to regulatory agencies and the food industry (Juneja and Marks, 2006). It causes salmonellosis, a major bacterial enteric illness in humans and animals. For example, it has been estimated that there are 1.4 million cases of salmonellosis every year in humans in the United States (Mead et al., 1999). The second pathogen to be examined in this study was verotoxin-producing *E. coli* O157:H7, a highly virulent serotype of *E. coli* which has emerged as an important foodborne pathogen of considerable public health concern. Although this serotype is associated with relatively few human infections in comparison with *Salmonella* and most other food borne pathogens, it poses particular concerns related to its very low infective dose (which may be as low as 10 organisms), and the severity of the resultant disease (Coia, 1998).

THE DECONTAMINATION APPARATUS

A decontamination apparatus (**Figure 1**) was designed and built by the Food Refrigeration and Process Engineering Research Centre (FRPREC) at the University of Bristol. The work presented here formed part of a European project entitled 'BUGDEATH' (EU QLK1-CT-2001-01415) co-ordinated by the University of Bristol, U.K., the aim of which was to produce a model that would predict the effects of thermal treatments, based on hot air or steam, on the surface decontamination of food products.

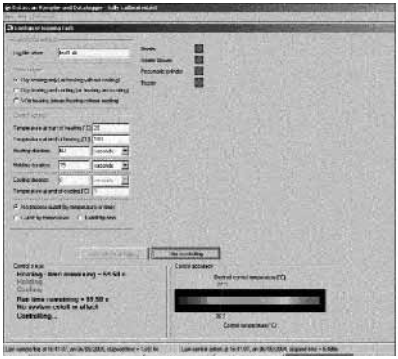
The apparatus was fully computer-controlled and was designed to produce repeatable and known heating time-temperature cycles on food surfaces using dry air or steam. The design criteria were that the apparatus should be capable of heating the surface of a sample from 5º C to a maximum of 100º C in a time selectable by the user. Samples were heated in an air stream at 20.0 m/s (range 15.0 to 25.0 m/s); the sample size used was a compromise

between the maximum size required to facilitate uniform heating and the minimum size required for microbiological analysis. Heating and holding times were entered into a graphical user interface (GUI) (Figure 2), written in Microsoft Visual Basic. Performing a test involved placing an inoculated sample in a heat-resistant glass dish, which fitted on top of a metal sample holder in the apparatus. By pressing a button connected to the GUI, a pneumatic actuator moved the sample holder into a heating chamber. In the chamber, a regenerative blower was used to blow air over the sample through a 3.3kW heater using a nozzle adjacent and perpendicular to the surface of the sample. The sample was rotated in the chamber using an electric motor to provide even surface heating. Surface temperatures in the chamber were monitored throughout a test period using an infra-red (IR) sensor; the heating profile for each sample was automatically recorded and stored on a PC.

When using the steam function of the decontamination apparatus, sample surfaces were heated in steam at approximately 100°C for pre-set times, using steam generated within the system. Performing a test using steam was exactly analogous to that described for samples heated using the dry air facility except that steam was introduced into the chamber 5 s prior to the introduction of the sample and surface temperature measurements were taken using thermocouples.

Figure 1: Heat treatment apparatus

Figure 2: Screenshot of graphical user interface during a treatment.



OBJECTIVES OF THE PROJECT

- To determine the effectiveness of steam decontamination of beef, pork and chicken inoculated with *Salmonella* Typhimurium DT104 and *Escherichia coli* O157:H7
- To determine the effectiveness of dry air decontamination of beef inoculated with *Salmonella* Typhimurium DT104 or *Escherichia coli* O157:H7.

STEAM PASTEURISATION OF MEAT SURFACES INOCULATED WITH *S.* TYPHIMURIUM DT104 OR *E. COLI* O157:H7

This study investigated the effectiveness of steam in the decontamination of beef, chicken and pork. Meat discs (beef, chicken (skin on and off) and pork) were point inoculated with 7-8 $\log_{10}$ cfu/cm^2 of *S.* Typhimurium DT104 or *E. coli* O157:H7. Meat discs were treated with steam for predetermined time intervals of up to 60 s in experimental heat treatment apparatus (Figure 1). Steam temperature was monitored using K-type thermocouples. Three temperatures were recorded over a 60 s period (1) the steam injection temperature at the nozzle, (2) the temperature of the steam/air mixture before sample heating, and (3) the temperature of the steam/air mixture after

sample heating. Surface temperatures of samples during steam treatment for 60 s were measured using T-type thermocouples which were placed just beneath the upper and lower surfaces and also inserted into sample centres. The temperature readings were recorded every second using a Grant Squirrel Data Logger 1000 Series (Grant Instruments, Cambridge UK) and readings from 5 replicate samples were stored. A heat and mass transfer model developed by Havet (2005) was also used to predict upper surface temperatures.

Following treatment, samples were placed into stomacher bags containing 100 ml of pre-cooled diluent. Treated discs were examined by a selective overlay resuscitation method to determine surviving pathogen numbers. The relationship between pathogen numbers and treatment times was examined using non-linear regression to fit the negative exponential equation:

$$Y = A + BR^t.$$

where Y = pathogen counts ($\log_{10}$ cfu/cm^2), t = time (s) and A, B, and R are parameters that must be estimated. The asymptote of the model is A, the count at time zero is A + B and the parameter R influences the rate of decay. The patterns of change in pathogen numbers on meat surfaces following steam treatments for 60 s are presented in Figures 3 – 6. In all cases, pathogen numbers declined rapidly during the first 10 seconds of steam treatment; this initial rate of decline slowed during the next 50 s so that considerable pathogen numbers were still present after steam treatment for 60 s. There were differences among the shapes of curves obtained during steam treatment which appear to be influenced by the pathogen investigated and the meat type. These shapes, based on estimates of R, B, and A together with goodness of fit (% variance) for the negative exponential model for the different substrates and pathogens, are shown in Table 2.1; they show a satisfactory fit between the counts for both organisms and treatment time for most substrates i.e. 68 to 88%. However, on chicken meat, the goodness of fit (% variance) relationship between *E. coli* O157:H7 counts and treatment times was lower (56%).

Standard deviations between replicates (SD_R) and between times (SD_T) are presented in Table 1. These data show that the standard deviations between replicates were generally large *i.e.* 1-2 logs and that different meat types had different SD_R values. In almost all cases, SD_T values were much smaller. SD_T values of zero indicate that the corresponding component of variance was negative. The predicted numbers of *E. coli* O157:H7 and *S.* Typhimurium DT104 recovered from the surface of beef, chicken meat, chicken skin and pork after steam pasteurisation for 30 and 60 s are presented in Table 2. The data show that there were no significant differences in pathogen numbers for either pathogens or meat types whether the samples were steam pasteurised for 30 or 60 s. Therefore, *E. coli* O157:H7 numbers were predicted to decline by 2.53, 3.13, 3.53 and 3.27 (log_{10} cfu/cm^2) for beef, chicken meat, chicken skin and pork respectively, by the end of the 60 s steam treatment. *S.* Typhimurium DT104 numbers declined by 3.65, 5.23, 6.15 and 2.64 (log_{10} cfu/cm^2) for beef, chicken meat, chicken skin and pork respectively after steam treatment.

For chicken skin samples after the 60 s steam treatment, there was a significant difference between *E.coli* O157:H7 and *S.* Typhimurium DT104 counts ($P<0.05$) but for all other meat types no differences were observed. There were no significant differences between meat types in relation to the number of *E.coli* O157:H7 cells recovered after 60 s of steam treatment. For *S.* Typhimurium DT104, however, significant differences were observed between chicken meat and pork ($P<0.05$) and chicken skin and pork ($P<0.001$).

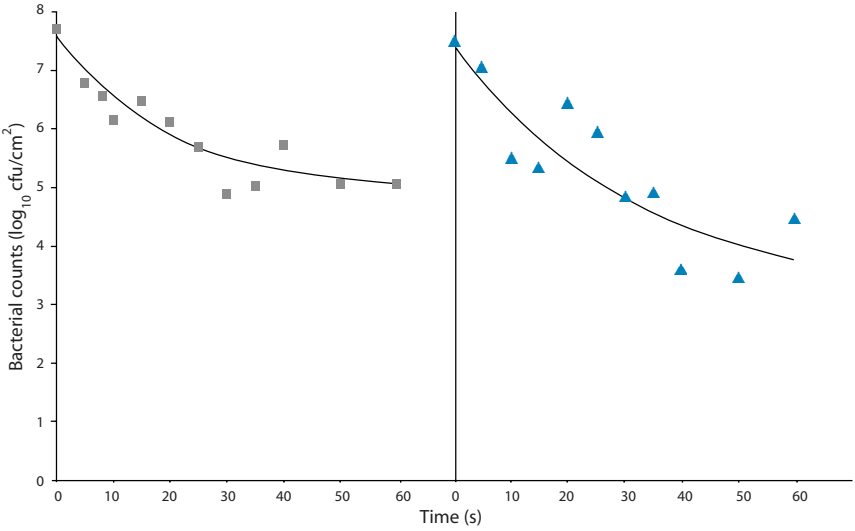


Figure 3: Relationship between the survival of (■) *E. coli* O157:H7 and (▲) *S.* Typhimurium DT104 and time on beef surfaces after steam pasteurisation.

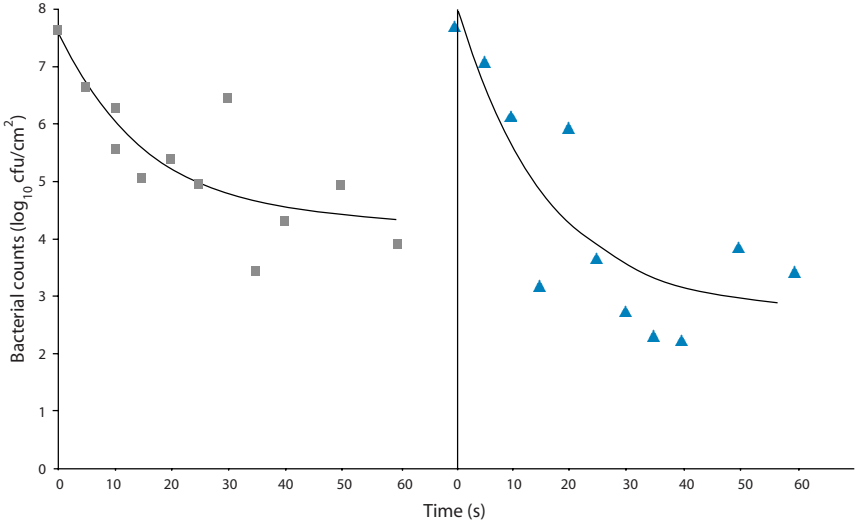


Figure 4: Relationship between the survival of (■) *E. coli* O157:H7 and (▲) *S.* Typhimurium DT104 and time on chicken meat surfaces after steam pasteurisation.

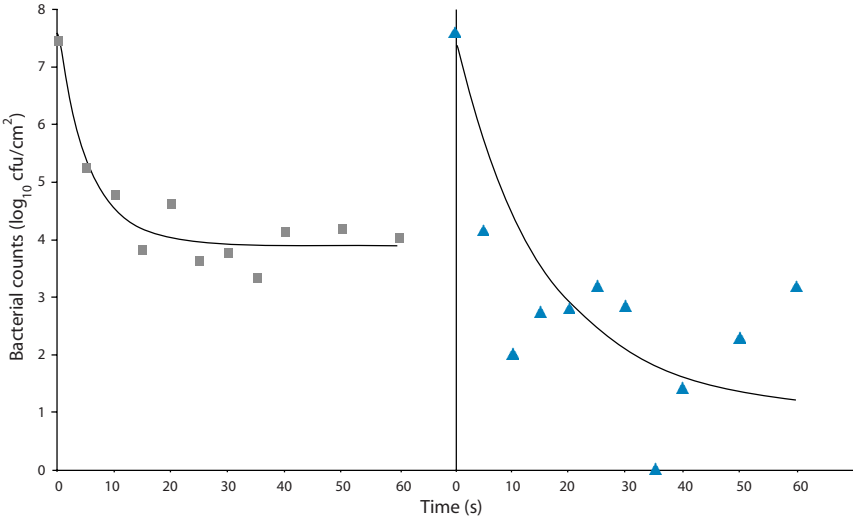


Figure 5: Relationship between the survival of (■) *E. coli* O157:H7 and (▲) *S.* Typhimurium DT104 and time on chicken skin after steam pasteurisation.

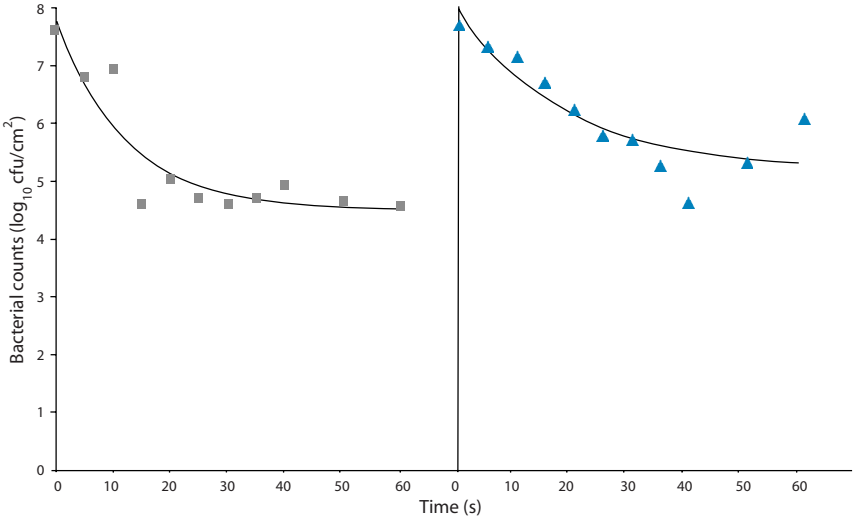


Figure 6: Relationship between the survival of (■) *E. coli* O157:H7 and (▲) *S.* Typhimurium DT104 and time on pork surfaces after steam pasteurisation.

Table 1: Estimates of R, B, A and of goodness of fit (% variance) for the negative exponential equation ($Y=A+BR^t$) and standard deviation between replicates (SD_R) and between times (SD_T)

Organism/ substrate	R	B	A	% Variance	SD_R	SD_T
E.coli O157:H7						
Beef	0.95	2.65	4.95	83	1.73	0
Chicken meat	0.94	3.24	4.29	56	0.92	0.42
Chicken skin	0.84	3.53	3.90	88	1.98	0
Pork	0.92	3.89	4.50	81	0.81	0.34
S. Typhimurium DT104						
Beef	0.97	4.35	3.06	70	1.22	0.48
Chicken meat	0.94	5.36	2.77	68	1.00	0.99
Chicken skin	0.94	6.30	1.08	87	2.37	1.03
Pork	0.95	2.77	5.16	76	2.70	0.68
R influences the rate of decay; A is the asymptote of the model; B: A+B equals the count at time zero.						

Table 2: Predicted numbers (log_{10} cfu/cm^2) of *E. coli* O157:H7 and *S.* Typhimurium DT104 recovered from the surface of beef, chicken skin, chicken meat and pork after steam treatment for 30 and 60 s.

	E.coli O157:H7 (Pasteurisation time (s))		*S.* Typhimurium DT104 (Pasteurisation time (s))	
Meat Type	(30)	(60)	(30)	(60)
Beef	5.43 ± 0.39*	5.04 ± 0.43	4.92 ± 0.77	3.85 ± 0.90
Chicken meat	4.73 ± 0.89	4.35 ± 0.98	3.49 ± 1.17	2.86 ±1.30
Chicken skin	3.91 ± 0.41	3.89 ± 0.41	1.94 ± 0.81	1.19 ±0.90
Pork	4.78 ± 0.52	4.53 ±0.57	5.73 ± 0.50	5.28 ±0.56
*Standard errors				

The data obtained in this study at time intervals following 60 s of steam treatment allow analysis of dynamic changes in bacterial numbers. Such data represent a significant step forward from those obtained in previous studies which estimated bacteria survival after a few, or in some cases only one, treatment period. This study showed a pattern of biphasic inactivation of *E. coli* O157:H7 in which there was an initial rapid (10 sec) linear decrease in pathogen numbers followed by a period of "tailing" (50 sec), during which no further reductions occurred. These results indicate the presence of a heat resistant sub-population which did not decrease over the time course of the experiment. The presence of this type of sub-population is of particular clinical and commercial interest since it represents a potential reservoir of infection, particularly given the very low infective dose of this pathogen.

SURFACE DECONTAMINATION OF BEEF INOCULATED WITH *S.* TYPHIMURIUM DT104 AND *E.COLI* O157:H7 USING DRY AIR

This study investigated the effects of dry air applied using a decontamination apparatus on numbers of the stationary phase of *S.* Typhimurium DT104 and *E.coli* O157:H7. Beef samples were surface inoculated with 7-8 log_{10} cfu/cm^2 of *S.* Typhimurium DT104 or *E. coli* O157:H7, surfaces were heated to 60, 75, 90

and 100º C using fast and slow heating rates and samples were subsequently held at these temperatures for up to 600 s. A temperature profile was recorded for each sample tested using the infrared sensor in the heat treatment apparatus. Beef surface water activities (a_w) were predicted during each treatment using the coupled heat–water model developed by Kondjoyan et al. (2006) for beef samples subjected to rapidly changing surface temperatures. Following treatment, samples were placed into stomacher bags containing 100 ml of pre-cooled diluent. Treated discs were examined by a selective overlay resuscitation method to determine surviving pathogen numbers.

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 treatment 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 heating rate were calculated. These reductions were analysed for the effects of heat up time (fast, slow) at each temperature (60, 75, 90, 100º C). In addition, the HUR, HTR and TR of the two pathogens were compared at each of the eight temperature/heat up time combinations. In each case, the data were structured as a 2 x 2 factorial. Three replicates were performed for each treatment and an analysis of variance was carried out using Genstat 5 (Statistics Department, Rothamsted Experimental Station, Hertfordshire, U.K). The data in Table 3 show 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 (1.97º C/s) and slow (0.33º C/s) heating were similar for each temperature tested.

At each temperature and heating rate, a heat up reduction (HUR) in counts was observed before the target surface 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 100º C, following 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 60º C, reductions in counts were higher following slow heating than fast for both pathogens but the differences were significant only at 90 and 100º C ($P< 0.05$). Holding time reductions (HTR)

following fast and slow heating for both organisms were generally small, not significant and did not exceed 3.07 $\log_{10}$ cfu/cm^2. Total reductions (TR) for the different heat treatments showed that the higher temperatures (90 and 100º 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 (HUR), significant differences were observed for fast heating at 75º C and slow heating at 90º C only ($P< 0.05$). During holding (HTR), significant differences in pathogen reductions were only observed for fast heating at 100º C; no significant differences were observed when the total reductions (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 difference between treatments.

The heating profiles and subsequent changes in pathogen counts of a representative selection of these treatments are shown in Figures 7-10. At 75º C, after the fast or slow heating of *S*. Typhimurium DT104 (Figure 7), 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 90º C, reductions in counts were linear during the holding phase (Figure 8a). During slow heating however, the change in counts was triphasic; this involved 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 (Figure 8b). Similar types of curves during fast and slow heating to 90º C were observed for *S*. Typhimurium DT104. Figures 9 and 10 illustrate the decline in cell numbers during fast and slow heating to 100º C for both pathogens; these show the same triphasic pattern of cell deactivation as that described for 90º C show.

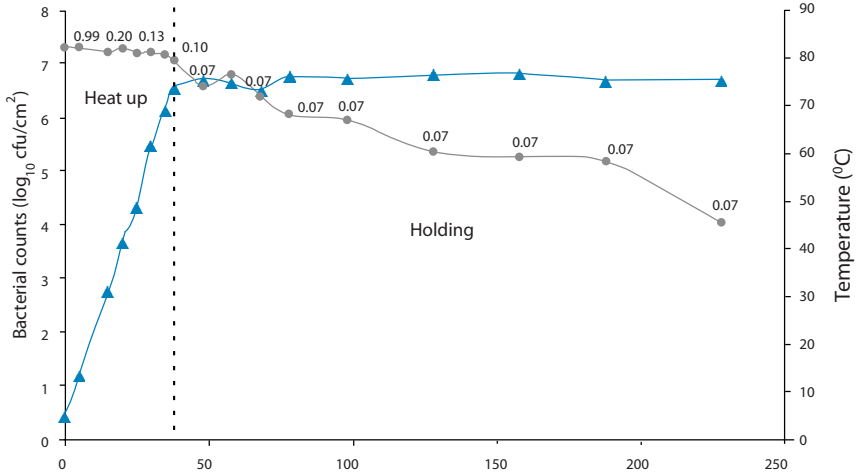
Using the coupled heat–water model, the predicted meat surface a_w values at all fast heating temperatures (60 to 100º C) declined rapidly (within 25–30 s) to very low values (0.1-0.2). Slow heating also achieved a_w values in this range but took much longer i.e. 150-200 s. These a_w reductions commenced when the meat surface temperature was approximately 20-30º C, during fast and slow heating.

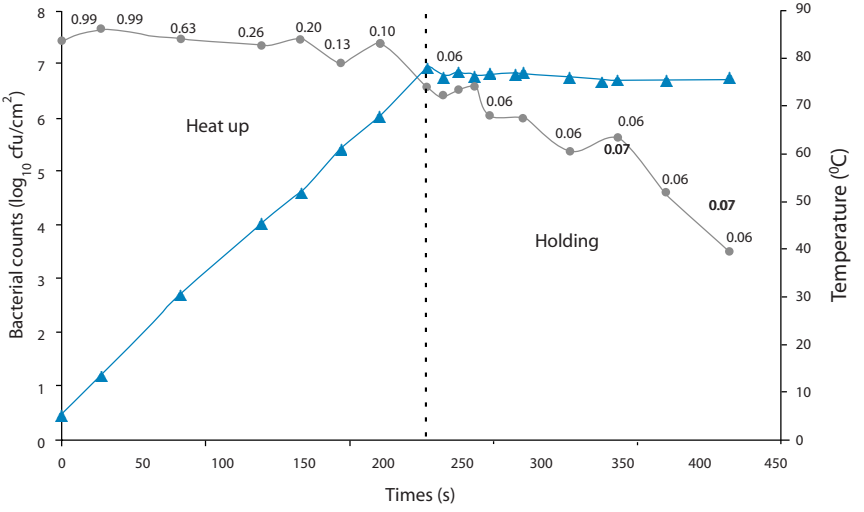
Table 3: Mean reductions (log_{10} cfu/cm^2) in *S.* Typhimurium DT104 and *E.coli* O157:H7 counts on beef after heat treatment.

Heating temp (º C)	Heat up time (s)	Holding time (s)	Rates of change (º C/s)	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[a]	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[b]	1.85	4.82
100	51	5	1.96	2.97 (0.93)	0.89[c]	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[a]	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[b]	1.02	6.06
100	51	5	1.96	2.33 (0.22)	3.63[c] (0.90)	5.95
	311	5	0.32	5.91	0.00	5.91

Key: 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 S.E.D value 0.20 [a], SED value 0.32 [b], SED value 1.05 [c]

(a)





(b)

Figure 7: Deactivation of *S.* Typhimurium DT104 on beef surfaces after heating for (a) 38 s (b) 229 s from 5 to 75º C and holding for 190 s. (▲) Beef surface temperature. (●) *S.* Typhimurium DT104 counts. Numbers on survival plot indicate beef surface a_w values.

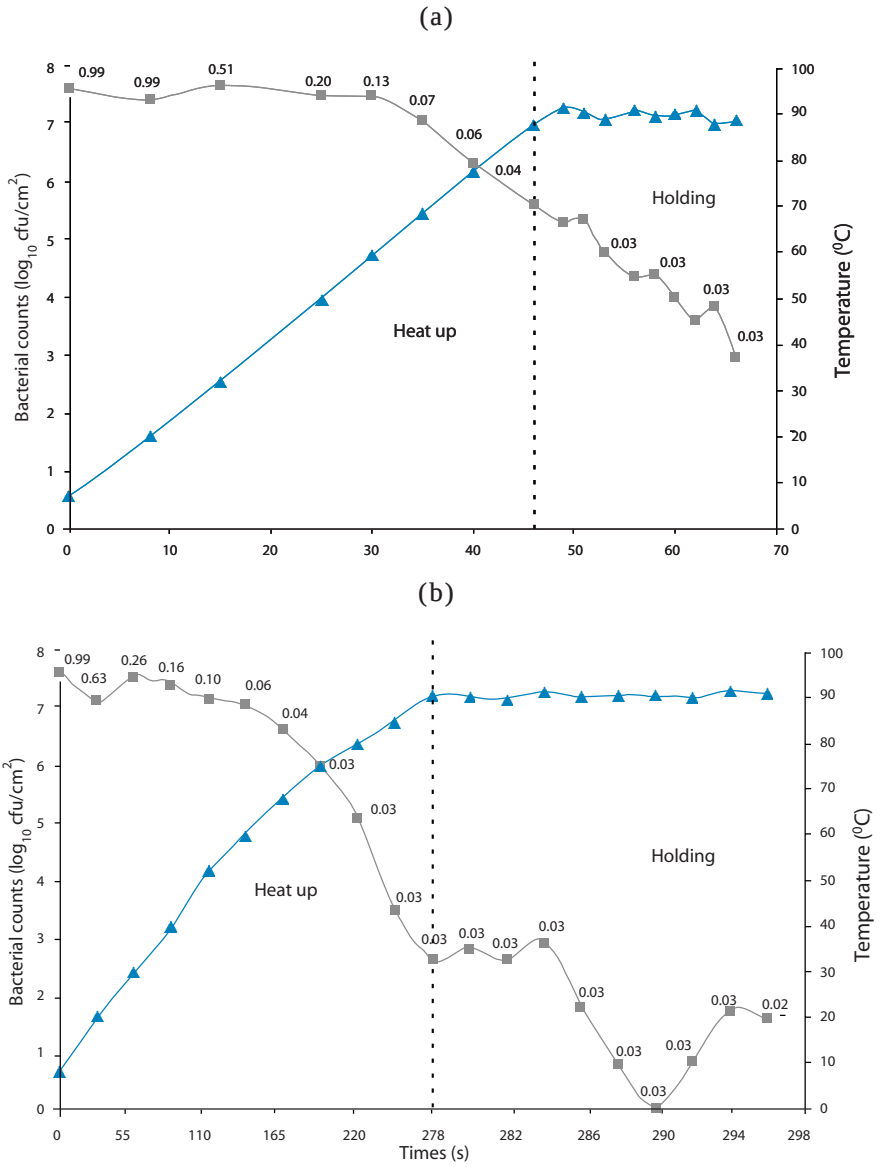


Figure 8: Deactivation of *E.coli* O157:H7 on beef surfaces after heating for (a) 46 s (b) 278 s from 5 to 90° C and holding for 20 s. (▲) Beef surface temperature (■) *E.coli* O157:H7 counts. Numbers on survival plot indicate beef surface a_w values.

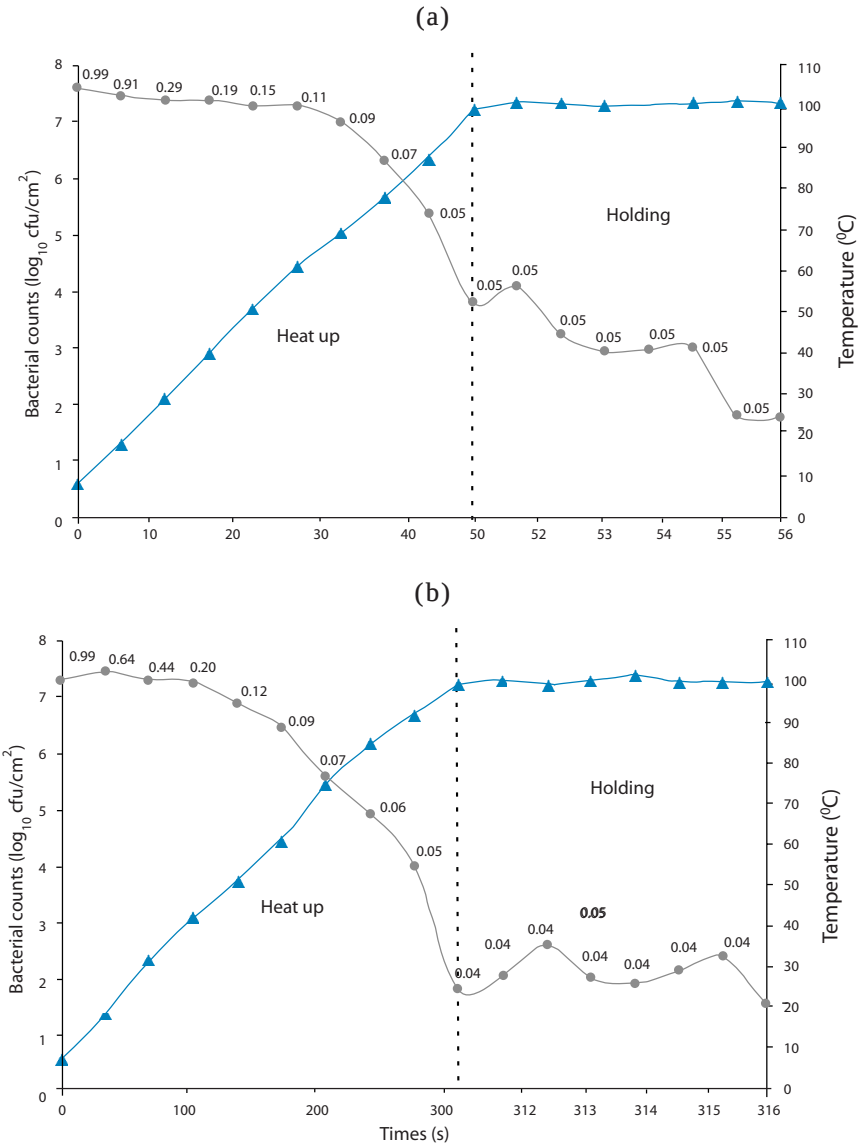


Figure 9: Deactivation of *S.* Typhimurium DT104 on beef surfaces after heating for (a) 51 s (b) 311 s from 5 to 100° C and holding for 5 s. (▲) Beef surface temperature (■) *S.* Typhimurium DT104 counts. Numbers on survival plot indicate beef surface a_w values.

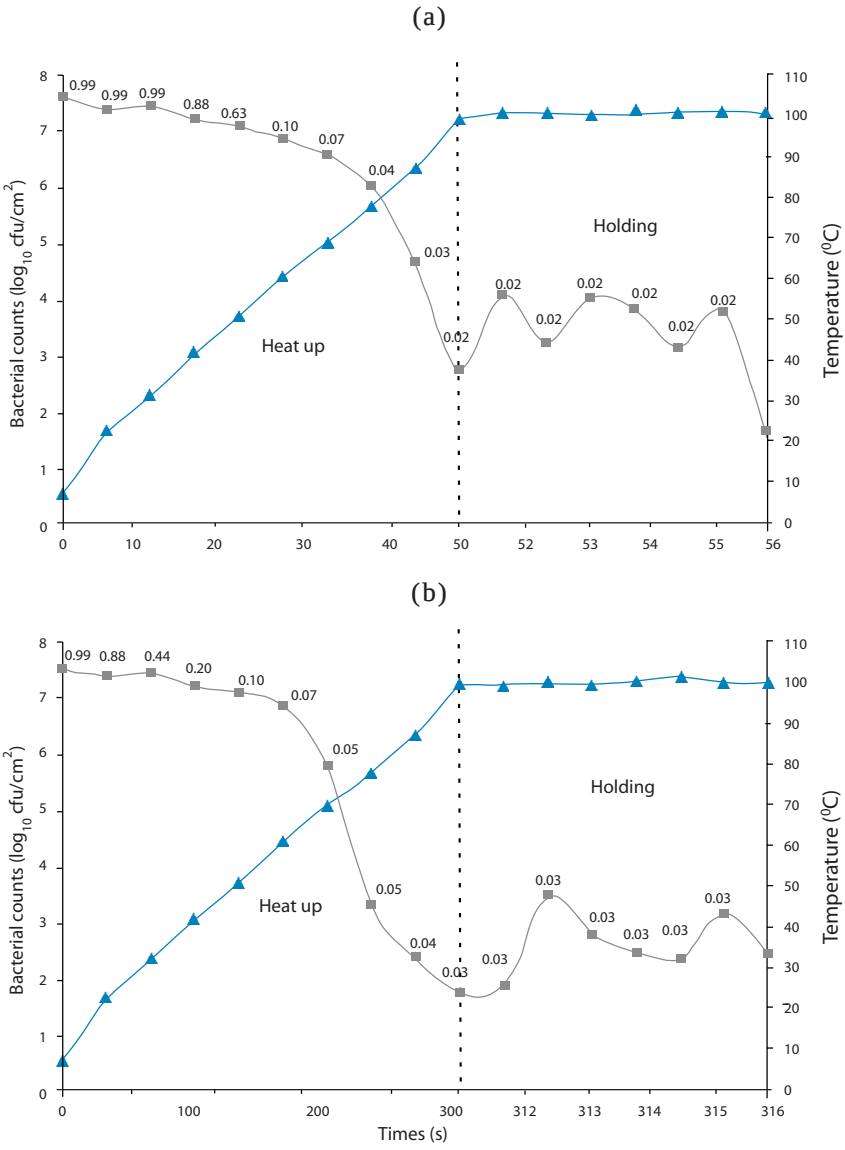


Figure 10: Deactivation of *E.coli* O157:H7 on beef surfaces after heating for (a) 51 s (b) 311 s from 5 to 100º C and holding for 5 s. (▲) Beef surface temperature (■) *E.coli* O157:H7 counts. Numbers on survival plot indicate beef surface a_w values.

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 (60-100º C) and 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 beef surfaces at different temperatures with fast or slow heating, revealed significant levels of cell death for both pathogen types, particularly at higher temperatures (90 and 100º 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 75º C; for example, at 60º C there was approximately a 6.00 $\log_{10}$ cfu/cm^2 survival after 10 min heating for both pathogens.

CONCLUSIONS AND RECOMMENDATIONS TO INDUSTRY

Steam pasteurisation of meat surfaces inoculated with ***S.*** Typhimurium DT104 or ***E. coli*** O157:H7:

This study showed that significant pathogen reductions on meat surfaces could be attained after steam pasteurisation using the test apparatus. These reductions in counts were dependant on the pathogen and meat type investigated. Pathogen reductions were generally represented using a bi-phasic curve.

Surface decontamination of beef inoculated with ***S***. Typhimurium DT104 and ***E.coli*** O157:H7 using dry air:

This study has shown that heating meat surfaces with dry air can achieve substantial reductions in *Salmonella* Typhimurium DT104 or *Escherichia coli* O157:H7. A difficulty arises however, in relation to the survival of significant numbers of organisms at high temperatures. In particular, the presence of resistant sub-populations 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.

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McCann, M.S., Sheridan, J.J., McDowell, D.A. and Blair, I.S. (2006). Effects of storage and the presence of beef microflora on the thermal resistance of *Salmonella* Typhimurium DT104: studies in beef and broth. *Journal of Applied Microbiology* (in press)

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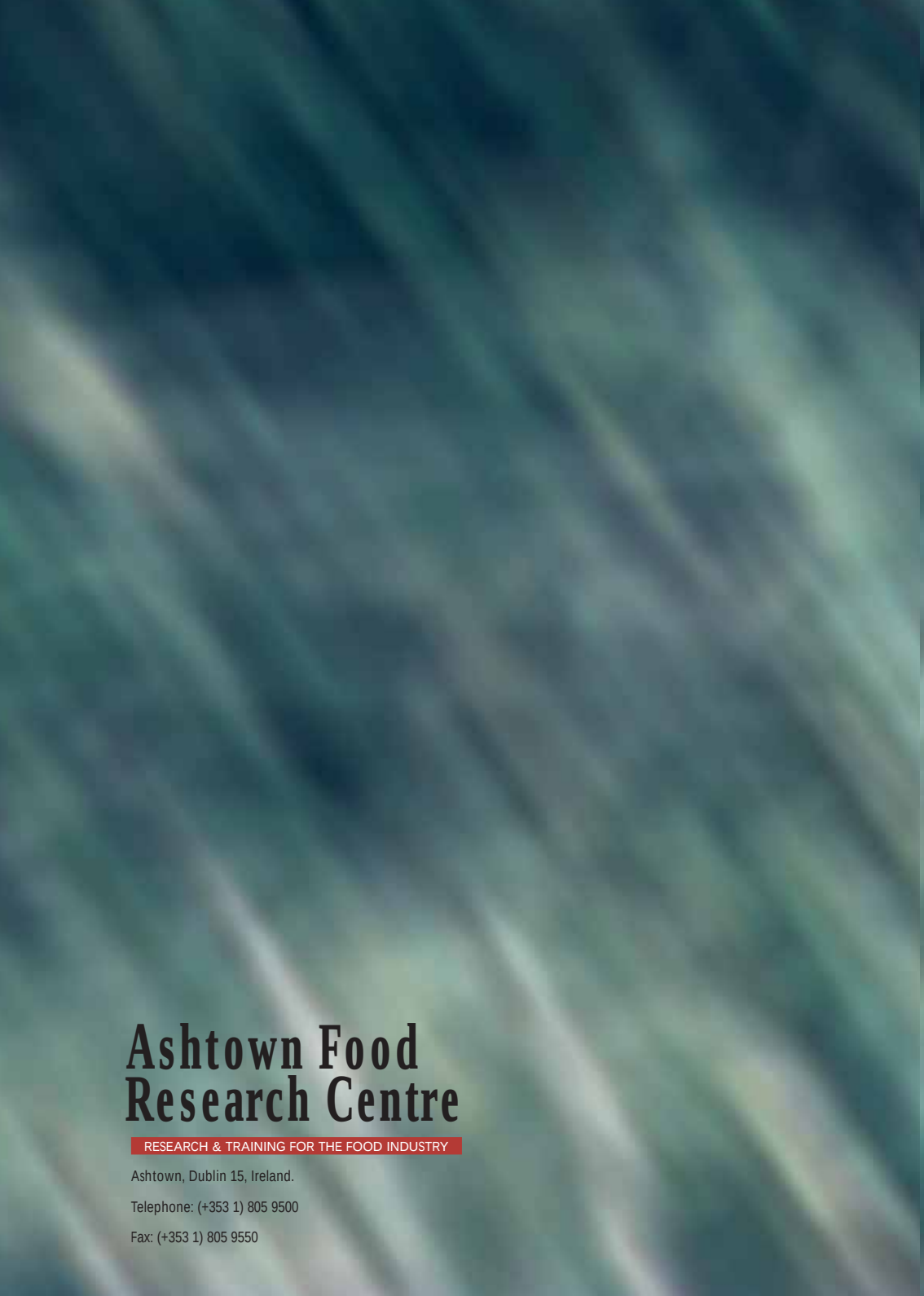

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