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Studies of steam decontamination of beef inoculated with *Escherichia coli* O157:H7 and its effect on subsequent storage

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ABSTRACT

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Aim: This study was carried out to determine the survival of *Escherichia coli* O157:H7 and subsequent shelf life of beef subjected to subatmospheric steam at differing temperatures.

Methods and Results: A specifically built, laboratory scale decontamination apparatus was used in decontamination trials to examine the effect of condensing steam at differing subatmospheric pressures on the survival of *E. coli* O157:H7 on meat. Beef slices were inoculated with a nontoxigenic *E. coli* O157:H7 strain and subjected to condensing steam at temperatures of 55, 65 and 75°C. Following treatment, the decontaminated meat was packaged and stored in air or under vacuum at temperatures of 10 or 0°C for up to 42 days. Microbiological analysis of the decontaminated and a control product (not subjected to any heat treatment) was carried out at regular intervals over the storage time of the product. Overall, significant reductions (*ca* 1·5 $\log_{10}$ CFU cm^{-2}) in pathogen numbers were observed at a steam treatment temperature of 75°C, however, postprocess storage conditions were important in ensuring no re-growth of the pathogen and this was best achieved by storage under vacuum at 0°C.

Conclusions: Steam had a significant impact in reducing *E. coli* O157:H7 populations, but storage conditions post-treatment were important for ensuring inhibition of the pathogen.

Significance and Impact of the Study: This study indicated that subatmospheric steam could have significant application in the decontamination of meat primals postfabrication, immediately prior to packaging thus ensuring a safer product for consumers.

Keywords: beef, decontamination, *E. coli* O157:H7, steam, subatmospheric pressure.

INTRODUCTION

At the time of slaughter, meat is essentially a sterile product however, during the slaughter process, events can occur which contribute to carcass contamination and ultimately, the underlying meat. Sources of such contamination have been identified as the skin, hair, hooves, feet and gut of an animal (Ayres 1955). Other sources implicated in carcass contamination include processing equipment, workers, and the slaughter environment (Dickson and Anderson 1992). The prime source of carcass contamination is the outer skin/hide of an animal, which is usually covered in dust, dirt and fecal material (Grau 1986; Gill 1998). During the hide/fleece removal stage of the slaughter process, the hide can come into contact with the underlying carcass and the meat. Any subsequent handling of the meat is also likely to increase the total bacterial contamination on the meat surface and result in cross-contamination of freshly exposed surfaces during indirect contact with previously contaminated surfaces.

Currently, one of the major food-borne pathogens of concern on raw meat is *Escherichia coli* O157:H7. This

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organism has been recognized as a significant cause of human illness since 1982 when it was first implicated in an outbreak of food-borne illness associated with ground beef (Riley *et al.* 1983). Since then, *E. coli* O157:H7 has also been linked to a number of illnesses and deaths, through the consumption of foods contaminated with the pathogen. These have included beef, ground beef (Riley *et al.* 1983; Hocker and Lior 1987; Ostroff *et al.* 1990; CDC 1993, 1996, 1997a) fruit juices, (Besser *et al.* 1993) organic foodstuffs (CDC 1997b), and fermented meat products such as salami and pepperoni (CDC 1995; Tilden *et al.* 1996). Beef cattle are considered a significant source of *E. coli* O157:H7 (Borcyzk *et al.* 1987; Chapman *et al.* 1989; Shere *et al.* 1998). Studies have shown that healthy cattle harbour the pathogen in their gut and shed it in faeces (Chapman *et al.* 1993; Hancock *et al.* 1994; Garber *et al.* 1999). Thus, the slaughter and processing of contaminated beef animals may pose a potential risk for meat contamination by ingesta/faeces present on the hide of such animals.

In view of these risks, much of the current research on decontamination has focused on whole animals, hides or carcass reduction methods (Castillo *et al.* 1998; Gill *et al.* 1999; McEvoy *et al.* 2001). These techniques have proved successful to some degree although, re-contamination during further processing and boning out operations can still pose a potential problem.

The current study was carried out to investigate the use of a novel decontamination method involving steam under subatmospheric pressures to decontaminate fabricated meat joints or primals that may become contaminated during the 'boning out' operation, resulting in contaminated product entering the food chain as retail cuts. Specifically, this study investigated the effects of steam decontamination on the reduction of *E. coli* O157:H7 and subsequent shelf life of the decontaminated product.

MATERIALS AND METHODS

Preparation of the meat

Beef striploins (*M. Longissimus dorsi*) were obtained from carcasses 3 days postmortem, from a local commercial abattoir (Kepak, Clonee, Co., Meath, Ireland). The striploins were cut into slices of 10·0 mm thickness, (size *ca* 180·0 cm^2) using a meat slicer (Krups, Solingen, Germany). The meat was held at 0°C until use, and screened for the presence of naturally occurring *E. coli* O157:H7 as described below.

Microbiological analysis/screening

A sample of meat (*ca* 10·0 g) was aseptically removed from each striploin, gravimetrically diluted 1 in 10 in maximum recovery diluent (MRD; CM 733 Oxoid, Unipath, Basingstoke, Hampshire, UK) using a gravimetric diluter (Watson and Marlow, Falmouth, Cornwall, UK) and homogenized for 1 min in a stomacher (Seward Medical, London, UK). A 10·0-ml volume of the homogenate was inoculated into 10·0 ml of double strength EE broth (CM 317; Oxoid) supplemented with novobiocin (0·02 g l^{-1}; Merck Chemicals, Darmstadt, Germany). The broth was incubated at 37°C for 18–24 h and streaked out on sorbitol MacConkey agar (SMAC, CM 813; Oxoid) supplemented with cefixime (0·05 g l^{-1}; Sigma Aldrich Chemicals, Dorset, UK) and potassium tellurite (2·5 mg l^{-1}; BDH, Dorset, UK). The SMAC plates were incubated at 37°C, for 18–24 h and examined for typical *E. coli* isolates (Doyle and Schenoi 1984). No naturally occurring *E. coli* O157:H7 were detected on any meat used in the study.

Escherichia coli O157:H7 culture

A nontoxigenic, antimicrobial resistant strain of *E. coli* O157:H7 (NCTC 12900) was used. This strain was originally isolated from a case of human diarrhoea but was found to have lost its ability to express verocytotoxin (Willshaw *et al.* 1994). The strain was made resistant to nalidixic acid (50 μg ml^{-1}; Sigma) and streptomycin sulphate (1000 μg ml^{-1}; Sigma) using the method described by Blackburn *et al.* (1994) in order to allow easier recovery from the meat. Stock cultures of the strain were maintained frozen on beads (Protect™; Technical Service Consultants Ltd, Heywood, Lancashire, UK) according to manufacturer's instructions.

Inoculum preparation

A single bead from frozen stock of the *E. coli* O157:H7 antimicrobial resistant strain was selected, inoculated into 9·0 ml of brain–heart Infusion broth (BHI, CM 225; Oxoid), and incubated at 37°C, for *ca* 8 h. Following incubation, a 0·1-ml aliquot of the culture was transferred to 100·0 ml of BHI and the culture incubated for a further 16 h at 37°C, to provide a stationary phase culture. Volumes of the culture (10·0 ml) were distributed into sterile Nalgene vials (Nalgene, Sartorius, Germany), centrifuged at 2000 *g* at 4°C for 10 min. The resulting pellet was washed once in 9·0 ml of MRD to remove secondary metabolites, and centrifuged for a further 10 min. The final pellet was re-suspended in 9·0 ml of MRD, the resultant suspension serially diluted in sterile filtered MRD and enumerated using the acridine orange epifluorescent staining technique described by Walls *et al.* (1989).

An inoculum with an initial level of *ca* 7·0 $\log_{10}$ CFU ml^{-1} was prepared by adding the required volume of enumerated suspension to 1000 ml of MRD. Plate counts of the enumerated suspension and inoculum were obtained on SMAC supplemented with nalidixic acid and streptomycin

sulphate and on tryptone soya agar (TSA CM 131; Oxoid) incubated at 37°C for 2 h (to allow recovery of potentially injured cells) and overlaid with supplemented SMAC (Doyle and Schenoi 1984; Riordan *et al.* 1998).

Inoculation of the meat

Prepared beef slices as described above, were inoculated with *E. coli* O157:H7 by immersion in the inoculum (described above) for 10 s (Logue *et al.* 1998). The slice was removed, allowed to drain, for *ca* 5 s placed in a sterile stomacher bag and held at 0°C until use. The slices were inoculated no more than 1 h prior to decontamination studies being carried out.

Construction and preparation of the decontamination rig

A decontamination rig designed and built at The Food Refrigeration, and Process Engineering Research Center (FRPERC) at the University of Bristol was used in these studies (Fig. 1). The rig consists of a vacuum vessel (bell jar and base plate) in which low pressures are achieved using a vacuum pump. Steam added at low pressures condenses at temperatures less than 100°C. Opening and closing the solenoid valve located on the base plate attached to the vacuum line achieves pressure control in the vacuum jar. When the valve is open, the pressure in the vacuum vessel is reduced, when closed the pressure rises as a result of steam introduction. On completion of the steam treatment, further evacuation of the vessel results in water in the vessel evaporating at low temperatures, removing heat added during the steam treatment. An ice filled condenser fitted to the vacuum line between the pump and the rig prevents water vapour and liquid reaching the vacuum pump oil and aids in achievement of the required vacuum cooling.

A control box (Edwards ADD, Surrey, UK) automatically adjusts the pressure in the bell jar by opening and closing the solenoid valve. The control box consists of two relay switches, which were set for the condensation temperature and the vacuum cooling pressure in the rig. Typical temperatures that can be achieved by setting the low and high pressure points of the controller are described in Table 1.

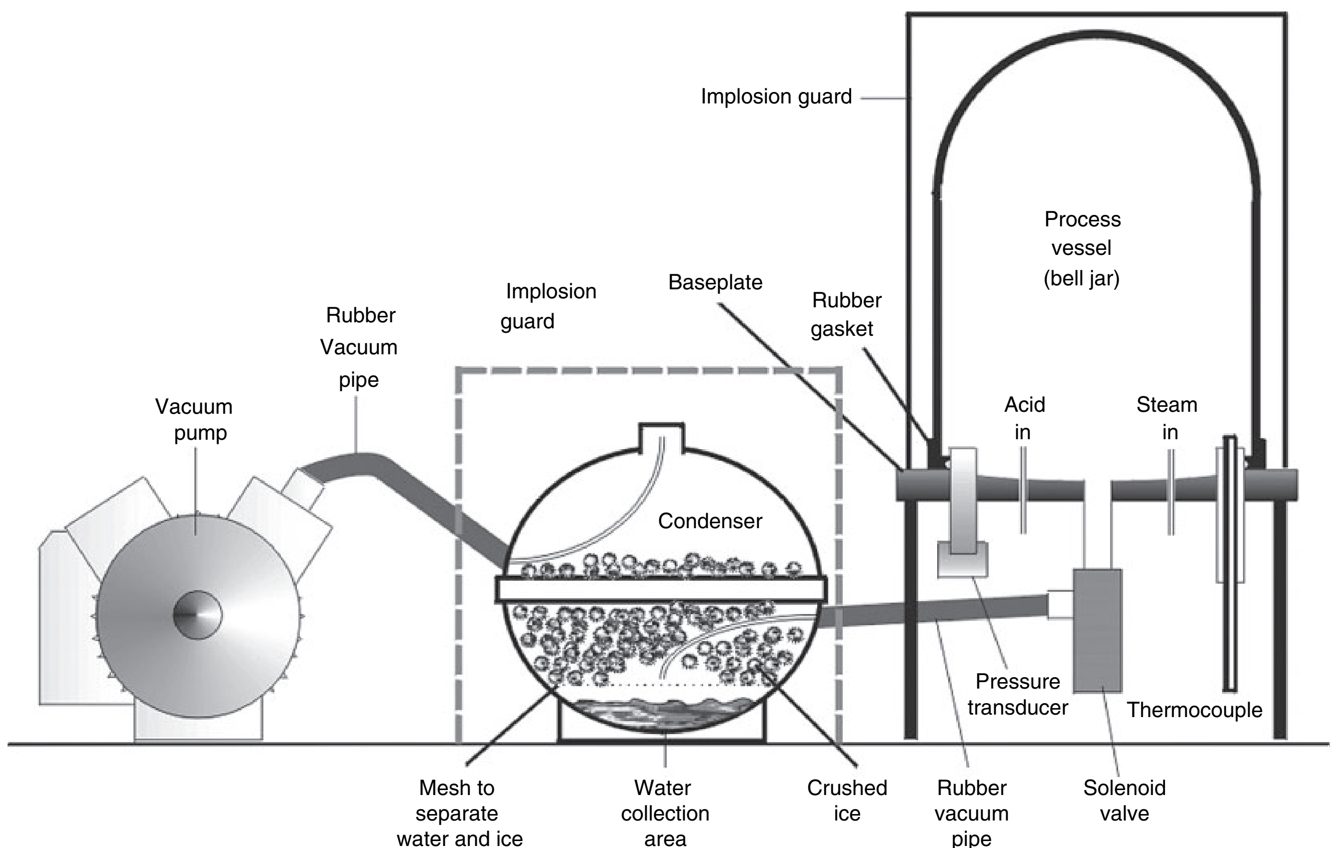

Fig. 1 Schematic of the decontamination rig assembly

Table 1 Pressures required for different steam condensation temperatures

Temperature (°C)	Pressure (KPa)	Controller set points (KPa)	
		Low	High
55	15·7	14·4	17·4
65	25·0	23·0	27·5
75	38·6	35·6	42·1

Preheating the decontamination rig

Preheating of the rig was carried out prior to any decontamination run to sterilize the rig and ensure no loss of latent heat, which would increase the time required to reach treatment temperature. The vacuum pipe from the solenoid valve to the condenser was disconnected, the solenoid valve opened, and a beaker placed under the open end of the vacuum pipe to catch condensate from the heating rig. The glass bell jar was positioned on the base plate and the steam generator switched on. The valve to the rig was opened once steam was visible from the purge valve of the generator. Steam treatment was continued for *ca* 10 min to ensure full heating of the base plate and rig. Temperature monitoring indicated that temperatures in the range of 100°C were achieved in the rig during the sterilization/prewarming cycle (results not shown). When complete, the steam inlet and solenoid valves were closed, the condenser was filled with ice, and the pipe from the rig to the condenser reconnected. The vacuum pump was switched on, the solenoid valve opened and a vacuum of 1·2–2·0 KPa drawn. Once the required vacuum pressure was achieved (2·0 KPa), the pump was switched off, and the vacuum in the vessel broken by opening the air inlet valve. Cooling to remove condensed moisture was usually achieved in 3–4 min and did not result in any significant heat losses from the rig.

Heat treatment of the meat samples

Following preheating and sterilizing of the rig, steam decontamination of the inoculated meat was carried out immediately in order to ensure as little heat loss as possible from the preheated rig. An inoculated beef slice as described above, was placed hanging in the rig. Temperature probes (Grant Instruments, Cambridge, UK; Grantware Squirrelwise Software, Cambridge, UK) were attached to the surface of the meat using bulldog clips.

With the rig preset to a condensing temperature of 55, 65 or 75°C on the control box (Table 1), the bell jar and implosion guards were positioned over the meat slice. A vacuum (pressure 2·0 KPa) was drawn in the vessel by opening the solenoid valve and switching on the vacuum pump. When the required vacuum pressure was reached, the solenoid valve was closed and switched to automatic control where the pressure in the bell jar was maintained by the pressure sensor in the control box. Steam from the steam generator was introduced into the bell jar through the steam inlet valve and the surface temperature of the meat slice monitored (Squirrelwise Software). When the required process temperature (55, 65 or 75 ± 5°C) was achieved on the temperature probes, the steam exposure interval (cooking time) was measured using a stopwatch (Laboratory Supplies, Dublin, Ireland). These were 55°C/10 min, 65°C/18 s and 75°C/10 s. At the end of the required exposure interval, the steam inlet valve was closed, the control box switched to manual mode, which opened the solenoid valve and allowed vacuum cooling to occur. Vacuum cooling was continued until a pressure of 1·2–2·0 KPa was achieved in the bell jar, at which point the vacuum pump was switched off and the vacuum in the bell jar broken by opening the air inlet valve.

The decontaminated meat slice was aseptically removed from the rig and cut into pieces of size *ca* 5 cm × 4 cm for storage and microbial analysis as described below. A total of three meat slices were decontaminated using the above procedure for each process temperature (55, 65 and 75°C) analysed.

In addition to the decontaminated meat described above, control meat slices (three) were also inoculated as described but were not subjected to any heat treatment. These were packaged and stored in a similar manner to the treated meat.

Packaging of the meat

Pieces of decontaminated or control meat were placed in small weighing boats (BDH; Lab Supplies, Dublin, Ireland) and overwrapped with kitchen grade cling film – oxygen transmission rate (OTR) 20 000 $cm^3\ m^{-2}\ 24\ h^{-1}\ atm^{-1}$ (Huntsman Film Products, Philippsburg, Germany). The meat for vacuum pack storage was placed in Cryovac BB4L bags (W.R. Grace Ltd, Dublin, Ireland) of *ca* size 8 cm × 6 cm. The bags had an OTR of 30 $cm^3\ m^{-2}\ 24\ h^{-1}$ at 23°C and 0% relative humidity (RH), and a CO_2 transmission rate of 150 $cm^3\ m^{-2}\ 24\ h^{-1}$ at 23°C and 0% RH. The packs were sealed using a vacuum packaging machine [Model 380; Swissvac, (GB) Ltd, Langley, UK]. All packs were labelled and stored at 0 or 10°C in chilled incubators.

Microbiological analysis of the stored meat

Microbiological analysis of the meat used in the study was carried out following initial inoculation with the pathogen, and during storage at the time intervals specified (see

Table 2 Sampling intervals used for stored packaged meat

Storage temperature (°C)	Packaging treatment	Sampling intervals (days)
0	Air	5, 10, 15
10	Air	1, 2, 3, 4, 5
0	Vacuum	14, 28, 42
10	Vacuum	7, 14, 21

Table 2). Where meat was steam decontaminated, additional analysis was carried out immediately following steam treatment. Samples of the control and decontaminated meat were analysed for *E. coli* and spoilage flora as described below. Pieces of meat were aseptically removed from their packages, diluted 1 in 10 in MRD, using a gravimetric diluter and homogenized for 1 min in a stomacher. Aliquots of the homogenate or serial dilutions, were plated out on the following media.

Escherichia coli O157:H7 were enumerated on SMAC supplemented with nalidixic acid (50 μg ml^{-1}) and streptomycin sulphate (1000 μg ml^{-1}), the plates were incubated at 37°C for 24 h.

Injured *E. coli* O157:H7 were allowed to recover on TSA and incubated for 2 h before a SMAC overlay (supplemented with nalidixic acid and streptomycin sulphate) was added. Overlaid plates were incubated at 37°C for a further 18–24 h.

Total counts were determined on Plate Count Agar (PCA, CM 325; Oxoid) incubated at 25°C for 3 days. *Pseudomonas spp*. were enumerated on *Pseudomonas* selective agar (cetrimide, fucidin, cephalosporin) (CFC, CM 559, SR 103; Oxoid) incubated at 25°C for 2 days.

Lactic acid bacteria were enumerated on deMann, Sharpe Rogosa Agar (MRS, CM 361; Oxoid) incubated anaerobically at 30°C for 3 days. *Mycobacterium thermosphacta* were enumerated on Streptomycin sulphate thallous acetate agar (STAA, CM 881, SR 151; Oxoid) incubated at 25°C for 2 days. Enterobacteriaceae were enumerated on Violet Red Bile Glucose agar (VRBGA, CM 485; Oxoid) incubated at 37°C for 24 h.

Bacterial counts obtained in this study were calculated as CFU g^{-1} and converted to CFU cm^{-2} using the conversion factor of Keeley (1988), i.e. count cm^{-2} = CFU g^{-1} × 0·3.

Statistical analysis

The design of the experiment consisted of randomized blocks of data with two meat types ('control' and 'heated'), three temperature treatments (55, 65 and 75°C), two storage temperatures (0 and 10°C) and two storage conditions (air and VP).

A single analysis was carried out for each storage temperature condition, i.e. the effect of air on untreated product stored at 0 and 10°C was analysed after 15 and 5 days storage respectively, while meat stored under vacuum was analysed at 42 and 21 days (Table 4).

In addition, the initial effect of heating on the recovery of *E. coli* was analysed for each treatment temperature of 55, 65 and 75°C (Table 5) and the effect of heating on the survival of *E. coli* following storage was analysed for each storage condition, i.e. air at 0 and 10°C after 5 and 15 days and in VP after 15 and 42 days (Table 6).

The effect of heating vs non heated samples stored at 0 and 10°C in air or vacuum pack was compared for each treatment temperature after storage at 21 and 42 days in VP at 0 and 10°C, and after 5 and 15 days in air at 0 and 10°C (Table 7).

Analysis of variance (ANOVA) using GENSTAT 5 (Rothamsted Experimental Station, Harpenden, Herts, UK) was carried out in all cases. Results presented are a mean of three replicates. The significance of the difference between the mean values was determined using the least significant difference approach (Steel and Torrie 1980).

RESULTS

The effect of heating beef pieces in the test apparatus showing the typical heat up and cool down times is illustrated in Fig. 2. Overall, the process consisted of a heating-up stage, during which the samples were heated to the desired treatment temperature. At this time, the treatment stage commenced which varied with the desired treatment temperature, i.e. 55, 65 or 75°C. The treatment stage was followed by a cool-down phase during which the temperature was returned to an ambient temperature of *ca* 40°C. The times for each of these treatment phases at the

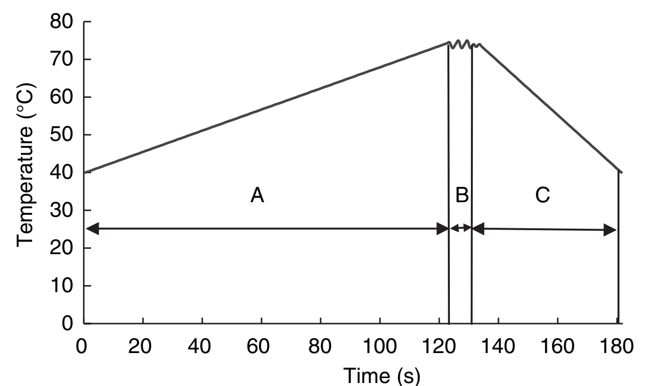

Fig. 2 Times and temperatures during treatment of beef at 75°C. (a) Heat-up stage: The steam inlet valve is opened and steam is introduced into the process vessel, causing the temperature to rise to 75°C. (b) Treatment stage: Timing begins and the temperature is maintained at 75 ± 2°C for 10 s. (c) Cool-down stage: The steam inlet valve is closed and the process vessel is evacuated resulting in cooling

Table 3 Process parameters during treatment of meat pieces with steam condensing at 55, 65 and 75°C

Treatment temperature (°C)	Treatment time* (s)	Heat-up time† (s)	Cool-down time‡ (s)	Overall duration§ (s)
55	600	173	66	839
65	18	126	64	208
75	10	123	48	181

*Treatment of the meat at the treatment temperature ±5°C.
†Time to raise the temperature at the meat surface from 40 ± 0·5°C to the treatment temperature ±0·5°C.
‡Time to cool-down the meat surface from the treatment temperature to 40 ± 0·5°C.
§The combined heat-up, treatment and cool-down times.

Table 4 The effect of storage in vacuum packs and in air at 0 and 10°C for different times (days) on the survival of *Escherichia coli* O157:H7 ($\log_{10}$ CFU cm^{-2}) inoculated on beef (no heat treatment)

Storage conditions	Vacuum packs		Air	
Temperature (°C)	0	10	0	10
Time (days)	42	21	15	5
Initial counts ($\log_{10}$ CFU cm^{-2})	5·59	5·59	5·59	5·59
Final counts ($\log_{10}$ CFU cm^{-2})	4·67	3·47	5·10	6·68
S.E.D.	0·55	0·56	0·47	0·83
d.f.	22	28	26	18

S.E.D., standard error of differences between mean values; d.f., degrees of freedom.

different treatment temperatures and the overall treatment time for each temperature are displayed in Table 3.

On examination of the microbiological data, initial analysis showed that there were no significant differences observed in the numbers of *E. coli* O157:H7 recovered from selective agar (SMAC) vs recovery media (TSA + SMAC). The numbers of injured cells following heat treatment were small, usually less than 0·5 $\log_{10}$ CFU cm^{-2}. As the differences in pathogen numbers recovered on both media were similar, the results presented in this study are for *E. coli* O157:H7 enumerated on SMAC media only.

When beef inoculated with *E. coli* O157:H7 (i.e. controls with no heat treatment) was stored at 0 or 10°C in vacuum pack, pathogen counts decreased but significant reductions were only observed at 10°C ($P < 0{\cdot}01$) (Table 4). Where meat was stored in air, *E. coli* counts decreased at 0°C and increased at 10°C but the changes observed were not significant.

The initial effect of heating beef samples inoculated with *E. coli* O157:H7 at 55, 65 and 75°C is shown in Table 5. At all these heating temperatures, reductions in *E. coli* O157:H7 counts were observed, however, these reductions were only significant at 75°C ($P < 0{\cdot}05$).

Table 5 The effect of heating at 55, 65 and 75°C on the survival of *Escherichia coli* O157:H7 ($\log_{10}$ CFU cm^{-2}) inoculated on beef

	Treatment temperature (°C)		
Sample treatment	55	65	75
Control (no heating)	5·65	5·53	5·59
Heated	4·75	4·12	3·48

S.E.D. = 0·83 (standard error of differences between mean values); d.f. = 18 (degrees of freedom).

Table 6 The survival of *Escherichia coli* O157:H7 ($\log_{10}$ CFU cm^{-2}) on inoculated beef samples after heating at different temperatures and subsequent storage in vacuum packs and in air

Heat treatment (°C)	55	65	75
Storage in vacuum packs			
Before storage	4·75	4·12	3·48
After storage			
0°C/42 days*	4·45	3·62	3·73
10°C/21 day†	5·32	4·83	5·52
Storage in air			
Before storage	4·75	4·12	3·48
After storage			
0°C/15 day‡	3·68	2·79	2·84
10°C/5 day§	7·24	4·01	4·28

S.E.D., standard error of differences between mean values; d.f., degrees of freedom.
*S.E.D. = 0·55, d.f. = 22.
†S.E.D. = 0·56, d.f. = 28.
‡S.E.D. = 0·47, d.f. = 26.
§S.E.D. = 0·83, d.f. = 18.

When inoculated beef samples were heated and stored at 0 or 10°C in vacuum packs or in air, changes in *E. coli* counts compared with similar samples prior to storage were observed. In VP stored at 0°C no significant changes in counts occurred at any of the heating temperatures (Table 6). *Escherichia coli* counts on meat stored at 10°C increased but increases were only significant on meat heated at 75°C ($P < 0{\cdot}01$). In contrast, when inoculated, heated meat was stored in air at 0°C significant reductions were observed for all heated meat treated at 55 ($P < 0{\cdot}05$), 65 ($P < 0{\cdot}01$) but not at 75°C. However, when the storage temperature was increased to 10°C significant *E. coli* increases only occurred on meat treated at 55°C ($P < 0{\cdot}01$), while changes were not observed or the numbers were not significantly different to numbers prior to storage for meat treated at 65 and 75°C (Table 6).

Finally, when unheated, stored vacuum packaged (VP) samples were compared with heated samples stored in a similar manner, there were no significant differences in pathogen counts at 0°C after storage for 42 days (Table 7).

Table 7 The effect of storage in vacuum packs and in air on the survival of *Escherichia coli* O157:H7 ($\log_{10}$ CFU cm^{-2}) on beef samples with or without heating

Sample treatment	0°C/42 day	10°C/21 day
Storage in vacuum packs		
No heating	4·67	3·47
Heated samples (°C)		
55	4·45	5·32
65	3·62	4·83
75	3·73	5·52
S.E.D.	0·54	0·51
d.f.	10	24
	0°C/15 days	10°C/5 days
Storage in air		
No heating	5·10	6·68
Heated samples (°C)		
55	3·68	7·24
65	2·79	4·01
75	2·84	4·28
S.E.D.	0·42	0·89
d.f.	19	11

S.E.D., standard error of differences between mean values; d.f., degrees of freedom.

However, significant growth occurred in VP samples stored at 10°C after heating at 55°C ($P < 0·01$), 65 ($P < 0·05$) or 75 ($P < 0·001$), compared with unheated samples over the same time interval. Storage in air at 0°C following heating revealed significant reductions in counts when samples were heated at 55°C ($P < 0·01$) and at 65 and 75°C ($P < 0·001$). At 10°C storage in air, heating significantly reduced growth at 65 and 75 ($P < 0·05$) or growth increased slightly although not significantly at 55°C.

When the counts in untreated samples were compared with those heated at 55, 65 or 75°C for the total counts (PCA), lactic acid bacteria (MRS), pseudomonads (CFC) or *M. thermosphacta* (STAA) stored at 0 and 10°C in vacuum packs and in air, no relationship could be established with the *E. coli* counts after inoculation.

It was possible to show some significant differences in control and heated samples for the CFC and PCA counts in vacuum packs stored at 0°C. For the PCA counts, significant reductions were noted at 65 and 75°C ($P < 0·05$) compared with controls and this was also noted for the CFC counts. When the vacuum packs were stored at 10°C, there were no differences in heated or control samples for any of the organisms tested, despite the large differences in *E. coli* O157:H7 counts as described previously (Table 7).

In air at 0°C the only significant difference was for the CFC counts where the controls were higher than the counts at 75°C ($P < 0·01$). Storage in air at 10°C showed significant differences ($P < 0·05$) for PCA, CFC and STAA counts between unheated and samples heated at 75°C.

Generally, therefore, differences in counts between the normal microflora for unheated samples stored in vacuum packs or in air at 0 and 10°C and heated samples were inconsistent and varied with the organism being tested, the storage temperature and the packaging treatment.

DISCUSSION

In studies of carcass decontamination, one of the most important factors influencing it is hide contamination levels. Hide removal is one of the most important critical control points (CCP) in the slaughter process, as it can be a significant source of contamination for the underlying carcass and ultimately the meat (Bell 1997; Elder *et al.* 2000). Hides have been reported as significant sources of soil and faecal material, with counts as high as 9·0 $\log_{10}$ CFU cm^{-2} observed (Jericho *et al.* 1996). Re-distribution of these organisms can occur during subsequent fabrication and dressing procedures, resulting in meat becoming a potential source of pathogens such as *E. coli* O157:H7 (Borczyk *et al.* 1987; Chapman *et al.* 1989, 1993).

Much of the current decontamination research has focused on methods to decontaminate animals/carcasses at either the pre hide removal stage with processes such as chemical dehairing, shaving or washing regimes (Schnell *et al.* 1995; Van Donkersgoed *et al.* 1997; Castillo *et al.* 1998; Byrne *et al.* 2000) or more commonly, at the post de-hiding stage at the end of the process line (postdressing) with trimming, washing, acid sprays, soap washes, chlorine, trisodium phosphate (Cabedo *et al.* 1996; Regan *et al.* 1996; Cutter *et al.* 1997), steam vacuuming (Dorsa *et al.* 1997; Kochevar *et al.* 1997) or steam pasteurization treatments (Nutsch *et al.* 1997, 1998; Phebus *et al.* 1997). All of these interventions have resulted in reductions in the numbers of organisms on the surfaces of hides or carcasses with varying degrees of success. However, there is still the potential risk of recontamination during cooling, grading, fabrication and boning out operations (Nutsch *et al.* 1997). In view of these potential risks, the current study focused on investigating subatmospheric steam decontamination of beef primals as a postfabrication process to complement other decontamination regimes.

The data generated from this study showed that subatmospheric steam decontamination had a significant effect in reducing the numbers of *E. coli* O157:H7 on inoculated meat. Generally, reductions of 0·5 $\log_{10}$ CFU cm^{-2} were recorded on meat treated at 55°C, 1·0 $\log_{10}$ CFU cm^{-2} at 65°C and 1·5 $\log_{10}$ CFU cm^{-2} at 75°C. Reductions similar to those described were evident in the studies of Abdul-Raouf *et al.* (1993) working with *E. coli* O157:H7 in inoculated beef slurries heated at 54°C where reductions

of *ca* 0·5 $\log_{10}$ CFU ml^{-1} occurred within 10 min. Another study by Benito *et al.* (1999) reported *E. coli* O157:H7 reductions of 0·5–2·0 $\log_{10}$ CFU ml^{-1} after 10 min exposure at 52°C in liquid culture, while reductions of 1–5 $\log_{10}$ CFU ml^{-1} were observed for similar strains studied at 57°C within 8 min. The disparity of these results and that of the current investigation are probably because of the protocols used, i.e. these studies were conducted in liquid media. This suggests that meat may provide a protective effect, thus reducing the effect of heat. Other workers have also reported that proteins and fats associated with heating menstra can provide a greater protective effect to cells during cooking/heating processes (Line *et al.* 1991; Ahmed *et al.* 1995; Duffy *et al.* 1995; Kaur *et al.* 1998). Strain difference and culture age may also influence thermotolerance. Duffy *et al.* (1999) reported differences in the heat resistance of three *E. coli* O157:H7 strains heated in a salami matrix. Reductions with a range of 1–2 $\log_{10}$ CFU g^{-1} were observed in the test strains when heated at 55°C for 10 min. While Kaur *et al.* (1998) reported late stationary phase cultures had greater heat resistance than logarithmic phase cultures. The current study used stationary phase (16 h) cultures in all heating trials carried out.

The rates of reduction in *E. coli* numbers in the present study were directly related to the treatment temperature used, i.e. reduction rates observed increased with increasing steam temperature. These reductions were relatively comparable with studies by other workers also using steam decontamination regimes. Phebus *et al.* (1997), reported reductions of 3·5 $\log_{10}$ CFU cm^{-2} at a process temperature of 91–93°C for 15 s in a specifically designed steam chamber. In other developmental studies of the same apparatus, Nutsch *et al.* (1997) reported the initial numbers of *E. coli* on carcasses were in the range of 0·6–1·53 $\log_{10}$ CFU cm^{-2}, and following a steam treatment of 90·5–94°C for 6–8 s no *E. coli* were detected. In additional studies, where contamination at specific points on carcass surfaces was examined and a steam treatment temperature of 82·2°C and 6·5 s exposure time were used, *E. coli* reductions of *ca* 0·5 $\log_{10}$ CFU 100 cm^{-2} were observed (Nutsch *et al.* 1998).

Other studies have examined steam vacuuming to decontaminate specific areas of a carcass where contamination occurs during processing as a result of splashing. Kochevar *et al.* (1997) reported reductions of 0·72 $\log_{10}$ CFU cm^{-2} in the total numbers of microflora present, and 0·26 $\log_{10}$ CFU cm^{-2} in the numbers of *E. coli* on carcasses where no visible faecal contamination was evident, while reductions of 1·05–2·3 $\log_{10}$ CFU cm^{-2} were observed for total counts and 1·23–2·15 $\log_{10}$ CFU cm^{-2} for *E. coli* on carcasses where faecal contamination was visible. Steam vacuum decontamination is however, limited to small areas because of the size of the vacuum head. Dorsa *et al.* (1997) reported total count reductions of *ca* 1·6 $\log_{10}$ CFU cm^{-2} observed using steam vacuuming, while reductions of 2·1 $\log_{10}$ CFU cm^{-2} were observed in the numbers of *E. coli* O157:H7. It should be considered that the decontamination effects of stream vacuuming are difficult to evaluate, as factors such as the speed of head movement, initial contamination levels, carcass surface types, etc. influence reductions.

Similar evaluation of the decontamination apparatus used in these studies by McEvoy *et al.* (2001) noted reductions in the numbers of *E. coli* O157:H7 on beef hides of *ca* 2 $\log_{10}$ CFU g^{-1} (0·6 $\log_{10}$ CFU cm^{-2} when converted using the Keeley (1988) method after exposure to steam at 80°C for 10 s, however once the exposure time was increased to 20 s, reductions of *ca* 5 $\log_{10}$ CFU g^{-1} (1·5 $\log_{10}$ CFU cm^{-2}) were noted.

In the present study, a change in the growth patterns of *E. coli* O157:H7 was observed during the initial storage of the decontaminated product postprocessing, with poor growth or reduction evident for a period of 1–2 days before growth occurred. In studies by others, Dorsa *et al.* (1997) noted that when decontaminated carcasses were stored, shifts in the growth patterns of *E. coli* were observed during the first two days of storage before growth gradually declined. Ingraham (1987) reported this response was because of temperature shifts influencing the behaviour of *E. coli*. When the organism is shifted to temperatures at or below its minimal growth temperature (i.e. a nongrowth temperature) growth continues for a short period of time usually 1–2 days before the growth rate gradually declines. It was interesting to note that in the present study the opposite effect was observed in that growth increased after the initial reductions suggesting recovery of the cells or ability to grow under temperature shifts.

In the present study, growth of the organism postprocessing was evaluated and found to occur most often where the decontaminated was stored under temperature abuse conditions, i.e. 10°C in either VP or air. In contrast, growth on untreated meat was only evident on meat stored in air at 10°C.

In similar studies, Nissen *et al.* (2001) evaluated post-process contamination of meat decontaminated using an identical steam decontamination apparatus and regime (75°C for 10 s) as that of the present study. An additional lactic acid wash step was incorporated into the procedure where the meat was sprayed with volumes of 0·2 M lactic acid after steam treatment, just prior to vacuum cooling. Increases of 3 $\log_{10}$ CFU cm^{-2} were observed by 5 days at 10°C on aerobically stored decontaminated meat vs 1 $\log_{10}$ CFU cm^{-2} on untreated meat. Some differences were also observed between the replicates an effect that was explained as being caused by residual lactic acid on the meat. In contrast, where the meat was stored in vacuum packs pathogen growth was slow on untreated meat but showed a significant increase of 3 $\log_{10}$ CFU cm^{-2} over a 21-day period.

In Nissen *et al.*'s (2001) study, the growth of *E. coli* was generally better on decontaminated meat stored at 10°C, suggesting that the destruction of other elements of the spoilage microflora may allow for selective growth of the pathogen and therefore promote increased multiplication, because of the extended shelf life particularly in combination with vacuum packaging.

Vold *et al.* (2000) suggest that background microflora can play a role in influencing the growth of *E. coli* under aerobic and anaerobic conditions, an effect that was noted in this study (Tables 4, 6 and 7), however, significant differences in the growth of *E. coli* in the presence of competitive natural microflora were only observed in vacuum packs at 10°C ($P < 0{\cdot}001$) with significant reductions occurring by 21 days (Table 4).

Overall, the data obtained in this preliminary study supports the view that steam under subatmospheric pressure can have a significant effect in reducing pathogen numbers on fabricated meat. The decontamination rig used in this study could have potential impact as a complimentary system to large-scale carcass decontamination units and be used to prevent contaminated primals entering the food chain. It could therefore have significant application in helping to further reduce the risk of pathogen contamination of primals destined for consumers. The method however needs to be used in conjunction with effective temperature controls to ensure no re-growth of contaminants. Further studies are, however, required to refine the decontamination regime and increase the efficiency of the process. One such method that will be investigated is the use of organic acids in combination with subatmospheric steam.

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