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A Test Bacterial Decontamination System for Meat Products

The National Food Centre

RESEARCH & TRAINING FOR THE FOOD INDUSTRY

RESEARCH REPORT NO 23

Contents

A TEST BACTERIAL DECONTAMINATION SYSTEM FOR MEAT PRODUCTS

Authors:

Oonagh C Ward, B.Sc., M.Sc.
Catherine M Logue, B.Sc., Ph.D.
Jim Sheridan, M.A., M.Sc., Ph.D.
The National Food Centre
Teagasc, Dunsinea, Castleknock, Dublin 15

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Teagasc 19 Sandymount Avenue Ballsbridge Dublin 4

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SUMMARY

A pilot scale apparatus was designed to allow meat samples to be treated with steam at sub-atmospheric pressures and correspondingly reduced temperatures. Experiments were carried out to determine the effectiveness of sub-atmospheric steam decontamination in eliminating bacteria on the surface of fresh beef. This type of treatment can have special advantages in that steam can be produced at temperatures well below 100°C. This means that the heat advantages of steam as a decontaminating agent can potentially be obtained at lower temperatures.

The steam system was combined with organic acids and the possible synergistic effects of these two treatments were assessed in the decontamination of fresh beef inoculated with *E. coli* 0157:H7. The effect of these treatments on the natural microflora of beef was also determined.

The results showed that the sub-atmospheric steam at a range of temperatures, such as 75°C for 10 sec., was unsuccessful in reducing contamination. Neither was there any advantage in combining acid with this heat treatment. The data indicated that all short time treatments were unsuccessful in reducing contamination significantly.

Highly significant reductions in pathogen and other bacterial numbers were obtained when the contact time for steam was increased to 4 mins. A synergistic effect with organic acids using this treatment could also be demonstrated. With steam at 65°C and 0.2 M lactic acid applied for 4 min., a 5 – 6 log reduction in bacteria counts could be obtained. A drawback with this treatment was a deterioration in the colour of the meat surfaces.

Subsequent storage trials, in air and vacuum packs, of meat using these treatments, indicated that the product needed to be stored at a low temperature to retain the observed reductions in pathogen and other counts. It was considered possible that this type of process could have applications in vacuum packaged beef where the colour of the meat surfaces is less important than for meat as carcasses.

INTRODUCTION

In the normal healthy animal the tissues which ultimately become meat or meat products are sterile. During slaughter and processing all edible tissues are subject to contamination from a variety of sources within and outside the animal. Microbial growth is generally confined to the outer surfaces where bacteria become irreversibly attached. The microbiological quality of raw meat is critical to the quality of the final product, as fresh meat presents an environment which is ideal for the growth of many microrobes. Contamination can easily result in spoilage or a hazard to the health of the consumer and one of the major pathogen concerns is *Escherichia coli* O157:H7.

Food poisoning outbreaks caused by *E. coli* O157:H7 in meat and meat products are common in many countries. Many of the outbreaks of *E. coli* O157:H7 have been directly or indirectly related to the consumption of undercooked beef products, especially ground beef. The purpose of the present study was to investigate decontamination treatments, such as steam, that would extend safety and shelf life, without changing the appearance or organoleptic characteristics of the meat.

Steam at 100°C has a substantially higher heat content than the same mass of water at that temperature. If steam is allowed to condense onto the surface of meat then it has the ability to rapidly raise the surface temperature. Steam can be produced under vacuum at temperatures below 100°C without substantially reducing its heat capacity. It has been shown that rapid heating, and subsequent cooling, has the potential to destroy surface microorganisms, without causing meat quality changes. A feature of condensing steam is its ability to penetrate cavities and condense on any cold surface.

Chemical methods of decontamination are currently receiving much attention. The majority of studies have been on the use of organic acids, which appear to be the most acceptable form of chemical decontamination. The general conclusions are that organic acids can reduce the numbers of pathogenic and spoilage organisms typically by 1 to 3 $\log_{10}$ cycles. There may be synergistic effects between two decontamination systems that individually have advantages.

In the present project the rapid heating and subsequent cooling of meat, in the presence of organic acids, was investigated. Condensing a vapour is a very efficient method of rapidly raising surface temperature and evaporating the condensed liquid is an equally efficient method of rapidly cooling the surface. The addition of an organic acid may provide an additional synergistic decontaminating effect without influencing the organoleptic qualities of the product.

METHODS

Test apparatus

The pilot scale test apparatus consisted of a vacuum vessel (bell jar and baseplate) in which low pressures could be achieved using a vacuum pump (Fig. 1). Steam added at these low pressures condensed on the meat surface at temperatures lower than 100°C. The required pressure was achieved by opening and closing a solenoid valve in the vacuum line. When the valve was opened pressure was reduced; when closed, the pressure rose due to steam introduction.

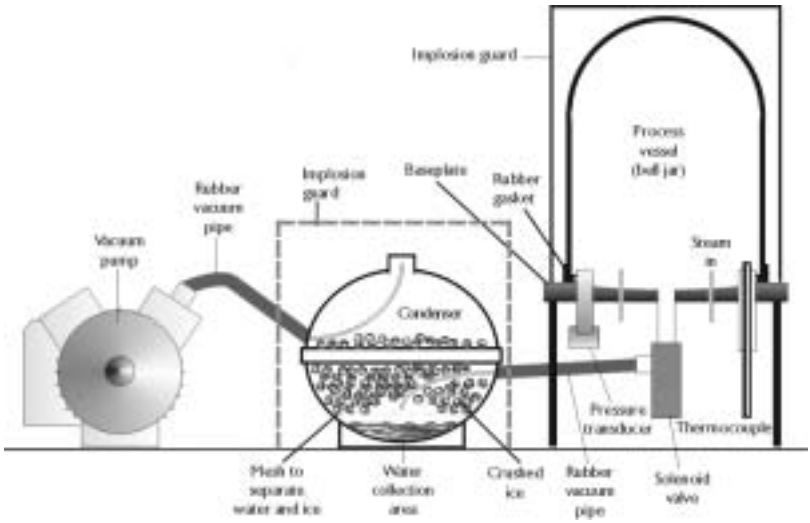

Figure 1: Test apparatus for decontamination of beef

Organic acids were sprayed onto the meat surface in the vessel before or after the steam treatment, using a variable speed peristaltic pump. When steam and acid treatments were complete, the vessel could be further evacuated. Water in the vessel evaporated at low pressures / temperatures (e.g. 10°C), removing the heat added during the steam treatment. To prevent vapour and liquid entering the vacuum pump oil and to enable the required vacuum cooling pressures to be achieved, an ice filled condenser was installed in the vacuum line between the vessel and the pump.

Beef samples

Beef striploins were obtained from carcasses in a commercial abattoir three days after slaughter. The striploins were cut using a Krups slicing machine (Krups, Germany) into slices of approximately 1 cm thickness. The meat was then held at 0°C until use and screened for the presence of naturally occurring *Escherichia coli* O157:H7.

Organic acids and concentration

The following acids were investigated in this study:

- lactic acid (food grade) (Purac, Netherlands) at a concentration of 0.2M
- glacial acetic acid (BDH, UK) at a concentration of 0.3M
- L-buffered lactic acid (Purac, Netherlands) as supplied
- mixture of 1:1 lactic and glacial acetic acid at a concentration of 0.2M:0.3M
- a control was prepared using sterile distilled water

Shelf-life experiments

Following decontamination treatment, steaks were cut into pieces (size approximately 5 x 4 cm) pooled and randomly assigned for packaging. The meat was packaged in either aerobic or vacuum packs and stored at 0°C or 10°C. Pieces of meat were placed in small weighing boats (BDH, Laboratory Supplies, Dublin) and over-wrapped with food grade cling film (Irish Merchants, Dublin) for aerobic packs. For vacuum packs meat was placed in cryovac BB4 bags (Cryovac, UK) of approximately 8 x 6 cm. The packs were

sealed using a cryovac vacuum sealer (Cryovac, UK). Control steaks were also inoculated and prepared as described above but were not decontaminated. Microbiological examination of all steaks was carried out after storage for the following time intervals:

- stored in air at 0°C and examined after 5, 10, 15 days
- stored in air at 10°C and examined after 1, 2, 3, 4, 5 days
- stored under vacuum at 0°C and examined after 14, 28, 42 days
- stored under vacuum at 10°C and examined after 7, 14, 21 days

Microbiological examination of meat

Maximum recovery diluent (MRD)

Pieces of meat were aseptically removed from their respective packages, diluted 1 in 10 in MRD (maximum recovery diluent) and homogenised for 1 minute in a stomacher. Aliquots of the homogenate, or serial dilutions, were plated out on the following media:

- *E. coli* O157:H7 was enumerated on Sorbitol MacConkey Agar plates (SMAC) supplemented with nalidixic acid (50µg/ml) and streptomycin sulphate (1000µg/ml) for the mutant, and plates were incubated at 37°C for 24 h.
- Injured *E. coli* O157:H7 cells were allowed to recover on Tryptone Soya Agar plates (TSA). The plates were incubated at 37°C for 2 h before being overlaid with a layer of supplemented SMAC.
- *Pseudomonads* spp. were enumerated on Pseudomonads Agar base (Oxoid, UK) supplemented with Pseudomonads CFC antibiotic supplement and incubated at 25°C for 48 h.
- Total counts were determined on Plate Count Agar plates incubated at 25°C for 72 hrs.

Six replicates were carried out for the inoculated control and decontaminated steaks in all studies unless stated otherwise.

RESULTS AND DISCUSSION

A trial was carried out to determine the effect of time of application of the organic acid i.e. initial vacuum, after initial vacuum or after the heat treatment, before the final vacuum cooling. Mean numbers of decimal reductions (log_{10}cfu/cm^2) for *E. coli* 0157:H7, Pseudomonads and the total counts on beef treated with 0.2 M lactic acid and 1% Tween 80 before and after heating at 75ºC for 10 sec are shown in Table 1. The data show that differences between the application of acid before or after the heat treatment gave greater reductions when acids were applied first but the increased effect was not significant.

Table 1: Effect of acid and heat treatment on bacterial counts on beef cuts

E. coli 0157:H7	Acid application before heat	Heat applied before acid
SMAC	1.63	0.99
TSA-SMAC	1.06	0.90
Pseudomonads	3.44	2.73
Total bacteria	1.24	0.90

Standard error of differences between means = 0.45
Degrees of freedom = 36

Effect of acid type and temperature

Table 2 shows mean numbers of decimal reductions (log_{10}cfu/cm^2) for *E. coli* 0157:H7, Pseudomonads and total bacteria on beef treated with steam at 75ºC for 10 sec, followed by acids applied at 55ºC. The data (Table 2) show that a water treatment was generally as successful as any of the acids in reducing bacterial counts. The only exception to this was for buffered lactic acid at 55ºC, which was significantly better in reducing the total bacteria than water ($P<.05$). The temperature of application of the acids or water did not appear to be a major factor in reducing bacterial counts, although in general

the reductions at 55°C were higher than at 10°C. These data also suggested that the acids were not effective in reducing the pathogen numbers and that the observed effects were from the 10 sec heat treatment alone. It was further observed that injury to *E. coli* 0157:H7 cells was not an important factor in this or subsequent experiments. In these experiments the extent of injury had to be determined since these cells can recover and grow normally causing infections.

The effect of addition of surfactants

The influence of adding Tween 80, a surfactant, to the lactic acid was investigated. Table 3 shows mean numbers of decimal reductions (log_{10}cfu/cm^2) for *E. coli* 0157:H7, Pseudomonads and total bacteria on beef treated with lactic acid, with and without Tween 80 before steam

Table 2: Effect of different acids following steam decontamination on bacterial numbers on beef cuts

	Acetic	Buffered lactic	Lactic	Mix of lactic and acetic	Water	SED[1]	DF[2]
				10°C			
E. coli 0157:H7							
SMAC	0.17	0.44	0.23	0.19	0.67	0.36	98
Pseudomonads	1.36	2.24	1.18	1.34	2.25	0.45	50
Total bacteria	0.67	1.85	1.12	0.73	1.39	0.48	48
				55°C			
E. coli 0157:H7							
TSA – SMAC	0.86	0.39	0.73	0.44	0.79	0.36	98
Pseudomonads	1.77	1.89	1.28	1.00	1.29	0.45	50
Total bacteria	1.61	2.05	1.12	1.34	0.92	0.48	48

[1] SED = standard error of differences between means

[2] DF = degrees of freedom

Table 3: Decontamination of beef cuts using lactic acid with a surfactant and steam

	Lactic + Tween	Lactic without Tween
E. coli 0157:H7		
SMAC	1.63	0.92
TSA - SMAC	1.06	0.91
Pseudomonads	3.44	2.10
Total bacteria	1.24	1.10

Standard error of differences between means = 0.40
Degrees of freedom = 28

decontamination at 75°C for 10 sec. The results show that there were greater reductions using the Tween 80, although the differences were significant only for *E. coli* 0157:H7 enumerated on SMAC and the Pseudomonad counts ($P<.05$).

Effect of contact time of acid on meat

The introduction of a suitable delay period of 4 min. upon addition of lactic acid and before steam treatment, gave a 5 log reduction in *E. coli* 0157:H7 counts. Mean numbers of decimal reductions ($log_{10}cfu/cm^2$) after treatment with 0.2 M lactic acid at 55°C, with and without Tween 80, and a contact time of 4 minutes with steam at 80°C for 10 sec. are shown in Table 4. The addition of the surfactant, Tween 80, increased the effect for all the organisms tested but the difference was not significant.

Additional trials established that the pre-heated base plate alone provided a temperature rise of 65°C for 4 min and in the presence of lactic acid this was sufficient to give a 5 log reduction in pathogen counts, similar to that when steam was used. Table 5 shows mean numbers of decimal reductions ($log_{10}cfu/cm^2$) after treatment of beef with 0.2 M lactic acid at 55°C, compared with water and a contact time of 4 min, with heat at 65°C from the base plate only (no steam).

Table 4: Use of lactic acid and a surfactant with a contact time of four minutes

	Lactic acid + Tween	Lactic acid without Tween
E. coli 0157:H7		
SMAC	5.57	5.05
TSA + SMAC	5.01	4.56
Pseudomonads	3.96	3.22
Total bacteria	3.95	2.65

Standard error of differences between means = 0.66
Degrees of freedom = 40

These data also show the synergistic effect of acid and heat when the contact time is sufficiently long. Although the use of heat alone (water) gave a 3 log reduction, this was increased to 5 logs in the presence of 0.2 M lactic acid. Table 6 shows a summary of decimal reductions ($log_{10}cfu/cm^2$) obtained using different combinations of heat and lactic acid on the survival of *E. coli* 0157:H7, Pseudomonads and total counts on beef. The data in Table 6 summarise the effect of heat and acid and in particular, the necessity for a sufficiently long contact time to obtain the desired decimal reductions in the

Table 5: Decontamination of beef cuts using lactic acid or distilled water at 65°C for 4 min

	Lactic acid	Distilled water
E. coli 0157:H7 (TSA + SMAC)	5.57	3.18
Pseudomonads	2.38	1.83
Total counts	5.66	2.62

Standard error of differences between means = 0.91
Degrees of freedom = 20.0

Table 6: Summary of different decontamination treatments for beef cuts

	Steam, 75°C for 10 sec with 0.2 M lactic acid at 55°C	0.2 M lactic acid at 55°C – 4 min contact, with steam at 80°C for 10 sec	0.2 M lactic acid at 55°C – contact 4 min with heat at 65°C from baseplate only	Distilled water at 55°C – 4 min contact, with heat at 65°C from baseplate only
E. coli 0157:H7				
TSA – SMAC	0.73	5.05	5.57	3.18
SMAC	-	4.56	-	-
Pseudomonads	1.28	3.22	2.38	1.83
Total counts	1.12	2.65	5.66	2.62

E. coli 0157:H7 counts. The synergistic effects of acid and heat in these conditions are also evident.

Storage trial for treated beef cuts

A storage trial was carried out with beef that had been treated with 0.2 M lactic acid at 55°C and subsequently heated at 65°C for 4 minutes. Table 7 shows the effect of storage at 0 and 10°C in air or vacuum packs. The data show that the reduction in bacteria obtained from this treatment was maintained only when the storage temperature was at 0°C. When stored at 10°C, increases were beginning to occur in samples stored in air, and in vacuum packs significant amounts of growth occurred from 7 days onwards.

CONCLUSIONS

- The objective of this project was to assess the effectiveness of sub-atmospheric steam in combination with organic acids in the decontamination of fresh beef. The system was assessed using a pilot scale plant in which atmospheric pressure and temperature were controlled.

Table 7: The effect of packaging method and storage time and temperature on bacterial counts in decontaminated beef cuts

Air						
	Day 0	*Day 5*	*Day 10*	*Day 15*		
0°C Control	4.85	4.61	4.32	4.68		
0°C Treated	0.00	0.34	0.00	0.80		
	Day 0	*Day 1*	*Day 2*	*Day 3*	*Day 4*	*Day 5*
10°C Control	4.85	5.22	5.07	5.57	5.33	5.86
10°C Treated	0.00	0.00	0.00	0.61	0.57	2.50
Vacuum pack						
Day 0	*Day 14*	*Day 28*	*Day 42*			
0°C Control	4.85	4.61	4.59	4.36		
0°C Treated	0.00	1.31	1.69	0.71		
Day 0	*Day 7*	*Day 14*	*Day 21*			
10°C Control	4.85	4.98	5.06	5.25		
10°C Treated	0.00	3.54	6.82	4.76		

- Short-term high temperature steam treatments, with or without organic acids, gave only small reductions in bacterial counts. These reductions were too small to be of any practical significance.
- A successful treatment was developed with steam and organic acids and a synergistic effect between the two was clearly demonstrated. The reduction in pathogen counts of *E. coli* O157:H7 and other organisms was $\log_{10}$ 5-6, which was considered very satisfactory. The treatment used a sufficiently long contact time, 4 min, to allow the destruction of the bacteria present.
- When treated meat was packaged and stored in air or vacuum packs at 0 or 10°C it was found that pathogen and other bacterial growth was prevented at 0° but not at 10°C.
- The use of a 'heat sink' in conjunction with the organic acid was a significant improvement and showed that a heated vapour was as effective a treatment as sub-atmospheric steam.

- A drawback of the system was that the meat surface was discoloured as a result of this treatment. Despite the discolouration it is considered that this type of decontamination procedure had commercial potential in vacuum packaged beef.
- While this type of technology is only at the pilot scale it could be developed for industry application. The optimum point for use of this technique would be in the boning hall where it could be used as a final decontamination step prior to packing. The most likely application would be in a manufacturing beef boning facility where visual effects would have minimal customer impact. The expression "manufacturing beef" is usually applied to lower grade beef, e.g. cow beef, which is destined for further processing. Typical examples of such processing are the manufacture of frozen hamburgers, ready cooked meals and patties for fast food outlets. The majority of such products involve mincing, in which case the visual impact of any discolouration on the surface would be eliminated.

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The National Food Centre

RESEARCH & TRAINING FOR THE FOOD INDUSTRY

Dunsinea, Castleknock, Dublin 15, Ireland.

Telephone: (+353 1) 805 9500

Fax: (+353 1) 805 9550

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