Epidemiol. Infect. (1992), **109**, 491–496

Destruction of bacteria on fresh meat by hot water

M. G. SMITH

CSIRO, Division of Food Processing, Meat Research Laboratory, PO Box 12, Cannon Hill, Queensland 4170, Australia

(*Accepted 23 June 1992*)

SUMMARY

Strains of different bacterial genera: *Escherichia coli* (7), *Salmonella* serotypes (7), Enteropathogenic *E. coli* O 157 (2), *Aeromonas hydrophila* (4), *Yersinia enterocolytica* (1), *Pseudomonas fragi* (5), and *Listeria monocytogenes* (5), inoculated onto the surface tissues of pieces of fresh beef were found to be susceptible to the lethal action of hot water. On average, more than 3 $\log_{10}$ (99·9%) of these organisms were destroyed when water at 80 °C was applied for 10 or 20 s. Therefore, a hot water decontamination cabinet would be effective for destroying *in situ* any viable cells of these bacteria which might be present on the surface tissues of fresh beef carcasses.

INTRODUCTION

Laboratory studies undertaken previously indicated that the application of hot water under controlled conditions would destroy up to 3 $\log_{10}$ (99·9%) of *Escherichia coli* or salmonella organisms inoculated onto the surface of fresh meat [1]. These preliminary results also indicated that it should be possible to develop the process for use on dressed animal carcasses in commercial abattoirs. Subsequently, a novel hot water cabinet was designed and built [2, 3] and tested using sides of beef from freshly slaughtered animals with excellent results [4, 5]. Using this data, a prototype hot water decontamination cabinet was constructed. However, intensive testing showed that modifications were necessary before it could be considered suitable for installation on the continuous rail system of a modern commercial abattoir. The design now developed has been simplified so the cabinet is cheaper to construct, install and maintain; the operating conditions can be easily monitored; and it is now more versatile, allowing variations to be incorporated to suit different requirements.

Immediately after treatment at 80 °C, the surface tissues of carcasses have a bleached, grey, 'cooked' appearance to a depth of about 0·5 mm. Therefore, it is very easy to ascertain by visual inspection whether any areas of a carcass have received inadequate treatment. However, the treated carcasses subsequently regain their original colour and appear quite normal after overnight chilling. Experienced personnel from a large metropolitan abattoir who inspected the carcasses after this time said they were quite acceptable commercially. The only small area of disagreement was that some of the observers thought the treated

sides looked better than the untreated ones, the fat cover appearing whiter against the redness of the lean meat tissue.

Calculations using two parameters, the time of treatment and the temperature of the water, allow accurate estimates to be made of the effectiveness of an operational cabinet for the destruction of contaminating bacteria *in situ* on the surface tissues of sides of beef. When properly adjusted the cabinet will give at least 99·9% coverage over the whole surface of a side of beef (including the internal body cavity) and at the highest recommended temperature (80 °C) will result in more than an average 3 $\log_{10}$ (99·9%) destruction of viable bacterial cells, rendering the surface tissues of commercial carcasses virtually sterile.

To date all tests have been carried out using *E. coli* and salmonella organisms inoculated onto pieces of meat, or of *E. coli* cells inoculated over the whole surface area of sides of beef from freshly slaughtered cattle. However, bacteria of other genera, namely *Aeromonas*, *Yersinia*, and *Listeria*, some strains of which have been implicated in human illness, and of *Pseudomonas* which in large numbers are the major cause of spoilage of fresh meat, can also occur on animal carcasses [6–10]. Strains of Enteropathogenic *E. coli* (O 157) are also known to cause human illness and have been isolated from meat [11].

The following experiments were undertaken to determine the destruction of representative strains of different species of these genera of micro-organisms inoculated onto pieces of fresh meat and treated with water at various temperatures.

METHODS

Bacterial cultures

Type cultures of *E. coli* K12, *Y. enterocolytica*, and *Ps. fragi* were obtained from the University of Queensland. The other strains of *E. coli* and the salmonellas were isolated originally from sheep and cattle. The two *A. hydrophila* strains, isolated from abattoir effluent, and the *E. coli* strains, were identified using API 20E test strips (La Balme Les Grottes, Montalieu Vercieu, France), and the salmonella serotypes were identified by the Salmonella Reference Centre, Adelaide, South Australia, as *S. typhimurium*, *S. anatum*, *S. seftenberg*, *S. adelaide*, *S. oranienberg*, *S. derby*, and *S. havana*. The two enteropathogenic *E. coli* strains were also isolated from abattoir effluent. Neither fermented sorbitol and both agglutinated using the latex O 157 test (Oxoid Diagnostic Reagents). The listeria cultures were obtained originally from the Centre for Disease Control, Food and Drug Administration, Atlanta, Georgia, USA, where they had been identified as *L. monocytogenes* strains V7, C194, SCTA, MURB and SE 31. The other pseudomonads were isolated from meat and identified biochemically as strains of *Ps. fragi*.

Preparation of inoculated meat

Briskets were obtained from freshly slaughtered cattle, cut into pieces *c.* 10 cm square, and placed on trays. The required number of meat pieces were inoculated by swabbing liberally the exposed top surface with a bacterial culture grown in Oxoid nutrient broth for 24 h. The pseudomonas strains were grown at 25 °C. All other strains were grown at 37 °C. The pieces of meat were allowed to drain for *c.* 30 min at room temperature (22 °C) before testing. This technique normally gave

c. 10^{6-7} colony-forming units (c.f.u./cm^2 on the surface of each sample of meat. This is greatly in excess of the numbers of bacterial cells which may have been originally present on the raw meat tissue (usually less than *c.* 10^2 c.f.u./cm^2). The pieces of meat were heavily inoculated with the bacterial cells in this manner to obtain counts after treatment which could be treated statistically.

Sampling

The sampling and testing procedures have been described previously [1] except that five samples (each 2×5 cm^2 surface area) were excised at each sampling time to give a more accurate estimate of the effectiveness of each treatment. Counts were made using surface plating on Tryptone Soya Agar (Oxoid) to which 2 g glucose (Univar AR) and 2 g yeast extract (Oxoid) were added per litre (TYSG). All plates were incubated at 37 °C for 24 h except those containing pseudomonas organisms which were incubated at 25 °C for 72 h.

RESULTS

The decrease after 10 or 20 s treatment with water at different temperatures (40°, 60°, 80 °C) in the $\log_{10}$ c.f.u./cm^2 of the various bacterial genera inoculated onto the surface of pieces of fresh beef are listed in Table 1.

The difference in the magnitude of the standard deviations are probably due to differences in the surface texture of the meat samples rather than any inherent difference in the heat resistant characteristics of the strains of these organisms.

A comparison of the average mean decreases found in the present study (Table 1) and those found in an extensive study carried out previously on whole sides of beef carcasses treated in a decontamination cabinet using only *E. coli* SF as the test organism, are shown in Table 2. The *E. coli* SF decreases were calculated from the original results which were previously published in graphical form (Fig. 3, reference 5].

DISCUSSION

Strains of all of the genera of micro-organisms tested in these experiments can contaminate fresh meat. The experiments showed that each was also susceptible to the lethal action of hot water when applied to the surface of pieces of fresh beef tissue. There may have been some difference in the susceptibility of different bacterial species due to this treatment (Table 1) but the variation in the results obtained might alternatively have been due to differences in the surface texture of the beef samples used.

The minimum temperature at which *E. coli* cells are destroyed on the surface of fresh meat has been estimated at *c.* 53–55 °C [5]. Therefore, the results using water at 40 °C may be regarded as estimating the 'wash-off' effect. Even though in the present experiments, there was an average decrease of 45·2% of the inoculated cells after 20 s treatment with water at 40 °C (Table 1) this was not statistically significant. When dealing with large numbers of bacterial cells percentages can be misleading and $\log_{10}$ numbers should be used [12]. At 60 and 80 °C the mean decreases were highly significant ($P < 0{\cdot}001$).

In previous, extensive experiments, no sub-lethal injury was detected in *E. coli*

https://doi.org/10.1017/S0950268800050482 Published online by Cambridge University Press

Table 1. *Mean survival log_{10} c.f.u./cm^2 of inoculated micro-organisms on fresh meat treated with water at different temperatures (40, 60, 80 °C) for 10 or 20 s*

	Treatment time (10 s)				Treatment time (20 s)			
Organisms	Initial count	40 °C	60 °C	80 °C	Initial count	40 °C	60 °C	80 °C
E. coli (7 strains)	6·72 (0·16)*	6·55 (0·19)	5·38 (0·47)	3·63 (0·23)	6·78 (0·31)	6·49 (0·34)	5·47 (0·33)	3·26 (0·22)
Salmonella (7 strains)	6·76 (0·28)	6·54 (0·29)	5·57 (0·35)	3·41 (0·30)	6·88 (0·35)	6·59 (0·40)	5·48 (0·23)	3·28 (0·34)
Listeria (5 strains)	5·97 (0·44)	5·84 (0·43)	5·07 (0·34)	3·14 (0·26)	6·20 (0·29)	6·02 (0·22)	4·97 (0·15)	3·32 (0·37)
Pseudomonas (5 strains)	6·82 (0·15)	6·54 (0·22)	5·61 (0·26)	3·86 (0·26)	6·80 (0·15)	6·45 (0·15)	5·17 (0·39)	3·41 (0·46)
Aeromonas hydrophila (4 strains)	6·84 (0·52)	6·56 (0·60)	5·20 (0·50)	3·64 (0·52)	6·87 (0·71)	6·68 (0·78)	5·27 (0·68)	3·56 (0·81)
Enteropathogenic *E. coli* (2 strains)	6·27 0·17)	6·07 (0·14)	5·47 (0·25)	3·68 (0·40)	6·25 (0·24)	5·99 (0·35)	5·27 (0·52)	3·22 (0·70)
Yersinia enterocolytica (1 strain)	5·63	5·60	3·63	2·06	5·82	5·64	3·63	2·16
Mean	6·57	6·37	5·34	3·49	6·62	6·37	5·26	3·27
Mean decrease		0·20	1·23	3·08		0·25	1·36	3·35
% Decrease		37·8	94·1	99·92		45·2	95·7	99·96
Significance (*t* test)		NS	†	†		NS	†	†

*, Standard deviation.
NS, Not significant ($P > 0·05$).
†, $P < 0·001$.

Table 2. *Decrease of* log_{10} *c.f.u./cm*^{2} *of* E. coli *SF* [*5*] *and the bacterial organisms listed in Table 1 after treatment with water at different temperatures* (*40, 60, 80 °C*) *for 10 or 20 s*

	Treatment time (10 s)			Treatment time (20 s)		
	40 °C	60 °C	80 °C	40 °C	60 °C	80 °C
E. coli SF	0·25	0·90	2·97	0·21	0·95	3·34
Table 1	0·20	1·23	3·08	0·25	1·36	3·35

treated on fresh cattle carcasses when counts were made 30 min after treatment on either Oxoid MacConkey Agar No. 3, and on TYSG [5]. Also, no further decreases were found when counts were made on the same carcasses after 24 and 48 h in a chiller (air temperature −1 to +2 °C). It appears that the treatment of bacterial cells on the surface of raw meat with hot water results in the immediate destruction of the cells affected and sub-lethal injury cannot be demonstrated. Also, in the present experiments, counts were made on TYSG plates, a non-selective nutrient medium, which would have allowed all the remaining viable cells to grow.

The lower numbers of cells of *Y. enterocolytica* on the meat samples appeared to be due to this strain of organism not growing to the same extent in nutrient broth. Thus, the inoculum was smaller. Also, only one strain was tested.

Although the pieces of fresh meat were heavily inoculated initially to obtain counts after treatment that could be treated statistically, it would be expected that equivalent reductions would occur in the numbers of viable cells from any initial degree of contamination.

At 40 and 80 °C there was no discernible overall difference (Table 2) between the results obtained in this series of experiments and of those obtained in experiments carried out previously on whole sides of beef carcasses treated in a decontamination cabinet [5]. At 60 °C there may have been a difference in heat susceptibility between the different groups of organisms but this possibility would best be resolved in further experiments specifically designed to investigate this question. Certainly, the results found in the present study do not indicate any overall diminished effect. Therefore, treatment in a hot water decontamination cabinet of sides of cattle carcasses contaminated with different strains of bacteria should be as effective as shown in previous trials in which *E. coli* SF was used as the test organism [5].

ACKNOWLEDGEMENTS

This work was funded in part by the Meat Research Corporation (formerly Australian Meat and Livestock Research and Development Corporation). I would also like to thank Mr G. Higgs for his excellent technical assistance.

REFERENCES

1. Smith MG, Graham A. Destruction of *Escherichia coli* and salmonellae on mutton carcasses by treatment with hot water. Meat Sci 1978; **2**: 119–28.
2. Davey KR. Theoretical analysis of two hot water cabinet systems for decontamination of sides of beef. Int J Food Sci Technol 1989; **24**: 291–304.

3. Davey KR. A model for the hot water decontamination of sides of beef in a novel cabinet based on laboratory data. Int J Food Sci Technol 1990; **25**: 88–97.
4. Davey KR, Smith MG. A laboratory evaluation of a novel hot water cabinet for the decontamination of sides of beef. Int J Food Sci Technol 1989; **24**: 305–16.
5. Smith MG, Davey KR. Destruction of *Escherichia coli* on sides of beef by a hot water decontamination process. Food Australia 1990; **42**: 195–8.
6. Roberts, Diane. Bacteria of public health significance. In: Brown MH, ed. Meat microbiology. London, New York: Applied Science Publishers Ltd, 1982: 319–86.
7. Buchanan RL, Palumbo SA. *Aeromonas hydrophila* and *Aeromonas sobria* as potential food poisoning species: A review. J Food Safety 1985; **7**: 15–29.
8. Grau FH. Microbial ecology of meat and poultry. In: Pearson AM, Dutson TR, eds. Advances in meat research, vol. 2. Meat and poultry microbiology. Westport, Connecticut: AVI Publishing Company Inc, 1986: 1–47.
9. Hird DW. Review of evidence for zoonotic listeriosis. J Food Protect 1987; **50**: 429–33.
10. Forsyth JRL. *Listeria* – update and future implications. Food Australia 1991; **43**: 99–104.
11. Doyle MP. *Escherichia coli* O 157:H7 and its significance in foods. Int J Food Microbiol 1991; **12**: 289–302.
12. Tanner FW. Microbiology of foods. Champaign, *Ill*: Garrard Press, 1944: 322.