Meat Science 126 (2017) 50–54

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

The fate of *Salmonella* Typhimurium and *Escherichia coli* O157 on hot boned versus conventionally chilled beef

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ARTICLE INFO

Article history:
Received 27 October 2016
Received in revised form 13 December 2016
Accepted 19 December 2016
Available online 21 December 2016

Keywords:
Hot boning
Beef
TVC
Salmonella
VTEC

ABSTRACT

This study investigated the fate of *Salmonella* Typhimurium and *Escherichia coli* O157 on hot boned versus conventionally chilled beef. Beef samples were individually inoculated with *S.* Typhimurium ATCC 14028, *S.* Typhimurium 844, *E. coli* O157 EDL 933 or *E. coli* T13. Half the samples were subject to the same time-temperature chilling profile used for conventionally chilling beef carcasses while the other half was subject to hot boned conditions. The surface pH (5.5) and a_w (0.95 to 0.97) were stable. *S.* Typhimurium and *E. coli* O157 counts, which decreased by up to 1.0 and 1.5 $\log_{10}$ cfu cm^{-2}, respectively, were statistically similar ($P > 0.05$), regardless of the chilling regime applied, with the exception of *E. coli* O157 EDL 933, where the counts on hot boned beef were significantly ($P < 0.05$) higher. It was concluded that any decrease in pathogenic bacteria during beef chilling may be significantly ($P < 0.05$) less for hot boned beef depending on the bacterial strain.
Hot boning may therefore result in an increased risk to the consumer.

1. Introduction

Beef carcasses are usually chilled immediately after slaughter for a period of at least 24 h before cutting into primals in the boning hall (Savell, Mueller & Baird, 2005). Hot boning, a process in which carcasses are deboned immediately after slaughter, has several advantages over conventional chilling, including a requirement for less chiller space, more flexible logistics and reduced costs (Pisula & Tyburcy, 1996). However, this technology has not been adopted in most countries, as a failure to immediately chill the surface temperature of the beef could promote bacterial growth (EFSA, 2014).

A recent scientific opinion by the European Food Safety Authority (EFSA) identified *Salmonella* and *Escherichia coli* O157 as high priority hazards for beef (EFSA, 2013a). In the European Union (EU) up to 60% of bovine hides have been reported to be *Salmonella* positive (Rhoades et al., 2009) and 0.6% of an estimated 6 million salmonellosis cases in Europe have been associated with beef (EFSA, 2011; EFSA 2014); with *E. coli* O157 prevalence rates ranging from 0.2 to 2.3%, 1.5% to 13.7% and 5.5% to 20.2% for individual animal, herd and slaughter batches, respectively (EFSA, 2013b). *Salmonella* and *E. coli* O157 are carried asymptomatically in the gastrointestinal tract of cattle and shed in the faeces. Although EC 853/2004 requires that cattle presented for slaughter should be clean and dry, soiled hides are still a major source of carcass contamination with these organisms which are readily transferred during dehiding (Koohmaraie et al., 2005). Control is dependent on the implementation of an effective prerequisite programme (PRP) and hazard analysis and critical control point as required by EC 852/2004 and EC 853/2004. Although lactic acid, applied as a spray or mist, at concentrations of 2% to 5% and temperatures of up to 55 °C, may be applied to beef carcasses (EC 101/2013) this is rarely used as it is not accepted by retail customers. In contrast, chilling has been incorporated into many beef slaughter HACCP plans (Lenahan et al., 2010), not least because several studies have shown that more efficient chilling results in improved carcass hygiene (Philips et al., 2006, Ruby, Zhu & Ingham, 2007; Sheridan, 2004).

Current legislation, Regulation EC 853/2004, requires that carcasses be immediately chilled after *post-mortem* inspection to ensure a temperature throughout of not >7 °C in the case of meat and not >3 °C for offal. Beef carcass surface temperatures range between 15 °C and 20 °C immediately after slaughter (Reid et al., 2017), temperatures that will support the survival and growth of both *Salmonella* spp. and *E. coli* O157. It is therefore important that the temperature be reduced to below 5 °C, their minimum growth temperature, as soon as possible (EFSA, 2014; James & James, 2004). However, in practice beef carcasses are usually chilled to not below 10 °C in the first 10 h (to prevent cold shortening) and to 0 to 2 °C thereafter. In contrast hot boning of carcasses employs boning out and cut up of carcasses into various pieces and primals followed by vacuum packaging and placement in cardboard boxes before chilling. It has been suggested that the higher

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http://dx.doi.org/10.1016/j.meatsci.2016.12.010
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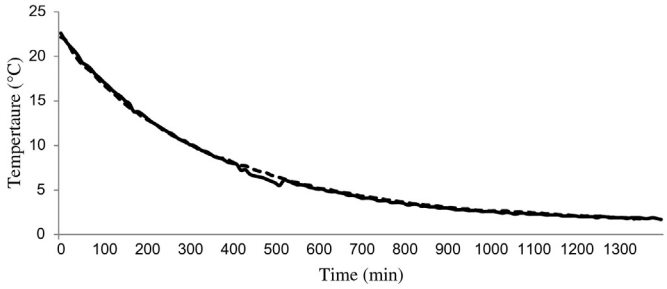

Fig. 1. Chill profiles of beef samples in 13 L of water at 2 °C (solid line) achieved the same time-temperature profile as chilling the hot boned primals in a commercial abattoir (dashed line).

temperatures associated with hot boned beef could promote bacterial survival and growth, including *Salmonella* and *E. coli* O157 (Sheridan & Sherington, 1982; Spooncer, 1993; Yang, Balamurugan, & Gill, 2011). The aims of this study were therefore; [1] to investigate the survival of bacteria used as general indicators of hygiene (total viable count, TVC and total Enterobacteriaceae count, TEC) during the chilling of beef and [2] to determine if the higher temperatures associated with hot boning versus conventional chilling could facilitate the survival and/or growth of *S.* Typhimurium and *E. coli* O157.

2. Materials and methods

2.1. Bacterial cultures

S. Typhimurium strains, reference strain ATCC 14028 and bovine isolate 844 (from the Teagasc culture collection) and *E. coli* O157 reference strain EDL 933 and bovine isolate (T13) also from our culture collection were used in these experiments. All strains were made resistant to 1000 μg mL^{-1} streptomycin sulphate (Sigma Aldrich, Ireland) according to the method described by Blackburn & Davies (1994) to facilitate selective recovery from beef samples. The growth rates of the mutant strains were compared to the wild type strains to ensure that there was no significant differences ($P < 0.05$) (data not shown). Stains were stored on Protect beads (Technical Service Consultants Ltd., UK) at −20 °C until required.

2.2. Inoculum preparation

One bead containing the a specific strain (ATCC 14028, 844, EDL 933 or T13) was spread plated onto Tryptone Soya Agar (TSA) plates (Oxoid) and incubated at 37 °C for 24 h. A single colony was then aseptically transferred to 10 mL Tryptone Soya Broth (TSB) (Oxoid) and incubated at 37 °C for 24 h. The culture was then centrifuged (Eppendorf centrifuge 5403) at 5000*g* at 4 °C for 10 mins. The recovered pellet was washed twice with 10 mL phosphate buffered saline (PBS) (Oxoid) and then resuspended in 10 mL PBS to produce a culture containing approximately 9 $\log_{10}$ cfu mL^{-1}. The suspension was then serially diluted 1:10 in Maximum Recovery Diluent (MRD) to create a suspension containing 6 $\log_{10}$ cfu mL^{-1}. Exactly 1 mL of this suspension was added to 1 L of MRD to create the final inoculum suspension with a final concentration of approximately 3 $\log_{10}$ cfu mL^{-1}, which was used to dip inoculate the beef samples. The final average concentration of the dip inoculum was determined by plating out 100 μl aliquots onto TSA supplemented with 1000 μg mL^{-1} streptomycin sulphate and incubating at 37 °C for 24 h.

2.3. Recording the time-temperature profiles for conventionally chilled (cold boned) and hot boned beef carcasses.

On 3 separate occasions, 2 heifers ($n = 6$) of commercial breeds were slaughtered in the Meat Industry Development Unit (MIDU) in Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland. After evisceration, each heifer was centrally split into left and right sides ($n = 12$). On the right side, the *M. longissimus thoracis et lumborum* (LTL) (striploin), *M. psaoa major* (fillet), *M. semitendinosus* (inside round), *M. quadriceps* (knuckle) and *Biceps Femoris* muscles were hot boned (HB) out within 90 min of slaughter and vacuum packaged (Pi-Vac Elasto-pack system, Nofima) to prevent cold shortening. These primals were then placed in a commercial chiller at 0 °C for 24 h. The left side was treated as the conventional side and was placed in a commercial chiller for 24 h. The surface temperature of the hot boned muscles (24 h chill at 0 °C) and the conventionally treated carcasses (10 °C for 10 h followed by 0 °C for the remaining 14 h) were monitored (every 10 min for 96 h) using Testo-T175 (Eurolec Instrumentation LTD) data loggers.

2.4. Development of a model system to mimic the time-temperature profiles of hot boned and conventionally chilled beef

The method of Hudson et al. (2013) was used to mimic the chilling curves of conventional and hot boned beef carcasses, samples (10 × 10 × 1 cm), prepared from the conventionally chilled *B. Femoris* muscle, placed in containers filled with 5 L of water (pre-heated to 22 °C) and were chilled in a programmable incubator that was set to 10 °C for 10 h followed by 0 °C to mimic the commercial chilling surface temperature. Hot boned samples were placed in containers of 13 L of water (pre-heated to 22 °C) and chilled at 2 °C for 24 h. Previous work has established that these volumes of water gave the best results for mimicking conventional beef carcass chilling and hot boning surface temperatures. Surface temperatures were monitored as described above.

2.5. Beef sample preparation

Exactly 140 beef samples (10 × 10 × 1 cm) were prepared from the conventionally chilled *B. Fermoris* muscle. From these, 14 samples were randomly assigned to each of the following treatment combinations; [1] *S.* Typhimurium ATCC 14028 – hot boned; [2] *S.* Typhimurium ATCC 14028 – conventionally chilled; [3] *S.* Typhimurium 844 – hot boned; [4] *S.* Typhimurium ATCC 844 – conventionally chilled; [5] *E. coli* O157 EDL 933 – hot boned; [6] *E. coli* O157 EDL 933 – conventionally chilled; [7] *E. coli* O157 T13 – hot boned and [8] *E. coli* O157 T13 – conventionally chilled. The remaining 2 sets of 14 were used as uninoculated controls to monitor psychrophilic TVC (TVCp), mesophilic TVC (TVCm) and TEC, set 1 being subject to the hot boned time-temperature profile and set 2 subject to conventional chilling. These control samples were also used to

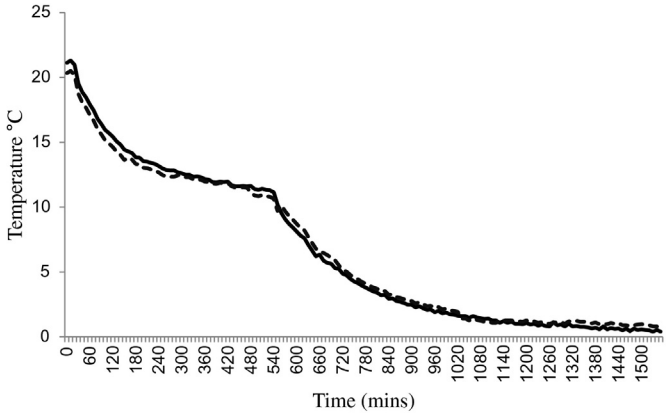

Fig. 2. Chill profiles of beef samples in 5 L of water (solid line) for 10 °C for 10 h and then to a target temperature of 0 °C for 38 h achieved the same time-temperature profile as chilling using the same time-temperature targets in a commercial abattoir (dashed line).