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Combined effect of chilling and desiccation on survival of *Escherichia coli* suggests a transient loss of culturability

L.A. Mellefont *, C. Kocharunchitt, T. Ross

Food Safety Centre, School of Land and Food/Tasmanian Institute of Agriculture, University of Tasmania, Private Bag 98, Hobart, 7001 Tasmania, Australia

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ABSTRACT

Dry air carcass chilling regimes used in some Australian meat works, which not only rapidly reduce the temperature of the carcasses but also dry the meat surface initially, are reported to cause reductions in the number of *Escherichia coli* present on carcasses after processing. This study used a laboratory broth model system to systematically investigate the basis of such reductions by simulating chilling and desiccation profiles observed on carcasses separately and, finally, in combination. Observed growth was compared to the predictions generated by a strain-specific modification of a validated *E. coli* growth model (Mellefont et al., 2003; Performance evaluation of a model describing the effects of temperature, water activity, pH and lactic acid concentration on the growth of *E. coli*). Good agreement between observed and predicted growth was evident when chilling or desiccation profiles were simulated individually. However, when chilling and desiccation profiles were applied simultaneously the observed population kinetics deviated from those predicted by the model. An initial reduction in cell numbers, not predicted by the model, was observed followed by an anomalously rapid increase in population density before growth resumed at a rate expected for the conditions imposed. From our analysis of the kinetics of the population changes, we suggest that the initial decrease in cell numbers was unlikely due to cell death, because conditions were growth permissive. Considering all possible explanations from the observed population kinetics, we propose that a temporary loss of the ability to produce colonies on agar plates may occur. These results may explain reports of increases in *E. coli* numbers two to three days after commencement of chilling, compared to those observed after 16–24 h, despite the imposition of growth-preventing temperatures.

1. Introduction

Australian meat works have traditionally used forced air chillers to cool carcasses. The rate of cooling is determined by the air temperature, velocity and humidity. A typical weekend cooling process employs fan speeds of ~1 m/s at 8.5 °C for the first 8 h of chilling, ~0.6 m/s at 9.5 °C for the following 12 h and ~0.4 m/s at 9.5 °C for the remainder of the time period (Ross, 1999; Salter, 1998). Chilling carcasses with cold air reduces meat temperature and also partially "dries" the meat surface (Eustace, 1981; Salter, 1998), as evidenced by a reduction in the water activity (a_w). The rate of a_w reduction of the surface tissues depends on the difference between the rate of evaporation at the surface and diffusion of moisture from the deeper layers of tissue. Drying of the carcass surface occurs mainly during the early part of chilling when the surface is warmer than the air. During storage, moisture will diffuse through to the surface from deeper tissues at a rate exceeding that of evaporation and, thus, the surface a_w increases again over time. Salter (1998) mapped the a_w of the carcass surface at different stages during chilling and reported that carcasses appeared to follow a similar pattern of large fluctuations over the first 20 h of chilling, with a_w falling as low as 0.929 at the rib site and 0.942 at the brisket site, and thereafter returning to, and stabilising at, levels in the range of 0.98 to 0.99. The increase in a_w after 20 h coincides with carcass surfaces reaching temperatures similar to that of the air in the chiller.

Eustace et al. (2004) suggested that air chilling significantly inhibits the growth of *Escherichia coli* on the carcass surface due to the combined hurdles of low water activity and chill temperature. Moreover, reductions of 0.43 and 0.40 $\log_{10}$ CFU have been observed after overnight (i.e., ~20 h) chilling (Eustace et al., 2004). Bacon et al. (2000) reported up to a 2 log reduction in *E. coli* over 24–36 h in some abattoirs in the United States. Similarly, in Ireland, McEvoy et al. (2004) reported reductions in *E. coli* counts of up to 2.13 log units after 24 h of chilling. Greig et al. (2012), based on an extensive analysis of the available literature on carcass chilling, concluded that chilling alone can cause inactivation of *E. coli*. However, studies on the performance of some carcass decontamination processes have shown that these reductions can be transient (Corry et al., 1995; Dickson and Anderson, 1992; Dickson et al., 1994) and that microbial loads on carcasses that had undergone a decontamination step returned to those of untreated carcasses after further

* Corresponding author. Tel.: +61 03 62 267469; fax.: +61 03 62 262642.
E-mail address: Lyndal.Mellefont@utas.edu.au (L.A. Mellefont).

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storage. McEvoy et al. (2004) postulated that the observed reductions were more likely due to cell injury as a result of low temperature and a_w rather than cell death. Prima facie, the temperature and a_w conditions experienced during air chilling, either on their own or in combination, are expected to permit growth of *E. coli*. For example, Presser et al. (1998) reported growth at 10 °C when $a_w \geq 0.965$.

This study initially aimed to determine whether reported reductions in *E. coli* populations subjected to non-lethal conditions on carcass surfaces during air chilling could be induced reliably in a laboratory model system. Levels of *E. coli* on carcasses are low, however, and make it impractical to accumulate enough information to study this question from naturally contaminated carcasses, and deliberate introduction of *E. coli* to carcasses in commercial processing plants is often precluded or logistically challenging. Preliminary laboratory studies had shown that populations of *E. coli* on the surface of spare-ribs at a_w 0.978 responded the same as *E. coli* suspended in a nutritious broth of equivalent water activity controlled by addition of NaCl (Ross and Mellefont, 2005). Accordingly, as a first approach, temperature and a_w changes relevant to carcass chilling were simulated in laboratory broths. Such model systems offer considerable advantages over in situ studies through the elimination of many uncontrolled variables (e.g. other contaminating bacteria, heterogeneity in meat tissue) that could confound the interpretation of results. Finally, if such inactivation could be demonstrated in the broth system, we hoped to gain insights into the mechanisms of inactivation.

Temporal changes in *E. coli* levels were determined by viable counts at close time intervals to examine, in detail, the population response to conditions similar to those experienced on beef carcasses during air chilling. The observed changes in *E. coli* were compared to the predictions of a model validated for *E. coli* growth on meat (Mellefont et al., 2003; Ross et al., 2003) and calibrated for the specific strain of *E. coli* used in this study. The model predictions were integrated over time, to include the effect of fluctuating water activity and temperature, and compared to the observed *E. coli* population changes.

2. Materials and methods

2.1. Overview

Several carcass chilling simulations were conducted. Initially the effects of temperature and a_w fluctuations were examined separately. The influences of physiological history and/or habituation were also investigated. Cooling conditions for a weekend chilling process were simulated. In that process carcass temperature fell to 15 °C in approximately 3 h and to <10 °C in a further 5 h (Eustace et al., 2004). Water activity profiles representative of either the brisket or cube roll site of a carcass derived from Salter (1998) were also simulated and investigated under static temperature conditions (25 and 13 ± 0.1 °C respectively). A final trial simulated combined chilling and water activity changes experienced on the carcass surface during weekend carcass chilling.

2.2. Bacterial strains

E. coli R31 (a verotoxigenic clinical strain originally provided by Ms. Silvana Bettiol, University of Tasmania Clinical School) was obtained from the School of Land and Food/Tasmanian Institute of Agriculture Culture Collection, University of Tasmania. This strain was selected because it did not visibly clump in broths supplemented with high levels of NaCl, with growth observed at 20 °C and a measured a_w of 0.949 (Salter et al., 2000). *E. coli* R31 stocks were maintained in multiple 10 μL suspensions of Nutrient Broth (Oxoid CM1; NB) with 15% glycerol and stored at −70 °C. Prior to the commencement of each experiment a 10 μL frozen culture was thawed at room temperature. A loopful was streaked onto Brain Heart Infusion Agar (Oxoid, CM375, BHA), then incubated at 37 °C for 24 h.

2.3. Preparation of inocula and test broths

2.3.1. Fluctuating temperature experiments

A "primary" culture was prepared by touching a sterile loop to 5 colonies of *E. coli* R31 and inoculating into 10 mL of pre-warmed (37 ± 0.1 °C) Brain Heart Infusion broth (Oxoid, CM225; BHI) in a 30 mL McCartney bottle. The broth was incubated without shaking at 37 ± 0.1 °C for 24 h. This culture was serially diluted in 0.1% Bacteriological Peptone (Oxoid, L37; PW). 50 μL of the 10^{-2} dilution was added to 50 mL of pre-warmed (37 ± 0.1 °C) BHI in a 125 mL side-arm flask. A second uninoculated side-arm flask containing BHI was used as a blank for spectrophotometric determinations. Growth was monitored turbidimetrically at 540 nm (Spectronic 20+, Milton Roy Co., USA) until absorbance had reached OD_{540} ~0.7. This "secondary" culture was used to prepare exponential and stationary phase inocula as follows.

100 μL of the secondary culture was removed and serially diluted in PW. 50 μL of the 10^{-2} dilution was added to 50 mL of pre-warmed (37 ± 0.1 °C) BHI in a 125 mL side-arm flask. Growth was monitored turbidimetrically until OD_{540} ~0.7, at which time cells were in the late-exponential phase of growth at a concentration of ~10^{7-8} cells/mL.

The remainder of the secondary culture was returned to the 37 ± 0.1 °C water bath and incubated until stationary phase. This method provided approximately 10^9 cells/mL in the stationary phase of growth after ~26 h at 37 ± 0.1 °C.

Inocula were added to 50 mL of BHI in a stoppered 125 mL conical flask. To enable direct comparison of responses of exponential and stationary phase cultures in various trials, the volume of inoculum added to the test broths varied to achieve similar initial cell concentrations. 0.5 mL of the 10^{-1} dilution of exponential phase cells or 0.5 mL of a 10^{-2} dilution of stationary phase cells was added to three flasks. The triplicate cultures were then subjected to a temperature profile simulating cooling on the brisket site of a beef carcass under commercial air chilling conditions as described in Section 2.4.1.

2.3.2. Fluctuating water activity experiments

2.3.2.1. Brisket site simulation. Stationary phase, non-habituated and NaCl-habituated inocula were prepared as follows. A primary culture was prepared as described in Section 2.3.1. The broth was incubated without shaking at 25 °C for 24 h. This culture was serially diluted in PW and 50 μL of the 10^{-3} dilution was added to 50 mL of pre-warmed (25 ± 0.1 °C) BHI at a_w 0.995, measured with an Aqualab CX2 dew point instrument (Decagon Devices, Pullman, USA), or BHI containing 3.5% w/v NaCl (measured a_w 0.972) in a 125 mL conical flask. The flasks were then incubated with shaking for 44 h at 25 ± 0.1 °C to provide approximately 10^9 cells/mL in the stationary phase of growth.

The stationary phase cultures were serially diluted in PW and 50 μL of the 10^{-1} dilution of appropriate stationary phase inocula was added to three flasks each containing 49.95 mL of BHI with 3% w/v NaCl added (measured a_w 0.979). The triplicate cultures were then subjected to a fluctuating a_w profile simulating changes at the brisket site as described in Section 2.4.2 and incubated at 25 ± 0.1 °C.

2.3.2.2. Cube roll site simulation. A stationary phase inoculum was prepared as described for the brisket temperature simulation experiments, except that 50 μL of the 10^{-3} dilution was added to 50 mL of pre-cooled (13 ± 0.1 °C) BHI in a 125 mL conical flask. The flask was then incubated with shaking for 88 h at 13 ± 0.1 °C to provide non NaCl-habituated or NaCl-habituated stationary phase inocula (approximately 10^9 cells/mL).

The pre-chilled, stationary phase culture was serially diluted in PW and 50 μL of the 10^{-2} dilution was added to each of three flasks containing 49.95 mL of BHI with 1.5% NaCl (w/v; measured a_w 0.987). The triplicate cultures were then subjected to a fluctuating a_w profile simulating changes at the cube roll site as described in Section 2.4.2 and incubated at 13 ± 0.1 °C.