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Effect of rapid surface cooling on *Campylobacter* numbers on poultry carcasses

Dean Burfoot [a, *], Jeremy Hall [b], Keith Nicholson [c], Kirsty Holmes [b], Cedric Hanson [c], Simon Handley [c], Elizabeth Mulvey [a]

[a] Campden BRI, Station Road, Chipping Campden, GL55 6LD, United Kingdom
[b] Bernard Matthews Limited, Great Witchingham Hall, Norwich, NR9 5QD, United Kingdom
[c] BOC Linde, Chinor Road, Thame, OX9 3NX, United Kingdom

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ABSTRACT

These studies examined the effect of rapid surface cooling on the numbers of *Campylobacter* on chicken carcasses. In two trials, chicken skins were immersed in liquid nitrogen. *Campylobacter* numbers were reduced by 1 $\log_{10}$ cfu/g by immersion for 20s. Immersion would not be practical for whole carcasses in commercial slaughterhouses. Twenty two trials investigated the effects of spraying liquid nitrogen towards whole carcasses either in a chamber or in a tunnel. The final four trials, with carcasses passing through a spray tunnel for 40s, caused average reductions in the numbers of *Campylobacter* of between 0.9 and 1.5 $\log_{10}$ cfu/g when tested the day after treatment and between 0.9 and 1.3 $\log_{10}$ cfu/g when tested a further six days later. The temperature of the flesh remained above −2 °C thereby showing that the flesh was not frozen by the process which offers a viable approach to reducing Campylobacter on chicken carcasses.

1. Introduction

Campylobacter continues to be the most common bacterial foodborne pathogen in humans in the EU, with broiler meat considered as the main source (EFSA and ECDC, 2014). The overall average prevalence of *Campylobacter* in poultry in the EU states is 75.8% (EFSA, 2010). This work assessed the potential for reducing the numbers of *Campylobacter* on poultry carcasses by rapid surface chilling (RSC) as described in a patent application (Hall & Normanton, 2012) and a granted UK patent (Hall, 2014).

Holding carcasses in a frozen state is known to reduce the *Campylobacter* counts on them. EFSA (2011) concluded that frozen storage will decrease the numbers of *Campylobacter* by 1 $\log_{10}$ after a few days and by approximately 2 $\log_{10}$ after 3 weeks. Although most research concludes that freezing reduces the *Campylobacter* counts on poultry it is not an option for birds to be labelled and sold as fresh in the EU because Regulation (EU) No 1308/2013 (European Council, 2013) requires that fresh poultry cannot have been previously frozen.

Crust freezing has also been studied. Zhao, Ezeike, Doyle, Huing, and Howell (2003) carried out trials with inoculated chicken to examine the effects of cooling and freezing temperatures on *Campylobacter jejuni*. They cooled chicken wings with liquid nitrogen at −80, −120, −160 and −196 °C such that the internal temperature quickly reached −3.3 °C. Cooling times ranged from 20 to 330s to achieve the required internal temperature. Reductions in *Campylobacter* were 0.5 $\log_{10}$ cfu/g at −80 °C, 0.8 $\log_{10}$ cfu/g at −120 °C, 0.6 $\log_{10}$ cfu/g at −160 °C, and 2.4 $\log_{10}$ cfu/g at −196 °C. Vapour-state liquid nitrogen was used to achieve temperatures between −80 °C and −160 °C and submersion in liquid nitrogen was used to achieve −196 °C.

Kennedy and Miller (2004) describe the use of rapid chilling to accelerate the maturation of poultry, the so called “Accelerated Inline Maturation” (AIM) process. They found a reduction in the number of *Campylobacter* positive birds from 14 positives out of a total of 15 carcasses at Day 0 to 2/15 at Day 5 and no positives at Day 7. By comparison, conventionally chilled birds had prevalence values of 11/15, 11/15, and 9/15 on the same days of testing.

Another study, by Boysen and Rosenquist (2009), used a commercial belt freezer running at −55 °C to cool chicken fillets to a surface temperature of −1 °C and achieved a reduction in *Campylobacter* of 0.4 $\log_{10}$ cfu/carcass. Other studies have used

* Corresponding author.
E-mail address: d.burfoot@campden.co.uk (D. Burfoot).

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inoculated samples and EFSA (2011) concluded that those studies show reductions closer to 1 $\log_{10}$.

However, a study by Lee, Smith, and Coloe (1998) concluded that *C. jejuni* 81116 on chicken skin could withstand repeated freezing and thawing and retained a high level of viability under all freezing conditions. However, that work was carried out with inoculated material. El-Shibiny, Connerton, and Connerton (2009) also found that rapid cooling or freezing does not always reduce *Campylobacter* counts. They concluded that rapid cooling (−20 °C/min) to 4 °C enhanced the survival of *Campylobacter* when compared to conventional cooling. Furthermore, rapid cooling (−30 °C/min) to −20 °C enhanced the survival of *Campylobacter coli* 99/367 compared to using a domestic refrigerator.

A review by Whyte, Hudson, and Turner (2005) concluded that fast freezing rates tend to increase bacterial survival by reducing the time over which they are exposed to osmotic stress. However, very fast freezing (>10 °C/minute) decreases the survival due to possible formation of intracellular ice crystals. Most of the reduction of *Campylobacter* numbers occurs during the freezing process rather than during storage. They also conclude that there is a marked variability in cold-tolerance between isolates during both chilling and freezing.

The studies reported here consisted of two trials to examine the effect on numbers of *Campylobacter* of dipping chicken skin in liquid nitrogen and 22 trials to examine the effect of rapidly cooling the surface of whole carcasses. Dipping carcasses in liquid nitrogen is not a practical option due to costs but the trials with skin were carried out to examine the effects of very rapid cooling.

2. Material and methods

2.1. Treatment equipment

In the two trials where poultry skin was dipped into liquid nitrogen, 200 ml of the liquid was held in a polypropylene beaker supported within a polystyrene beaker. In the other trials, liquid nitrogen was sprayed onto carcasses either inside a cabinet (Trials 3 to 15) or inside a tunnel (Trials 16 to 21). These processes are referred to as “RSC Cabinet” and “RSC Tunnel” later in this paper. The inside of the insulated cabinet measured 2.0 m (high) by 0.8 m (wide) by 1.5 m (deep). Carcasses were hung on shackles hanging from a mobile support frame that was pushed into the chamber. Liquid nitrogen was directed towards the carcasses through nozzles in spray bars located in the cabinet. The number and location of the nozzles was changed between trials to the most appropriate arrangement.

The inside of the tunnel measured 5.0 m (long) by 1.5 m (high) by 1.0 m (wide) and spray bars with nozzles were again used to direct liquid nitrogen towards the carcasses but, in this case, the carcasses passed continually through the tunnel on shackles mounted on a process rail. The continuous line of shackles passed though the tunnel, outside, and then back into the tunnel. Carcasses were located by hand on to the shackles and carefully removed by the legs, using sterile gloved hands, after leaving the tunnel.

2.2. Microbiological testing

Mesophilic aerobic plate counts (APC) testing was carried out based on to BS EN ISO 4833 (2003). Testing for *Campylobacter* was based on BS EN ISO 10272-1 (2006) for detection and BS EN ISO/TS 10272-2 (2006) for enumeration. The methods are repeated here for completeness.

Aerobic plate counts testing required each skin sample to be weighed, made up to a 1:10 dilution with buffered peptone water (Oxoid CM1049T, Hants. UK), and then stomached (Colworth 400 stomacher; A.J. Seward, London, UK) for 60 s. One millilitre of undiluted stomached solution was then spread on the surface of pre-poured plate count agar (PO0158A; Oxoid) using the spread plate technique or spiral plater. Further serial dilutions were diluted using maximum recovery diluent (Oxoid, CM0733T). The plates were incubated at 30 ± 1 °C for 48 ± 4 h and colonies were then counted.

When testing for *Campylobacter*, an aliquot of 1 ml from the same initial dilution was spread between 3 plates, then 0.1 ml from the subsequent serial dilutions were inoculated onto pre-poured modified Cefoperazone - Charcoal - Desoxycholate Agar (mCCDA: Biomerieux 33627 plates), and the inoculum was spread over the surface of the agar using the spread plate technique and allowed to soak into the agar before the plates were incubated microaerophilically (Campygen 3.5 L Oxoid CN0035A, Oxoid Thermofisher Ltd) at 41.5 °C ± 1 °C for 40–48 h. Following incubation, the number of colonies present in each Petri dish was counted. For confirmation, five colonies were taken covering the range of morphologies which were considered to be typical or suspect. If there were fewer than five typical or suspect colonies of each morphology type on each plate, all the typical or suspect colonies were taken for confirmation. The selected colonies were confirmed by preliminary confirmation using visual appearance of colonies and oxidase test, then Microgen Latex *Campylobacter* kit.

Testing was carried out on skin samples excised from the breast, breast and neck, thigh and neck, or back and neck. The type of sample used in a specific trial is noted later in the tables of results. For aerobic plate counts and *Campylobacter* spp enumeration, the measures of uncertainty were $\log_{10}0.03$ (3.25%) and $\log_{10}0.09$ (8.82%), respectively.

2.3. Trial 1 (immersion)

Forty carcasses were removed from the process line just before the chiller and then weighed. A breast skin was carefully removed from one side and the skin placed in a sterile bag and put into a cool box with covered ice packs. The other breast skin sample was removed and carefully dropped into 200 ml of liquid nitrogen in an insulated polypropylene container. The immersion time was either 1 or 8s. The nitrogen and skin samples were tipped into a sieve funnel. The total treatment times, including time in the sieve, for the 1 and 8s treatments were between 2 and 3s and 10s. The breast skin was removed with sterile tweezers and put in to a sterile bag and then into the cool box. For every tenth bird, the temperature of the skin was measured before and after immersion in the nitrogen using a digital thermometer (Model SuperFast Thermapen, Electronic Temperature Instruments Ltd, Worthing, BN14 8HQ, UK). These procedures were repeated for 20 carcasses using the 2–3s treatment and 20 carcases using the 10s treatment, alternating between left and right hand side breast as to which was the untreated control or treated breast. The samples were transported by refrigerated van (4 °C) to the microbiology laboratory where testing for APC and *Campylobacter* was carried out the next day.

2.4. Trial 2 (immersion)

This trial was similar to Trial 1 but carried out over 2 days. On the first day, 20 carcasses were removed from the production line immediately after evisceration (before the inside-outside washer) and 20 carcasses were removed from the same batch of carcasses pre-chill (as in Trial 1). Breast skin samples were removed and immersed for 20s in liquid nitrogen and matching skins from each bird were held as untreated controls. Samples were placed in cool boxes, transported to the microbiology laboratory, and tested as in