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Use of chemical treatments applied alone and in combination to reduce *Campylobacter* on raw poultry

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ABSTRACT

Research focussing on the use of chemicals to decontaminate poultry carcasses to reduce pathogenic and spoilage organisms has increased in recent years. The objective of this study was to evaluate the efficacy of 12% (w/v) trisodium phosphate (TSP), 2% (w/v) citric acid (CA) and 5% (w/v) capric acid sodium salt (CP) in reducing *Campylobacter*, total viable counts and total *Enterobacteriaceae* counts on poultry. These chemicals were also used in various combinations (TSP + CA, TSP + CP and CA + CP) to determine if sequential treatments would enhance microbial reductions. TSP (1.9–2.3 $\log_{10}$ cfu cm^2) and CP (2.2 –2.4 $\log_{10}$ cfu cm^2) gave the largest *Campylobacter jejuni* reductions while TSP was the most effective at reducing TVC (0.9 $\log_{10}$ cfu cm^2) and TEC (0.9 $\log_{10}$ cfu cm^2). TSP + CP was the most effective combination treatment (2.9 $\log_{10}$ cfu cm^2) for reducing *C. jejuni* counts and was significantly ($P < 0.05$) greater than any of the single chemical treatments, with the exception of CP treatment against strain 1146 (2.4 $\log_{10}$ cfu cm^2). The TVC and TEC populations proved more resistant to combination treatments as only TSP + CP showed a significantly ($p < 0.05$) enhanced reductive efficacy in comparison to single CP treatment. This study provides further data on the efficacy of a number of potential chemicals used alone and in combination for the decontamination of raw poultry.

1. Introduction

Campylobacteriosis, primarily caused by the foodborne pathogen *Campylobacter jejuni*, has been recognised as a leading cause of bacterial gastroenteritis in the world (FAO/WHO, 2009). While the main symptoms of disease include watery or bloody diarrhoea with fever and cramps, in rare circumstances serious complications may arise such as Guillian-Barré syndrome (Rees, Soudain, Gregson, & Hughes, 1995), an autoimmune disease characterised by weakness and possible paralysis of the limbs. Poultry has been identified as the main source of *Campylobacter* transmission to humans (Humphrey, O'Brien, & Madsen, 2007) with an estimated 20–30% of infections resulting from contaminated broiler meat and 50–80% resulting from the entire chicken reservoir (EFSA 2011). Ensuring that raw poultry consistently has either low levels of contamination or no contamination remains challenging. The highest concentrations of *Campylobacter* are found in the caecum yet it can also be isolated from carcasses as a result of cross-contamination during the slaughter process (Berrang, Smith, Windham, & Feldner, 2004; Whyte et al., 2004). A recent European Food Safety Authority (EFSA) baseline survey concluded that approximately 75.8% of poultry carcasses within the EU were contaminated with *Campylobacter* (EFSA 2010a; EFSA 2010b). This high contamination rate, along with other risk factors such as poor handling and hygiene practices in domestic and catering kitchens, has resulted in a year-on-year increase of campylobacteriosis in many countries within the EU (EFSA, 2012). It is widely accepted that controlling the levels of contamination on the surface of the carcass could significantly reduce the number of human infections. Rosenquist, Nielsen, Sommer, Norrung, and Christensen (2003) estimated that a reduction of 2-log of *Campylobacter* on poultry carcasses would result in a 30-fold reduction of human cases each year. It has been shown that the treatment of broiler carcasses with various chemicals can reduce the levels of campylobacters and may also affect spoilage organisms and increase product shelf life (Bolton, Meredith, Walsh, & McDowell, 2014; Meredith, Walsh, McDowell, & Bolton, 2013). Risk assessment models evaluating the effectiveness of treating carcasses with decontaminants based on organic acids and phosphates suggest this could reduce the risk to consumers of infection with *Campylobacter* by 90% (Havelaar et al., 2007).

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The Food and Drug Administration (FDA) has approved the use of a number of chemicals as decontaminants for poultry in the United States. Trisodium phosphate, in solutions of 8–12%, or a number of organic acids used at concentrations of up to 2.5%, applied as either sprays or dips, are classified as GRAS (Generally Recognised As Safe) for use in poultry processing plants (Capita, Alonso-Calleja, García-Fernández, & Moreno, 2002; Del Río, Panizo-Morán, Prieto, Alonso-Calleja, & Capita, 2007). Chlorinated ice water is also used in many processing plants in the United States for carcass chilling (Jacob-Reitsma, 2000). In the EU, the use of potable water is the only application that is currently permitted for use as a poultry decontaminant (EFSA 2011). The reason that the European Commission has not approved decontamination treatments is due to the concern that such treatments may mask unhygienic slaughtering practices or may lead to antimicrobial resistance in bacteria. However, EC Regulation 853/2004 allows decontamination treatments to be used as a supplement to good hygiene practices (European Commission, 2004). Risk managers in processing plants must demonstrate that a substance is both safe and effective at reducing the microbial load before it can be authorised. The EFSA BIOHAZ panel published an opinion on four chemicals (chlorine dioxide, acidified sodium chlorite, trisodium phosphate and peroxyacids) which could be used as decontaminants, and concluded that each of the chemicals posed no safety concern (EFSA, 2005; EFSA 2014) and that there was no indication that antimicrobial resistance would develop (EFSA 2008; EFSA 2014) although further studies were recommended. Despite these reports, and with the exception of EC Regulation 101/2013 (European Commission, 2013) which allows the application of lactic acid to be used on bovine carcasses, there are currently no chemicals authorised for use as carcass decontaminants in the EU. However their approval and use in broiler processing remains under review within the European Commission.

The objective of this study was to evaluate the effectiveness of 12% (w/v) trisodium phosphate (TSP), 2% (w/v) citric acid (CA) and 5% (w/v) capric acid sodium salt (CP) at reducing *Campylobacter* contamination on artificially inoculated drumsticks. In addition, total viable counts (TVC) and total *Enterobacteriaceae* counts (TEC) were also monitored to assess if treatments could reduce the overall bacterial load present on carcasses. Finally, the application of combinations of chemical treatments was also evaluated to determine if enhanced reductions in microbial levels could be achieved.

2. Materials and methods

2.1. Bacterial cultures and preparation of inocula

Two strains of *C. jejuni* were used for this study (*C. jejuni* NCTC 11168 and *C. jejuni* 1146). *C. jejuni* NCTC 11168 is a well characterised human clinical isolate while *C. jejuni* 1146 is a poultry strain isolated from retail chicken (Whyte et al., 2004). The strains were maintained in defibrinated horse blood (Oxoid Ltd., Basingstoke, Hampshire, England, SR0050) and stored at −80 °C. Inocula were prepared as described by Haughton, Lyng, Cronin, Fanning, and Whyte (2012). Briefly, a loopful of culture was inoculated into 20 ml Mueller-Hinton Broth (MHB; Oxoid Ltd., England, CM0405) with *Campylobacter* growth supplement (Oxoid Ltd., England, SR0232E) and incubated under microaerobic conditions for 48 h at 42 °C. Enriched cultures were streaked onto Columbia Blood Agar (CBA; Oxoid Ltd., England, CM0331) and incubated for a further 48 h under microaerobic conditions at 42 °C. Following growth, 20 ml aliquots of MHB containing *Campylobacter* growth supplement were inoculated with a single colony of *C. jejuni* and incubated for 24 h at 42 °C under microaerobic conditions. Aliquots were then combined to 200 ml volumes to give a final cell suspension of approximately 7 $\log_{10}$ cfu ml.

2.2. Inoculation of drumsticks

A total of 48 chicken drumsticks were collected from an Irish poultry processing plant immediately after carcass dressing and chilling and delivered to the laboratory at ≤4 °C within 24 h. Drumsticks were dipped in 200 ml of the bacterial suspension containing 7 $\log_{10}$ cfu ml for 30 s before removal from the solution and storage at room temperature for 30 min to allow for drainage and bacterial cell attachment.

2.3. Chemical treatment

Trisodium phosphate (TSP, Sigma–Aldrich, St. Louis, MO, USA, 222003, 12% w/v, pH 12.8), citric acid (CA, Sigma–Aldrich, USA, C0759, 2% w/v, pH 2.4) and capric acid sodium salt (CP, Sigma–Aldrich, USA, C4151, 5% w/v, pH 9.5) were prepared in 500 ml sterile distilled water. All chemicals were stored at room temperature for no more than 24 h before use. Twenty seven drumsticks were inoculated with *C. jejuni* NCTC 11168, divided into 9 groups of 3 drumsticks and labelled group 1 to group 9. Group 1 was used as the uninoculated control; group 2 as the inoculated control; group 3 drumsticks were treated with sterile distilled water; group 4 with TSP (12%, w/v); group 5 with CA (2%, w/v); group 6 with CP (5%, w/v); group 7 with the combination of TSP followed by CA; group 8 with the combination of TSP followed by CP and group 9 with the combination of CA followed by CP. All chemical treatments involved dipping in the chemical solution for 1 min at ambient temperature. After each treatment and between treatments (applies to combination treatments only, groups 7–9) the drumsticks were rinsed in sterile distilled water by immersion for 15 s. This experiment was repeated on 3 separate occasions. The entire experiment was then repeated with *C. jejuni* strain 1146.

2.4. Microbiological analysis

Following chemical treatment, the drumsticks were stomached for 30 s in 90 ml Maximum Recovery Diluent (Oxoid Ltd, England, CM0733) (MRD). The stomached rinsates were then diluted 1:10 in MRD before 50 μl volumes were spread plated in duplicate onto mCCDA (Oxoid Ltd, England, CM0739) for *Campylobacter* (37 °C, 48 h), standard plate count agar (Oxoid Ltd, England, CM0463) (SPCA) for total viable counts (30 °C, 72 h) and violet red bile glucose agar (Oxoid Ltd, England, CM0485) (VRBGA) for total *Enterobacteriaceae* counts (37 °C, 24 h) (Haughton et al., 2012).

2.5. Statistical analysis

All experiments were repeated on three separate occasions and all microbiological analysis was performed in duplicate. Microbial counts were converted to $\log_{10}$ cfu cm^2. The surface area of 25 drumsticks was measured with graph paper and an average value was obtained. Mean bacterial levels were then analysed by 1-way ANOVA followed by Tukey's Multiple Comparison test to compare the mean of each treatment group. Significance was determined at the $p < 0.05$ level. Data was analysed using the GraphPad Prism 5 software (GraphPad Software, San Diego, California, USA, www.graphpad.com).

3. Results

3.1. Single chemical treatment (Campylobacter)

This study tested the effect of chemical decontamination treatment using two strains of *C. jejuni*. The two *Campylobacter* strains were inoculated onto the drumsticks during separate trials. Table 1