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Evaluation of the efficacy of multiple physical, biological and natural antimicrobial interventions for control of pathogenic *Escherichia coli* on beef

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ABSTRACT

Antimicrobial effects of multiple physical, biological and natural interventions on pathogenic *Escherichia coli* in raw beef were assessed. A cocktail of *E. coli* strains was inoculated onto gamma-irradiated beef and enumerated immediately after each intervention and during storage at 4 °C for 7 days. Of the physical interventions, silver-containing antimicrobial packaging and ozone gas treatment did not show significant antimicrobial effects, however cold plasma treatment reduced *E. coli* levels by 0.9 and 1.82 $\log_{10}$ CFU/cm^2 after 2 and 5 min treatments, respectively. A phage cocktail reduced *E. coli* counts by 0.63 and 1.16 $\log_{10}$ CFU/g after 24 h storage at 4 and 12 °C, respectively. Of the natural interventions, vinegar and lactic acid (5%) washes for 5 min caused reductions of ~1 $\log_{10}$ CFU/g immediately after treatment, whereas lactoferrin and nisin treatments, separately or in combination, had insignificant antimicrobial effects. Nanoemulsions containing carvacrol or thyme essential oils caused immediate *E. coli* reductions of 1.41 and 1.36 $\log_{10}$ CFU/g, respectively, plus a progressive reduction in viable numbers during storage at 4 °C. Our findings suggest that cold plasma, bacteriophages, vinegar, lactic acid, or carvacrol and thyme essential oil nanoemulsions could potentially be of use to the beef industry for controlling pathogenic *E. coli* contamination.

1. Introduction

Foodborne illness is a major concern for industry, public authorities and consumers, with the global impact reaching 600 million cases and 420,000 deaths on an annual basis (World Health Organisation, 2015). Over the past few decades, the food producing sector has been experiencing an increase in the demand for meat products. Nevertheless, the meat sector has also been found to be the least trusted by consumers, probably due to the increase in the occurrence of foodborne outbreaks associated with meat (European Commission, 2010; Misra and Jo, 2017). *Escherichia coli* O157 is considered a worldwide health threat and is the serogroup of *E. coli* most commonly associated with illnesses and deaths in humans with clinical manifestations ranging from abdominal pain and diarrhoea to potentially fatal haemolytic-uraemic syndrome (Food Standards Agency, 2014). Although many food products have been implicated in foodborne outbreaks, foods of bovine origin are the most frequently reported as vehicles for human *E. coli* O157 infection (European Food Safety Authority, 2011). Initial *E. coli* O157 contamination of beef products occurs mainly at the de-hiding stage of slaughtering because of bacterial transfer and adherence to the carcasses (Chagnot et al., 2013). Hazard analysis and critical control point systems have been introduced in many countries aiming to reduce or eradicate these pathogens, but even with these systems in place, the absence of *E. coli* O157 from meat cannot be guaranteed and there are still outbreaks of this pathogen that can be traced back to beef and beef products. Due to the potential meat safety concerns, researchers and the industry are continuously investigating different strategies to tackle this issue. The use of antimicrobial interventions on animal tissues with the use of hot water washing and steam pasteurization, organic acids, chlorine dioxide trisodium phosphate and cetylpyridinium chloride has been extensively studied (Mohan and Pohlman, 2016). However, the frequent foodborne disease outbreaks associated with ground beef necessitates further research. Organic acids have been approved for meat decontamination in the United States (USDA Food Safety and Inspection Service, 1996) and in 2013 lactic acid was approved for decontamination of beef carcasses by the European Commission (2013). Thermal treatments have been found to be effective in inactivating pathogenic *E. coli* and other pathogens; however they can also result in unwanted physical and chemical changes. Non-thermal processing technologies have also been investigated as substitutes for thermal processes to reduce microbial contamination while increasing quality and nutrient retention (Wheeler et al., 2014). Food irradiation,

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specifically electron-beam irradiation, has been found to significantly reduce *E. coli* O157 on beef, without negative effects on the sensory characteristics of the meat (Arthur et al., 2005). However, negative consumer opinion regarding food irradiation hinders its widespread adoption. Ultraviolet radiation and ozone treatments are also of interest to the meat industry since they do not result in chemical residues or damage (Khadre et al., 2001). High pressure processing (HPP) is another non-thermal technology with high antimicrobial efficacy which has been gaining increasing importance and has been used under commercial conditions in many countries (Patterson, 2005; Hsu et al., 2015). HPP in a range 400–600 MPa has been shown to be effective in controlling most major foodborne pathogenic bacteria (e.g. *E. coli* O157:H7, *Salmonella* spp.) present in meat products such as beef and ground chicken, but is can also cause detrimental changes in meat quality (Chien et al., 2016). Among the non-thermal technologies, the application of cold plasma to improve the microbiological safety and quality of meat and meat products is very new. A few recent studies have demonstrated the potential of cold plasma technology as a novel intervention for ensuring the safety of ready-to-eat beef jerky, chicken and pork (Dirks et al., 2012; Kim et al., 2013, 2014). Essential oils have also been gaining importance as food preservatives, since many studies have found that they possess significant antimicrobial properties against a broad range of foodborne pathogens (Zhang et al., 2016). The antimicrobial efficiency of the essentials oils has been attributed to the high content of phenolic compounds they possess, such as carvacrol, eugenol and thymol, which can also be extracted, isolated and used as food antimicrobials (Burt, 2004). Furthermore, many studies have shown that the concept of combined decontamination treatments (hurdle approach) could be a more efficient strategy for reducing or eliminating pathogens than the application of single interventions (Sofos, 2005).

The aim of this study was to assess and compare the antimicrobial effects of different non-thermal physical (antimicrobial packaging, cold plasma, and ozone), biological (bacteriophages) and natural (vinegar, lactic acid, encapsulated essential oils, lactoferrin and nisin) interventions, as well as combinations of some treatments, against pathogenic *E. coli* present on beef cuts. The effect on *E. coli* was determined immediately after application of each intervention and throughout a 7-day storage period at refrigeration (4 °C), and in some cases mild abuse (12 °C) temperatures, after vacuum packaging.

2. Materials and methods

2.1. Bacterial strains used and inoculum preparation

A cocktail of four *E. coli* strains was used for inoculation of beef samples. This cocktail consisted of three *E. coli* strains, ATCC BAA 1427, ATCC BAA 1428 and ATCC BAA 1429, designated by the USDA Food Safety and Inspection Service (2015) as surrogate indicator organisms *for E. coli* O157, and a fourth *E. coli* strain, NCTC 12900, which is a shigatoxin negative serotype O157:H7 strain. For each strain a loopful of a fresh Tryptone soya agar plus 0.6% yeast extract (TSAYE, both Oxoid Limited, Basingstoke, UK) slope culture was inoculated into 10 ml of Brain heart infusion broth (BHI, Oxoid) and incubated at 37 °C for 24 h. Subsequently, 100 μl of a 10^{-4} dilution of this broth culture in maximum recovery diluent (Oxoid), was inoculated into another 10 ml BHI broth and incubated at 37 °C for 24 h, until the stationary phase of growth was reached. The final 10 ml cultures were harvested by centrifuging at 3600 × *g* for 30 min, washed twice in phosphate-buffered saline (PBS), and the pellet re-suspended in a final volume of 10 ml PBS to give approximately 10^8–10^9 CFU/ml. To produce the *E. coli* cocktail, equal volumes of suspensions of the four separate strains were combined and mixed well.

2.2. Preparation and inoculation of beef samples

Bulk packs (10 kg) of beef cuts were obtained from a local producer. Before use in challenge studies, rectangular beef cuts (approx. 5 cm × 5 cm) were aseptically weighed as 25 g ± 0.2 g samples into stomacher bags and sterilized by gamma radiation (15 kGy dose) at a nearby ^{60}Co Gamma beam 650 facility, in order to inactivate any naturally occurring *E. coli* cells. Irradiated beef samples were kept frozen at −20 °C until required for experiments.

The *E. coli* cocktail was spot inoculated (250 μl) onto the surface of irradiated beef samples, to simulate surface contamination with faeces (McCann et al., 2006). The final inoculum level was approximately 5 $\log_{10}$ CFU/g or CFU/cm^2. Inoculated, unpackaged beef samples were stored at 4 °C for 1 h before application of the antimicrobial intervention (Poimenidou et al., 2016). After application of each intervention, beef samples were vacuum packed, using a FoodSaver® vacuum sealing system and associated bags (Sunbeam Products, Inc., Boca Raton, USA), before storage at 4 °C (and 12 °C in certain cases only) for 7 days; samples were tested for viable *E. coli* at day 0, 1, 3, 5 and 7. Samples were stored at 4 °C as this is the storage temperature used by the beef industry.

2.3. Antimicrobial interventions

The antimicrobial effects of a range of different physical, biological and natural antimicrobial interventions were studied. The specific concentrations or processing conditions employed for each intervention were selected on the basis of relevant previously published studies and/or preliminary trials.

2.3.1. Physical interventions

To investigate the effect of antimicrobial packaging, a commercial antimicrobial polyethylene terephthalate film incorporating silver nanoparticles (kindly provided by LINPAC Packaging, Featherstone, UK) was used to wrap the meat before vacuum packing using a conventional film. Control samples were vacuum packed using a conventional food grade film.

To investigate the effect of cold plasma treatment, a dielectric barrier discharge cold plasma jet, as described by Alkawareek et al. (2012), was used. Briefly, the plasma source consisted of a quartz dielectric tube with an inner diameter of 4 mm and an outer diameter of 6 mm and it operated at voltage amplitude of 6 kV and a repetition frequency of 20 kHz. The plasma jet configuration was encased in solid acrylic tubing. The plasma jet was produced using a mixture of helium (99.5%) and oxygen (0.5%) at flow rate of 2 standard litres per min. The temperature of the produced plume was 39 °C. Beef samples were placed on a Petri dish at a distance of 15 mm from the plasma source during treatment. Based on preliminary trials (results not shown), 2 and 5 min exposure times were used as they showed promising antimicrobial activity without affecting the organoleptic properties of the beef. An untreated control (no exposure to plasma) was also tested. After treatment, beef samples were vacuum packed and stored under refrigeration (4 °C).

Ozone was applied to beef samples as a gaseous treatment in a hermetically closed transparent cylinder. Ozone was generated using an ozone generator (ESCO, Labozone model, UK). Two ozone concentrations were tested (7.2 and 32 g O_3/m^3) with an exposure time of 5 min in both cases. Ozone concentration was recorded using an ozone gas analyzer (GM-6000-OEM Ozomat, Germany). An untreated control was also tested.

2.3.2. Biological intervention

A commercially available bacteriophage cocktail (EcoShield™, Intralytix, USA) against *E. coli* O157 was purchased for this study. The bacteriophage cocktail contained three lytic phages (ECML-4, ECML-117, and ECML-134) belonging to the family *Myoviridae*. Phage cocktail