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Evaluation of chemical immersion treatments to reduce microbial populations in fresh beef

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ABSTRACT

The aim of the current study was to assess the ability of a number of chemicals (acetic Acid (AA), citric acid (CA) lactic acid (LA), sodium decanoate (SD) and trisodium phosphate (TSP)) to reduce microbial populations (total viable count, *Campylobacter jejuni*, *Escherichia coli*, *Salmonella typhimurium* and *Listeria monocytogenes*) on raw beef using an immersion system. The following concentrations of each chemical were used: 3 & 5% for AA, CA, LA, SD and 10 & 12% for TSP. Possible synergistic effects of using combinations of two chemicals sequentially (LA + CA and LA + AA) were also investigated. L*, a* and b* values were measured before and after treatments and ΔE* values were calculated in order to determine any changes in the color of meat due to the use of these chemicals. In general, all chemical treatments resulted in significantly ($p < 0.05$) reduced bacterial counts when compared to untreated controls. The greatest reductions were obtained by using LA3%, SD5%, AA5%, LA5% and SD3% for TVC, *C. jejuni, E. coli, S. typhimurium* and *L. monocytogenes*, respectively. However, no significant difference in microbial load was observed between the different concentrations of each chemical used ($p > 0.05$). The application of combinations of chemical immersion treatments (LA3% + AA3% and LA3% + CA3%) did not result in further significant reductions in microbial populations when compared to single chemical treatments ($P < 0.05$). Assessment of color changes in meat following the application of chemical immersion treatments indicated that using AA or CA at either concentration and LA at 5% led to an increase in the ΔE* value of > 3 immediately after treatment and after 24 h storage. The remaining treatments did not result in significant changes to the color of raw beef.

1. Introduction

Foodborne disease is a global health issue causing significant morbidity and mortality. It has been estimated that, globally, 1 in 10 people fall ill every year from eating contaminated food and 420,000 die as a result, with children comprising a substantial proportion of this estimate (WHO, 2015). The European Food Safety Authority (EFSA) reported campylobacteriosis, salmonellosis, listeriosis and *E. coli* (VTEC) infection as the main bacterial foodborne diseases for humans in 2015, with the number of cases at 229,213, 94,625, 2206 and 5901 respectively (EFSA, 2016). These pathogens (*Campylobacter*, *Salmonella*, *E. coli* and *Listeria*) are frequently associated from meat and meat products (Kramarenko et al., 2016; Tafida et al., 2013; Whyte et al., 2004; Yang et al., 2016). Due to potential food safety concerns associated with meat products, the food industry has continued to assess potential risk mitigation strategies to reduce pathogen populations on raw meat. The application of organic acids has been investigated as a possible technology to reduce bacterial levels in many foods especially meat and meat products (Lucera et al., 2012). EFSA has stated that lactic acid treatments can result in significant reductions in microbial counts when used to treat beef carcasses (EFSA, 2011). The mechanism of action of organic acids is dependent on the ability of undissociated acid to permeate through the cell membrane and dissociate inside the bacteria causing a decrease in internal pH, which may interrupt ATP and RNA synthesis, DNA replication and cell growth (Rajkovic et al., 2010).

Organic acids have been approved for use in the area of meat decontamination in the United States (USDA-FSIS, 1996) and are now routinely used in many countries to reduce bacterial contamination

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(Theron and Lues, 2010). However, to date, European authorities have preferred the application of strict hygiene measures during processing as the primary risk management approach. More recently the use of lactic acid for the decontamination of beef carcasses has been approved by the European Commission (2013). In addition to organic acids, many other chemicals have been assessed for meat decontamination such as trisodium phosphate (TSP) (Dickson et al., 1994). Trisodium phosphate has been used in the United States for decontamination of chicken carcasses using concentrations of 10–12%; this chemical also has generally recognized as safe (GRAS) status and does not require labeling (Lianou and Koustsoumanis, 2012). Many studies have investigated the effect of organic acids at concentrations of between 1 and 5% and TSP between 8 and 12% to decontaminate beef carcasses or beef cuts using spray methods (Barboza de Martinez et al., 2002; Cutter and Siragusa, 1994; Gill and Badoni, 2004; Gorman et al., 1995). However, few studies have investigated these chemicals on beef cuts using immersion treatments while also assessing their impact on organoleptic properties. These chemical treatments could be used to dip whole carcasses or, for example, on beef trimmings either before their use as a raw material for ground beef or prior to packing as cuts for direct consumption. Beef trimmings are frequently contaminated with pathogenic bacteria due to mixing of meat from different animals (Pohlman et al., 2002b). Furthermore, levels of contamination in beef trimmings can directly affect the bacterial quality of ground beef (Dorsa et al., 1998). Treatment with chemical or physical interventions may result in the survival of a population of bacteria some of which may be sub-lethally injured (Wesceie et al., 2009). However, injured cells may repair and remain viable if allowed maintained in non-stressful conditions (Jasson et al., 2007). Therefore, it is important to consider the presence of sub-lethally injured cells when estimating the effectiveness of bacterial deactivation methods in order to prevent the generation of inaccurate results (Wu, 2008).

Avoiding substantive changes in the color of raw beef is also an important consideration when assessing the suitability of individual chemical compounds as potential microbial decontaminants (Hunt et al., 2012). This is a key sensory property used by consumers to decide whether they should accept or reject meat products (Mancini and Hunt, 2005). Certain organic acids may cause a permanent discoloration (dull gray color) when applied to raw meat (Wenham and Locker, 1976).

A review by EFSA of a number of studies on the use of organic acids for the decontamination of beef concluded that concentration may influence the efficacy of bacterial reduction (EFSA, 2011). Furthermore, to the author's knowledge few studies have determined the effect of a water rinsing step treatment following treatment of beef (EFSA, 2011).

This study was carried out to:

(i) compare the effect of different concentrations of various chemical immersion treatments and water immersion on microbial populations in fresh beef (ii) assess the efficacy of using combinations of two of these chemicals on microbial reductions
(ii) investigate any potential undesirable color changes in meat due to these chemical treatments
(iii) estimate the level of sub-lethally injured cells following treatments.

2. Materials and methods

2.1. Preparation of bacterial suspensions and inoculation of samples

Salmonella typhimurium (DT104), *Campylobacter jejuni* (1146 chicken isolate), *Listeria monocytogenes* (NCTC11994) and *Escherichia coli* (ATCC25922) were used in the study. Suspensions of *C. jejuni* were prepared by inoculating 20 ml aliquots of Mueller-Hinton Broth (MHB) (Oxoid, UK, CM0405) containing *Campylobacter* growth supplement with a single colony of the isolate and incubated for 24 h at 42 °C under microaerobic conditions. A total of ten of the 20 ml aliquots were then combined to make up 200 ml volumes, and diluted with 300 ml of maximum recovery diluent MRD, (OxoidCM0733) to give a 500 ml volume containing a cell concentration of approximately 7 $\log_{10}$ cfu/ml. Individual colonies of *S. Typhimurium*, *E. coli* and *L. monocytogenes* were inoculated in 10 tubes each containing 20 ml of MHB and were then incubated for 24 h at 37 °C. Liquid from the 10 tubes were then pooled to give a 200 ml volume and made up to a final volume of 500 ml by adding 300 ml of sterile MRD. This corresponded to final cell concentrations of 8–9 $\log_{10}$ cfu/ml. Fresh beef was purchased from retail outlets and cut into 10 g pieces. Three samples were used for each treatment and dipped for 60 s in the 500 ml volumes of each bacterial suspension and left for 30 min prior to applying the various treatments to allow for attachment.

2.2. Chemical treatments

Each experiment was repeated in triplicate on three separate occasions. All samples ($n = 3$) were dipped in appropriate 500 ml chemical solutions for 60 s (stirring for 10 s) at room temperature. Samples were treated with either 3% or 5% of acetic acid (AA) (Sigma-Aldrich, USA, 320099), citric acid (CA) (Sigma-Aldrich, USA, C0759), lactic acid (LA) (Sigma-Aldrich, USA, W261114), sodium decanoate (SD) (Sigma-Aldrich, USA, C4151) and 10% or 12% of trisodium phosphate (TSP) (Sigma-Aldrich, USA, 222003) respectively. Following treatment, samples were immersed in 500 ml distilled water for 15 s to rinse off any residual chemical. Washed control (WC) samples were treated similarly, but in distilled water only prior to microbiological analysis. Untreated control samples (UC) were microbiologically analyzed directly without any treatment to determine the background microflora. For combined chemical treatments (LA + CA and LA + AA), samples were immersed sequentially in the first chemical solution and rinsed in water before immersion in the second solution to limit any potential chemical interaction. Samples were immersed for 60 s in each of the chemical solutions.

2.3. Microbiological analysis

Samples were stomached (Colworth Stomacher 400 series, UK) for 30 s in 90 ml MRD, and serially diluted (1:9) in MRD before being plated in duplicate onto modified Charcoal Cefoperazone Deoxycholate (mCCDA) (Oxoid, UK, CM0739) containing a selective supplement (Oxoid, UK, SR0155E) and incubated microaerobically at 42 °C for 48 h for *Campylobacter* enumeration. Samples were also plated in duplicate for total viable counts on plate count agar (PCA) (Oxoid, UK, CM0325) and incubated at 30 °C for 48 h), Violet Red Bile Agar (VRBA) + MUG (Oxoid, UK, CM0978) for *E. coli*, Xylose Lysine Desoxycholate Agar (X.L.D.) (Oxoid, UK, CM0469) for *S. typhimurium* and *Listeria* selective agar base (Oxford formulation), (Oxoid, UK, CM0856) with *Listeria* selective supplement (Oxford formulation) (Oxoid, UK, SR0140E) for *L. monocytogenes. E. coli, Salmonella* and *Listeria* plates were incubated at 37 °C for 24 h.

2.4. Meat color analysis

Three fresh meat samples were dipped in each chemical as previously described. Color measurements were then taken for each sample from three different locations directly before and after chemical treatment as well as following storage for 24 h at 4 °C. Color measurement was carried out using a Konica Minolta device (model CR-400) according to the CIELAB international system of color measurement. The device was calibrated with a white ceramic tile, in accordance with the manufacturer's instructions. The device reads three color parameters (L* (+ = lighter, − = darker), a* (+ = redder, − = greener) and b* (+ = yellower, − = bluer)). Overall differences in color (ΔE*) were calculated using these three parameters in the following formula: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]1/2$ (Tobergte and Curtis, 2013).

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