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The effect of chemical treatments in laboratory and broiler plant studies on the microbial status and shelf-life of poultry

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ABSTRACT

Poultry products are highly perishable with a short shelf-life of approximately 4–5 days. Chemical treatments have the potential to inhibit microbial spoilage and extend shelf-life. Preliminary tests were carried out on broiler skin samples in the laboratory and the most promising treatments tested on carcasses in the broiler processing plant. In the laboratory, the immediate and storage (3 days at 4 °C) effect of trisodium phosphate (TSP, 10 & 14%, w/v), lactic acid (LA, 1 & 5% v/v), citric acid (CA, 1 & 5%, w/v), peroxyacids (POA, 100 & 200 ppm) and acidified sodium chlorite (ASC, 500 & 1,200 ppm) on TVC (mesophiles and psychrotrophs), *Enterobacteriaceae*, *Pseudomonas*, lactic acid bacteria and yeasts/moulds were investigated. In general, TSP and CA were the most effective immediate treatments and in the broiler processing plant, the effect of dipping and spraying carcasses with these chemicals on microbial shelf-life was examined over 15 days at 4 °C. Although a significant ($P < 0.05$) immediate reduction in TVC (mesophiles and psychrotrophs) was observed with TSP in the laboratory experiment, there was no further inhibition and after 3 days the treated and control samples had similar counts. The organic acids also caused a significant ($P < 0.05$) immediate reduction in TVC (mesophiles and psychrotrophs) but, in contrast to TSP, a mild inhibition of subsequent growth was also observed. In the processing plant, a microbial shelf-life of approximately 4 days was obtained at 4 °C on the control (water-treated) samples which was extended by 1–2 days after treatment with TSP (14%, dip) and by up to 4 days with CA (5%, dip). It was concluded that the microbial quality and microbial shelf-life of poultry may be considerably enhanced using selective chemical treatments.

1. Introduction

Approximately one third of global meat consumption is poultry (FAO, 2012). However, poultry meat spoils after 4–5 days under refrigerated conditions (Morshedy & Sallam, 2009), limiting trade in fresh product and causing considerable financial loss to the poultry industry (Jimenez et al., 1997; Patsias, Chouliara, Paleologos, Savvaidis, & Kontominas, 2006). Shelf-life is the period of time a product may be stored without becoming unfit for human consumption. The sensory shelf life is define by organoleptic parameters and the product may be considered as spoilt when discolouration, off-odours and/or slime develop (Nychas, Skandamis, Tassou, & Koutsoumanis, 2008). These organoleptic changes are caused by psychrotropic microorganisms, especially *Pseudomonas* spp. which produce slime and off-odours when levels reach 10^{7-8} cfu per gram (Charles, Williams, & Rodrick, 2006; Nychas et al., 2008; Russell, Cox, & Bailey, 1997). Thus the microbial shelf-life of poultry may be defined by the TVC and the product is generally spoilt when bacterial counts reach 10^{7-8} cfu per gram. The time to spoilage, and therefore shelf-life, depends on the initial carcasses counts. This, in turn, is influenced by cross-contamination during immersion scalding, de-feathering and evisceration as well as from equipment and the general processing environment (Meredith, Walsh, McDowell, & Bolton, 2013; Morshedy & Sallam, 2009; Sampers et al., 2008). Psychrotrophic (cold tolerant) TVC are used as an indicator of shelf-life for poultry (Nychas et al., 2008) while mesophilic (organisms that grow between 20 and 45 °C) TVC, *Enterobacteriaceae*, *Pseudomonas* spp., lactic acid bacteria and yeast/moulds are used in the poultry industry as indicators of processing hygiene and microbiological quality (Alonso-Calleja, Martínez-Fernández, Prieto, & Capita, 2004; Álvarez-Astorga, Capita, Alonso-Calleja, Moreno, & García-Fernández, 2002).

Minimising microbial contamination on meat, including poultry, is dependent on the strict application of good farming

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practices (GFP) and hygienic processing. The latter is documented in the prerequisite (GMP/GHP) programme and hazard analysis and critical control point (HACCP) plans. HACCP includes critical control points (CCP), where an intervention may be used to prevent, reduce or eliminate microbial contamination. Chemical treatments have the potential to reduce microbial counts and may provide the basis for an effective intervention CCP. They may also inhibit subsequent microbial growth thereby extending shelf-life. Although widely used in the USA, the use of substances, other than potable water, for microbial decontamination of poultry products is banned in the European Union, despite the legal framework being in place since 2004 (Loretz, Stephan, & Zweifel, 2010). This situation may change in the near future. The recent removal of the ban on the use of lactic acid as a pathogen reduction treatment on beef carcasses (Commission Regulation (EC) No 101/2013) coupled with an assessment by the European Food Safety Authority (EFSA) that broiler TSP treatment poses no toxicological risk to human health (EFSA, 2005) has renewed interest in chemical treatments to improve the microbial quality of poultry meat.

While different chemical treatments, primarily based on organic acids, chlorine and phosphates have been investigated as potential decontaminants on poultry (Loretz et al., 2010), few have been tested against the natural flora on carcasses (del Rio, Panizo-Moran, Prieto, Alonso-Calleja, & Capita, 2007) and in the limited studies available the findings have been inconclusive (Gill & Badoni, 2004). Furthermore, the majority of these studies have been undertaken outside of Europe where broiler production and processing may be different. This is reflected in a recent EFSA report, which identified the need for data on the potential beneficial effect of chemical decontamination, including during chilled storage, where the birds are naturally contaminated and the treated poultry carcasses are rinsed in water immediately after treatment (EFSA, 2011). The objective of this study was therefore to investigate the immediate and storage effects of trisodium phosphate (TSP), citric acid (CA), lactic acid (LA), peroxyacids (POA) and acidified sodium chlorite (ASC) on naturally occurring TVC (mesophiles and psychrotrophs), *Enterobacteriaceae*, *Pseudomonas*, lactic acid bacteria and yeast/moulds, where all treatments were immediately followed by a water rinsing. The effect of the most effective chemical treatments on the sensory attributes of poultry has been reported elsewhere (Meredith et al., 2013).

2. Materials and methods

2.1. Antimicrobial preparation

Tri-sodium phosphate (TSP, VWR International, 10 and 14% w/v of the hydrated compound); lactic acid (LA, Sigma Aldrich, 1 and 5% v/v); citric acid (CA, Sigma Aldrich, 1 and 5% w/v); peroxyacids (POA, Ecolab, Bray, Ireland, 100 and 200 ppm v/v) and acidified sodium chlorite (ASC, Sigma Aldrich 244155, pH 2.4, 500 and 1200 ppm v/v) were prepared in 500 ml sterile distilled water. The ASC was freshly prepared and all other treatments were stored at 20 °C for no more than 24 h before use.

2.2. Sample preparation and treatment in the laboratory (experiment 1)

Exactly 72 skin samples (approximately 5 × 5 cm^2) were aseptically excising from the breast of freshly processed broiler carcasses from the same flock and divided into 12 groups × 6 skin samples. Groups 1–10 (inclusive) were treated with TSP (10%, w/v), TSP (14%, w/v), LA (1%, v/v), LA (5%, v/v), CA (1%, w/v), CA (5%, w/v), POA (100 ppm), POA (200 ppm), ASC (500 ppm) or ASC (1200 ppm), respectively, by sequential immersion of each of the 6 inoculated skin samples for 15 s in 500 ml volumes followed by immediate rinsing in sterile distilled water (SDW). Group 11 were dip-treated with SDW while group 12 were the untreated control. This procedure was repeated with a second set of samples but using a mist spray of approximately 3 mls of solution, on all surfaces, from a distance of 15 cm for a total of 15 s instead of dipping. Samples were tested in duplicate for TVC (mesophiles and psychrotrophs), *Enterobacteriaceae*, *Pseudomonas*, lactic acid bacteria and yeasts & moulds immediately and after 1 and 3 days aerobic storage at 4 °C.

2.3. Sample preparation and treatment in the processing plant (experiment 2)

During processing, 90 carcasses (from the same flock) were removed immediately after evisceration from the slaughter line in a commercial plant slaughtering approximately 11,700 birds daily. These were randomly assigned to 5 groups × 18 carcasses and the left hand side of each carcass (an average area of 438 cm^2) was swabbed using a pre-moistened (MRD) 10 × 10 cm sterile cellulose acetate sponge (Sydney Heath & Sons Ltd., Staffordshire, UK). Group 1 carcasses were immediately treated with sterile distilled water (SDW) applied as a spray (3 ml) and immersed in a bucket of approximately 5 l of SDW at approximately 20 °C for 2 s. The right hand side of 3 of the carcasses were then immediately swabbing as described above. The remaining 15 treated carcasses were stored aerobically at 4 °C and 3 carcasses were tested after each of 1, 3, 5, 10 and 15 days. Group 2 and 3 carcasses were immediately treated with TSP (14%, w/v) applied as a dip and as a spray (3 ml), respectively, dipped in SDW for 15 s (approximately 5 l at 20 °C which was replaced after each treatment) and tested using the same schedule as group 1. Group 4 and 5 carcasses were immediately treated with CA (5%, w/v) applied as a dip and as a spray, respectively, dipped in SDW as above and also tested using the same schedule as group 1.

2.4. Microbiological analysis

Exactly 90 ml of maximum recovery diluent (MRD, Oxoid, CM 0733, Basingstoke, UK) was placed into the stomacher bags containing the skin samples or sponge swabs and pulsified for 15 s (Pulsifier, Microgen Bioproducts). (Pulsifier, Microgen Bioproducts). Serial dilutions were prepared in MRD and plated onto the appropriate media. TVC were enumerated using standard plate agar (SPCA; Oxoid, CM 0463) and incubated at 30 °C for 72 h (mesophiles) or 6.5 °C for 10 days (psychrotrophs). Total *Enterobacteriaceae* counts (TEC) were obtained on violet red bile glucose agar (VRBGA, Oxoid, CM 0485) and incubated at 37 °C for 24 h. *Pseudomonas* were enumerated on Pseudomonas agar (Oxoid, CM 0559) with CFC selective supplement (Oxoid, SR103) and incubated at 25 °C for 48 h. Lactic acid bacteria were cultured using de Man Rogosa sharpe agar (MRS, Oxoid, CM 0361) at 30 °C for 72 h. Yeasts and moulds were estimated using oxytetracycline glucose yeast extract agar base (OGYE; Oxoid, CM 0545) with OGYE selective supplement (Oxoid, SR 0073) and incubated at 25 °C for 5 days.

2.5. Statistical analysis

All experiments were repeated on 3 separate occasion and all microbiological analysis conducted in duplicate. Microbiological counts obtained from each sample were averaged and converted to $\log_{10}$ cfu cm^{-2}. The microbiological data was subject to a least significant difference analysis, performed using GENSTAT ver. 12.1 (VSN International Ltd, Hemel Hempstead, UK).