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Effectiveness of several chemical decontamination treatments against Gram-negative bacteria on poultry during storage under different simulated cold chain disruptions

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ABSTRACT

Five chemical compounds – 12% trisodium phosphate (TSP), 1200 ppm acidified sodium chlorite (ASC), 2% citric acid (CA), 220 ppm peroxyacids (PA) and 50 ppm chlorine dioxide (CD) – were analyzed to determine their effectiveness against Gram-negative bacteria (*Salmonella enterica* serotype Enteritidis, *Yersinia enterocolitica*, *Escherichia coli* and *Pseudomonas fluorescens*) artificially inoculated on skinless chicken legs. Samples were stored for 120 h under three different simulated cold chain disruptions: T1 (12 h at 1 ± 1 °C, followed by 6 h at 15 ± 1 °C and then 102 h at 4 ± 1 °C), T2 (18 h at 1 ± 1 °C, 6 h at 15 ± 1 °C and 96 h at 10 ± 1 °C) or T3 (18 h at 4 ± 1 °C, 6 h at 20 ± 1 °C and 96 h at 7 ± 1 °C). Microbiological analyses and pH determinations were carried out at 0, 24, 72 and 120 h of storage. TSP, ASC and CA significantly ($P < 0.05$) reduced microbial populations, relative to control (untreated) samples. TSP and ASC caused higher ($P < 0.05$) reductions of *S. enterica* than CA under moderate temperature abuse conditions (T2 and T3). TSP and ASC (T2) or TSP and CA (T1 and T3) were the most effective compounds against *E. coli*. TSP and ASC showed the highest antimicrobial effect ($P < 0.05$) at mild temperature abuse conditions (T1) for *Y. enterocolitica*, while ASC was the most active compound against this pathogen under T2 and T3 conditions. TSP caused the largest average reductions of *Ps. fluorescens* at T1. PA and DC originated scant microbial reductions under all of the conditions proposed. These findings extend current knowledge about these chemical decontaminants, and may help the Regulatory Authorities in their decisions about the poultry decontamination authorization process within the European Union.

1. Introduction

Worldwide meat consumption in 2011 was estimated at an average of 42 kg per capita, and global meat output reached 295 million tonnes. Poultry meat output (approximately 101.1 million tonnes) was only exceeded by pork (FAO, 2011). Due to its relatively low price, poultry consumption is expected to surpass that of pork and beef in the next few years, mainly at the expense of beef (FAO, 2011). Consumers usually perceive poultry as a healthy, cheap and "easy to prepare" food, although its consumption is often related to food-borne diseases and occasionally to severe outbreaks, mainly caused by *Campylobacter* spp., *Salmonella* spp. or even *Listeria monocytogenes* (EFSA, 2012).

Meat producers must observe strict hygiene measures and implement the principles of the compulsory Hazard Analysis and Critical Control Points (HACCP) system in order to ensure that marketed products fulfill the microbiological criteria established by current food legislation (Del Río, Panizo-Morán, Prieto, Alonso-Calleja & Capita, 2007). In spite of this, the processes entailed in obtaining raw meat in slaughterhouses lead to unavoidable microbial contamination of carcasses (Álvarez-Astorga, Capita, Alonso-Calleja, Moreno & García-Fernández, 2002; Tsola, Drosinos & Zoiopoulos, 2008), which is the ultimate determinant of shelf-life and product safety. To reduce the growth rate of spoilage microorganisms and prevent the growth of most of the pathogenic bacteria on the meat surface, a chilling stage must be carried out immediately after slaughter and a specific low temperature must be maintained to the end of the meat's shelf-life in order to guarantee its quality and safety (James, Vincent, De Andrade Lima & James, 2006). Regulation (EC) N° 853/2004 of the European Parliament and of the Council, laying down specific hygiene rules for food of animal origin, establishes for poultry that "after inspection and evisceration, slaughtered animals must be cleaned and chilled to not more than 4 °C as soon as possible, unless the meat is cut while

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warm". Moreover, the same regulation indicates that during cutting, boning, trimming, slicing, dicing, wrapping and packaging operations, the temperature of the meat must be maintained at not above 4 °C, "as to prevent or minimize contamination" (OJEC, 2004).

In several countries outside the European Union (EU), the application of physical or chemical decontamination treatments to poultry carcasses is permitted after slaughtering and prior to or during the chilling stage, thus combining their separate antimicrobial effects. Chemical decontamination techniques such as the application of organic acids, phosphates or chlorine-based compounds, among others, have proved effective in reducing the microbial load of chicken meat stored at low temperatures (Loretz, Stephan & Zweifel, 2010). Nevertheless, current European regulations on meat hygiene do not allow any treatment other than potable water or steam. In 2008, the European Council finally rejected a proposal from the European Commission for a new Council regulation implementing the Regulation cited above, (EC) 853/2004 (OJEC, 2004), as regards the use of four antimicrobial substances to remove surface microbial contamination from poultry carcasses (OJEC, 2009). The lack of sufficient and accurate scientific data in relation to the hazards posed by the application of these chemicals led the Commission to adopt the "precautionary principle" (OJEC, 2002) until a complete risk assessment could be conducted.

In this context, the cooling stage at the end of the slaughter process, and maintenance of appropriate storage temperatures during the entire poultry supply food chain, appear to be the most effective means to prevent the food-borne illnesses associated with this product and to prolong its shelf-life. Since refrigerated storage at retail points and subsequent preservation in household fridges are the weakest points in the cold chain (Likar and Jevšnik, 2006; Nychas, Skandamis, Tassou & Koutsoumanis, 2008), an additional means, such as the use of chemical decontamination treatments, would be of great interest to prevent the undesirable growth of microorganisms if an interruption in the cold chain occurs.

The main objective of this study was to analyze the behavior of five compounds (trisodium phosphate, acidified sodium chlorite, citric acid, chlorine dioxide and peroxyacids), when used as chemical decontamination treatments against several Gram-negative species artificially inoculated on chicken legs that were subsequently stored under temperature abuse conditions, thus simulating three different types of cold chain disruption.

2. Material and methods

2.1. Food samples

A total of 360 chicken legs was collected from a local poultry processing plant immediately after evisceration. Samples were transported to the laboratory in an ice chest and stored at 3 ± 1 °C for no longer than 1 h before use. The skin of all chicken legs used in this experiment was aseptically removed immediately before applying any inoculation procedure or chemical treatment to them.

2.2. Bacterial cultures

The bacterial cultures used to inoculate legs were *Salmonella enterica* serovar Enteritidis – Spanish Type Culture Collection – CECT 556, *Escherichia coli* ATCC 12806, *Yersinia enterocolitica* subsp. *enterocolitica* NCTC 11174 and *Pseudomonas fluorescens* ATCC 13525. Strains were maintained at 3 ± 1 °C in tryptic soy agar (TSA; Oxoid Ltd., Hampshire, UK) slants. Before inoculation, the cultures were transferred to tryptic soy broth (TSB; Oxoid) and incubated at 25 ± 1 °C (*Y. enterocolitica* and *Ps. fluorescens*) or 35 ± 1 °C (*S. enterica* and *E. coli*) for 24 h. TSB cultures were streaked onto TSA plates supplemented with 0.6% (wt/vol) yeast extract (YE; Oxoid) and incubated at 25 °C or 35 °C for 48 h. One individual colony from each strain was placed into 10 ml tubes containing TSB at pH 6.2 (adjusted with 1 mol/L of HCl or NaOH; Del Río, González de Caso, Prieto, Alonso-Calleja & Capita, 2008) and incubated for 18–20 h at 25 ± 1 °C or 35 ± 1 °C according to each individual strain's requirements. Previous research has indicated that 18-h cultures would grow to 10^9 CFU/ml (Del Río, Panizo-Morán, et al., 2007).

2.3. Inoculation procedure

Two consecutive decimal dilutions were carried out on sterile 0.1% (wt/vol) peptone water (Oxoid) to yield about 10^7 CFU/ml. Inocula were prepared by diluting 5 ml of bacterial suspensions with 495 ml of sterile 0.1% (wt/vol) peptone water (Oxoid) to yield 10^5 CFU/ml. All chicken legs to be analyzed were randomly divided into four batches, each containing 90 legs. Samples in each batch were individually immersed for 30 min in a different bacterial suspension at room temperature (20 ± 1 °C). The ratio of chicken legs to suspension volume was 1:2 (wt/vol). Following dipping, the legs were kept for 30 min at room temperature to allow draining and bacterial attachment to the meat surface.

2.4. Chemical treatments

Samples in each batch were randomly divided into six groups, each containing 15 legs. The samples from five of these groups were individually dipped for 15 min into 500 mL of different aqueous sterile solutions: trisodium phosphate (TSP, 12% wt/vol; Merck, Darmstadt, Germany), acidified sodium chlorite (ASC), which was prepared by acidifying sodium chlorite (1200 ppm wt/vol; Fluka, Madrid, Spain) to pH 2.7 by adding citric acid (CA; Panreac, Barcelona, Spain), citric acid (CA; 2% wt/vol; Panreac), peroxyacids (PA, 220 ppm vol/vol; Inspexx 100, Ecolab, St. Paul, Minn.) and chlorine dioxide (CD; 50 ppm vol/vol; Tecsa® Clor, 5% vol/vol stabilized solution, Productos Técnicos Protecsa, Chile), respectively. Legs in the remaining group were not treated (controls). All treatments were applied at room temperature (20 ± 1 °C). The pH values of chemical solutions measured at the time of application were similar to those described by Del Río, Panizo-Morán, et al. (2007): 13.03 ± 0.05 (12% TSP), 2.70 ± 0.02 (ASC), 2.15 ± 0.04 (CA), and 3.75 ± 0.03 (PA). The pH value of the 50 ppm CD solution was 9.84 ± 0.02. After treatments, the chicken legs were drained on absorbent paper for 15 min at 20 ± 1 °C, with the external side of the legs facing upwards. The samples were individually placed in food-grade, 96° ethanol-cleaned lidded plastic recipients, carefully avoiding contact between the external surface of the meat and the inner surface of the plastic lid. Samples in each group were randomly divided into three subgroups of five units. After the inoculation/dipping treatment had been completed, legs in each subgroup were immediately stored at a different refrigeration temperature for 120 h, as will be described below.

2.5. Simulated abuse temperature conditions

Three different simulated temperature abuse conditions (denominated T1, T2 and T3, respectively) were implemented during storage of the samples. Condition T1 (mild temperature abuse) consisted of 12 h at 1 ± 1 °C, followed by 6 h at 15 ± 1 °C and then 4 ± 1 °C for the remaining time until the end of storage (102 h). Condition T2 (moderate temperature abuse) consisted of 18 h at 1 ± 1 °C, followed by 6 h at 15 ± 1 °C and then 96 h at 10 ± 1 °C. Finally, condition T3 (moderate temperature abuse) consisted of 18 h at 4 ± 1 °C, followed by 6 h at 20 ± 1 °C and 96 h at 7 ± 1 °C. These conditions were chosen in order to simulate different cold chain disruptions in one or more