



## Evaluation of Disinfectants Used in Pre-Chilling water Tanks of Poultry Processing Plants

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### ABSTRACT

In poultry processing plants, disinfectants are often added to pre-chilling water tanks to reduce microbial contamination. The present study aimed at evaluating the effect of five disinfectants (acidified sodium chlorite, alkyl dimethyl benzyl ammonium chloride, chlorine dioxide, peracetic acid, and sodium hypochlorite) on the populations of food quality indicator microorganisms and on *Salmonella* Enteritidis (SE) in the presence and absence of organic matter. The results showed that chlorine dioxide and sodium hypochlorite did not reduce microbial carcass counts. On the other hand, acidified sodium chlorite, alkyl dimethyl benzyl ammonium chloride and peracetic acid reduced total and fecal coliform counts. Peracetic acid reduced the number of psychrotrophic microorganisms. All products were effective in reducing SE counts only in the absence of organic matter. Acidified sodium chlorite, alkyl dimethyl benzyl ammonium chloride and peracetic acid could be candidates for the replacement of sodium hypochlorite (commonly used in Brazil) in pre-chilling tanks.

### INTRODUCTION

During broiler carcass processing, evisceration, carcass wash, pre-chilling and cooling steps can favor the dissemination of microorganisms and carcass contamination (USDA, 2002). One infected broiler arriving at the processing plant may be the source of contamination of many other carcasses (Lillard, 1989; Rasschaert *et al.*, 2008). Some microorganisms present in the broiler intestine, such as *Salmonella* spp., *Campylobacter* spp., and thermotolerant coliforms, represent a threat to public health (Scallan *et al.*, 2011). Carcasses can be also contaminated with spoilage microorganisms that reduce the shelf life of meat products (Silva *et al.*, 2006).

Outbreaks of foodborne diseases in Brazil remain largely uninvestigated, and consequently, few data are available. For instance, from 1999 to 2012, the Brazilian Health Surveillance Division reported 1,525 outbreaks of human foodborne salmonellosis, most of them related to food of poultry origin (Brazil, 2013). Other causes of foodborne diseases (e.g. campylobacteriosis) are rarely diagnosed in Brazil. In 2013, campylobacteriosis represented about 6.5% of all cases of human foodborne diseases in the United States of America (Crim *et al.*, 2014) and contaminated poultry meat was the main source of this pathogen. The Brazilian legislation establishes that *Salmonella* spp. must be absent in broiler carcasses after processing (Brazil, 2001), but no requirements are established for the presence *Campylobacter* spp.

Disinfectant compounds are added to the pre-chilling and chilling water tanks with the purpose of minimizing carcass microbial



contamination, particularly of pathogens and spoilage bacteria (Brazil, 1998; HACCP, 1995). According to the Brazilian legislation, only chlorine compounds, at a maximum level of 5 ppm, can be added to the pre-chiller water in poultry processing plants (Brazil, 1998). However, other countries allow the use of other substances, such as sodium methyl sulfate and peracetic acid (peroxyacetic acid) (USA, 1977). Recently, alkyl dimethyl benzyl ammonium chloride was also recommended for pre-chilling tanks (Busch *et al.*, 2013).

The present study aimed at evaluating alternative compounds that could replace the current chlorine-based compounds commonly used in Brazil. The effect of five disinfectant compounds (acidified sodium chlorite, alkyl dimethyl benzyl ammonium chloride, chlorine dioxide, peracetic acid and sodium hypochlorite) on the counts of food quality indicator microorganisms and *Salmonella enterica* subsp. *enterica* serovar Enteritidis (*Salmonella* Enteritidis) were evaluated in the presence and absence of organic matter.

## MATERIAL AND METHODS

Two separate experiments were performed to evaluate the effect of selected disinfectants on chicken carcass microbial counts: one with meat quality indicator microorganisms, and the second with *Salmonella* Enteritidis. All assays were performed at 25°C.

### 1. Experiment 1

#### 1.1. Sampling

Seven samplings were performed. In each sampling, 18 broiler carcasses were purchased from processing plants located in the state of São Paulo, Brazil state. Carcasses were taken in isothermal containers to the Avian Pathology laboratory of the Department of Veterinary Pathology, School of Agriculture and Veterinary Sciences (FCAV/UNESP), São Paulo, Brazil.

#### 1.2. Treatment of broilers carcasses

Six plastic buckets were filled with 30 L of cold water (14-16°C). Acidified sodium chlorite, alkyl dimethyl benzyl ammonium chloride, chlorine dioxide, peracetic acid, and sodium hypochlorite at the concentrations of 50, 175, 5, 50 and 5 ppm, respectively, were added to one bucket each. The sixth bucket contained only water (negative control). Three carcasses were immersed in each bucket for 25 minutes in order to simulate the pre-chilling processing step.

### 1.3. Microbiological analyses

Upon removal of the buckets, carcasses were placed in sterile plastic bags containing 400 mL of 0.1% Buffered Peptone Water (BPW) (Difco™, Detroit, Michigan, USA) and 400 mL of a mixture of neutralizers, consisting of 64.5 g of Tween 80, 20% sodium bisulfite, 7.84 g of sodium thiosulfate pentahydrate, 5 g of sodium thioglycolate, and 1.5 g of L-cysteine, all diluted in 1,000 mL of tryptone-salt solution (pH 7.2) (Espigares *et al.*, 2003). The neutralized BPW was further processed in order to obtain the most probable number (MPN) of total and fecal coliforms and counts of viable strict and facultative aerobic mesophylls, both according to the methodology described by Downes & Ito (2001). Additionally, the presence of aerobic psychrotrophic microorganisms was determined according to Downes & Ito (2001).

#### 1.3.1. Determination of the Most Probable Number (MPN)

The neutralized BPW resulting from carcass washing was serially diluted, and 1 mL of each dilution inoculated into three inverted Durham culture tubes containing 10 mL of Lauryl Tryptose Broth (LTB) (Difco™, Detroit, Michigan, US). The tubes were incubated at 37°C for 48h. Cultured tubes presenting turbidity and gas production or effervescence by subtle shaking, were considered presumptively positive.

In order to determine the MPN of total coliforms/mL, a sterile bacterial loop was plunged into the presumptively LTB-positive culture tubes followed by inoculation in 10 mL Brilliant Green Bile 2% (BGB) (Difco™, Detroit, Michigan, USA) also contained an inverted Durham tube, and incubation at 37°C for 48h. When gas production was observed on upper section of the inverted tube, the sample was considered positive.

The MPN of fecal coliforms/mL was surveyed similarly to the total coliforms, although *Escherichia coli* (EC) medium (Difco™, Detroit, Michigan, US) in an inverted Durham tube instead of BGB was used. The tubes were incubated in water bath at  $45.5 \pm 0.2^\circ\text{C}$  for 48h and the positive samples were identified as described above, according to the methodology of Silva *et al.* (2007). Finally, the MPN/mL was determined based on Hoskins' table (Hoskins, 1934).

#### 1.3.2. Standard count of viable strict and facultative aerobic mesophylls

This bacterial count was carried out using the pour-plate technique. Neutralized BPW obtained from



carcass washing was decimally diluted in a new sterile 0.1% BPW up to  $10^{-6}$ . Then, 1 mL of each dilution was poured on disposable sterile bacteriological Petri dishes (90 x 15 mm) containing 15 mL of Plate Count Agar (PCA) (Difco™, Detroit, Michigan, USA) at approximately 45°C. Cultures were mixed and after agar solidification, Petri dishes were incubated at 37°C for 48h. Thereafter, colony-forming units per milliliter (CFU/mL) were enumerated.

### 1.3.3. Standard count of aerobic psychrotrophic microorganisms

A volume of 0.1 mL from the neutralized BPW and its decimal dilutions were inoculated onto PCA medium (Difco™, Detroit, Michigan, USA). Colonies were counted according to Downes & Ito (2001) after 10 days of incubation at 7°C.

## 2. Experiment

### 2.1. *In-vitro* assessment of disinfectant activity on *Salmonella* Enteritidis

A spontaneous mutant of *Salmonella* Enteritidis resistant to nalidixic acid and to spectinomycin (SE Nal<sup>r</sup>Spc<sup>r</sup>), stored in the bacterial culture collection of the Avian Pathology laboratory of the Department of Veterinary Pathology of FCAV/UNESP, was used in this trial. This strain was inoculated into 10 mL of Lysogeny broth (LB) (Difco™, Detroit, Michigan, US) and incubated at 37°C for 16h under constant shaking (150 rpm). After that, the culture was centrifuged at 4,000 rpm for 20 min at 4°C and suspended in 10 mL phosphate buffered saline (PBS, pH 7.2). This wash step was repeated three times. Bacterial counts were performed in bacteriological Petri dishes (90 x 15 mm) containing Lysogeny agar (Difco™, Detroit, Michigan, US) with nalidixic acid (100 µg/mL) and spectinomycin (100 µg/mL) after incubation at 37°C for 24h (Hoskins, 1934). A volume of 0.1 mL SE Nal<sup>r</sup>Spc<sup>r</sup> inoculum, containing about  $4 \times 10^8$  colony-forming units (CFU), was added to each disinfectant solution at 14 to 16°C with or without organic matter (carcass wash solution). Each disinfectant was tested in the presence and in the absence of organic matter (carcass wash solution), as described in Table 1. Bacterial colonies were counted 5 and 25 minutes after disinfectant neutralization with 10 mL of the neutralizing solution described above. Colony-forming units (CFU) were converted to  $\log_{10}$  for statistical analyses.

**Table 1** – Experimental design of experiment 2.

Disinfectants	Treatments performed 5 and 25 minutes afterincubation*
**A, B, C, D and E	(T1) 9.9 mL of PBS + 0.1 mL of SE Nal <sup>r</sup> Spc <sup>r</sup> (T2) 9.9 mL (PBS + disinfectant) + 0.1 mL of SE Nal <sup>r</sup> Spc <sup>r</sup> (after each time point, 10 mL of neutralizer were added followed by bacterial counting) (T3) 9.9 mL of organic matter + 0.1 mL of SE Nal <sup>r</sup> Spc <sup>r</sup> (T4) 9.9 mL (disinfectant + organic matter) + 0.1 mL of SE Nal <sup>r</sup> Spc <sup>r</sup> (after each time point, 10 mL of neutralizer were added followed by bacterial counting)

\* T1: suspension containing the test microorganism and control PBS, with no organic matter (control or carcass wash solution); T2: suspension containing the test microorganism and the evaluated disinfectant, with no organic matter; T3: suspension containing the test microorganism and organic matter; T4: suspension containing the test microorganism, the evaluated disinfectant solution, and organic matter.

\*\* A – acidified sodium chlorite (50 ppm); B – alkyl dimethyl benzyl ammonium chloride (175 ppm); C – chlorine dioxide (5 ppm); D – peracetic acid (50 ppm); E – sodium hypochlorite (5 ppm). The final volume of each disinfectant solution in the test was 10 mL.

### Statistical analyses

Bacterial count results (CFU and MPN) were transformed in  $\log_{10}$  and means compared by Tukey's test at 5% ( $p < 0.05$ ) significance level. Analyses were performed using Statistical Analysis System (SAS) software version 9.1. In the second experiment, disinfectant substances were considered efficient when the SE Nal<sup>r</sup>Spc<sup>r</sup> counts decreased at least to  $10^5$  CFU/mL, according to the recommendations of the European Committee for Standardization (CEN, 2009).

## RESULTS

The results of the effects of the tested disinfectants on the counts of meat quality indicator microorganisms in broiler carcasses are shown in Table 2. None of the disinfectant tested reduced mesophyll counts ( $p > 0.05$ ). Peracetic acid reduced the psychrotrophic population (4.01 CFU/mL) compared with the control solution (4.73 CFU/mL) ( $p < 0.05$ ). The other evaluated disinfectants did not demonstrate any activity on psychrotrophic microorganisms ( $p < 0.05$ ). According to the statistical analysis, acidified sodium chlorite, alkyl dimethyl benzyl ammonium chloride and peracetic acid reduced total coliform counts from 2.47 (untreated control) to 1.67 MPN/mL, 1.74 MPN/mL, 1.75 MPN/mL and ( $p < 0.05$ ), respectively. These products also reduced the contamination of carcasses with thermotolerant coliforms from 2.32 MPN/mL (control) to 1.49 MPN/mL, 1.53 MPN/mL and 1.61 MPN/mL ( $p < 0.05$ ), respectively.



**Table 2** – Average counts ( $\log_{10}$ ) of meat quality indicator microorganisms in broiler carcasses washed with different disinfectant compounds.

Disinfectant substances	Microbial group ( $\log_{10}$ )			
	Mesophylls (CFU/mL)	Psychrotrophic bacteria (CFU/mL)	Total coliforms (MPN/mL)	Thermotolerant coliforms (MPN/mL)
Acidified sodium chlorite (50 ppm)	4.15 <sup>a</sup>	4.43 <sup>ab</sup>	1.67 <sup>b</sup>	1.49 <sup>b</sup>
Alkyl dimethyl benzyl ammonium chloride (175 ppm)	4.00 <sup>a</sup>	4.20 <sup>ab</sup>	1.75 <sup>b</sup>	1.61 <sup>b</sup>
Chlorine dioxide (5 ppm)	4.34 <sup>a</sup>	4.55 <sup>ab</sup>	2.08 <sup>ab</sup>	2.02 <sup>ab</sup>
Peracetic acid (50 ppm)	4.01 <sup>a</sup>	4.01 <sup>b</sup>	1.74 <sup>b</sup>	1.53 <sup>b</sup>
Sodium hypochlorite (5 ppm)	4.39 <sup>a*</sup>	4.45 <sup>ab</sup>	2.24 <sup>ab</sup>	2.05 <sup>ab</sup>
Group control	4.53 <sup>a</sup>	4.73 <sup>a</sup>	2.47 <sup>a</sup>	2.32 <sup>a</sup>

\* Means followed by the same letter in the same column do not differ by Turkey's test at significance level of 5% ( $p < 0.05$ ).

Peracetic acid showed the best results in reducing meat quality indicator microorganisms, whereas sodium hypochlorite and chlorine dioxide did not affect these populations when applied at the concentration allowed by Brazilian legislation (5 ppm).

The *in-vitro* assessment of the action of the tested compounds on SE Nal<sup>r</sup>Sp<sup>c</sup><sub>r</sub> is presented in Table 3.

**Table 3** – Average *Salmonella* Enteritidis (SE Nal<sup>r</sup>Sp<sup>c</sup><sub>r</sub>) counts in the presence and absence of organic matter 5 and 25 minute after disinfectant addition.

Treatment*	Time	
	5 minutes	25 minutes
Acidified sodium chlorite (50 ppm)		
T1	7.08 <sup>a</sup>	7.09 <sup>a</sup>
T2	≤ 1.00 <sup>b</sup>	≤ 1.00 <sup>b</sup>
T3	7.11 <sup>a</sup>	7.15 <sup>a</sup>
T4	7.11 <sup>a</sup>	6.98 <sup>a</sup>
Alkyl dimethyl benzyl ammonium chloride (175 ppm)		
T1	7.08 <sup>a</sup>	7.10 <sup>a</sup>
T2	≤ 1.00 <sup>b</sup>	≤ 1.00 <sup>b</sup>
T3	7.11 <sup>a</sup>	7.14 <sup>a</sup>
T4	6.98 <sup>a</sup>	7.13 <sup>a</sup>
Chlorine dioxide (5 ppm)		
T1	7.22 <sup>a</sup>	7.25 <sup>a</sup>
T2	≤ 1.00 <sup>b</sup>	≤ 1.00 <sup>b</sup>
T3	7.18 <sup>a</sup>	7.23 <sup>a</sup>
T4	7.15 <sup>a</sup>	7.21 <sup>a</sup>
Peracetic acid (50 ppm)		
T1	7.00 <sup>a,**</sup>	7.03 <sup>a</sup>
T2	≤ 1.00 <sup>b</sup>	≤ 1.00 <sup>b</sup>
T3	7.07 <sup>a</sup>	7.42 <sup>a</sup>
T4	7.14 <sup>a</sup>	7.02 <sup>a</sup>
Sodium hypochlorite (5 ppm)		
T1	7.15 <sup>a</sup>	7.18 <sup>a</sup>
T2	≤ 1.00 <sup>b</sup>	≤ 1.00 <sup>b</sup>
T3	7.18 <sup>a</sup>	7.20 <sup>a</sup>
T4	7.16 <sup>a</sup>	7.28 <sup>a</sup>

SE Nal<sup>r</sup>Sp<sup>c</sup><sub>r</sub> = *Salmonella* Enteritidis resistant to nalidixic acid and to spectinomycin;  
\*Treatments are detailed in Table 1; \*\*For each disinfectant solution, means followed by the same latter in the same column or row are not different by Turkey's test at 5% ( $p < 0.05$ ) significance level.

When compared with the PBS control group (T1), all tested disinfectants were efficient to reduce SE Nal<sup>r</sup>Sp<sup>c</sup><sub>r</sub> counts in the absence of organic matter (T2) ( $p < 0.05$ ), according to the European Committee for Standardization (CEN, 2009). Under the conditions of the present experiment, no SE Nal<sup>r</sup>Sp<sup>c</sup><sub>r</sub> was detected 5 and 25 minutes after disinfectant addition.

## DISCUSSION

Microorganisms are considered good indicators of the hygienic conditions present during food manufacturing process (Hoffmann *et al.* 2004). For example, high coliform counts indicate post-processing contamination and/or unsuitable sanitization (Kottwitz *et al.* 2010). *Salmonella* spp., *Escherichia coli* and *Staphylococcus aureus* are examples of food-contaminating microorganisms that cause foodborne diseases in human beings. These bacteria are frequently found in contaminated chicken meat (Jakobsen *et al.* 2012; Kottwitz *et al.* 2010; Silva *et al.* 2010), and therefore, should be eliminated or, at least, reduced in broiler carcasses.

The addition of disinfectants in pre-chilling tanks is an important tool to minimize the presence of microorganisms in chicken carcasses during processing. The addition of peracetic acid and many other chemical compounds in pre-chilling tanks is allowed in the United States of America (USA, 1977). On the other hand, the Brazilian legislation only allows the use of chlorine-based compounds at a maximum concentration of 5 ppm (Brazil, 1998). Therefore, studies assessing the efficacy of other disinfectants added in pre-chilling tanks may encourage Brazilian public health authorities to review the current legislation. According to Voidarou *et al.* (2007), the pre-chilling step itself without any disinfectant has little effect on reducing microorganisms in broiler carcasses.

In the present study, sodium hypochlorite and chlorine dioxide did not show any effect on the



evaluated meat quality indicator microorganisms. These results are consistent with the findings of Lopes *et al.* (2007), who did not observe any effect of those compounds in broiler carcass bacterial counts when added to pre-chilling tanks in processing plants. On the other hand, another study reported 1.5% and 70% reduction of coliform and *Campylobacter* spp. populations, respectively, using sodium hypochlorite and chlorine dioxide (Kameyama *et al.*, 2012). However, in this study, sodium hypochlorite was added at 10 ppm and chlorine dioxide at 50 ppm (USA, 1977), which are higher than the 5 ppm currently allowed in the Brazilian legislation. Bashor *et al.* (2004) also reported that addition of chlorine at 25 to 35 ppm reduced *Campylobacter* spp. populations in broiler carcasses in 0.5 log CFU/mL of washing solution. Therefore, it is clear that the current Brazilian legislation regarding the use of sodium hypochlorite in pre-chilling tanks needs to be reviewed.

Russell & Axtell (2005) reported that utilization of sodium hypochlorite at 50 ppm in pre-chilling tanks reduced carcass contamination by *Salmonella* spp. and *Pseudomonas fluorescens*, but it was not successful in reducing *Escherichia coli*. In an trial carried out by Mergonsi *et al.* (2007), the addition of chlorine to a pre-chilling tank, where organic matter was present, did not reduce the number of carcasses contaminated with *Salmonella* spp. Consistent results were obtained in the present experiment, where no effect of the evaluated disinfectants on *Salmonella* Enteritidis counts in the presence of organic matter was detected.

The results of the present study showed that peracetic acid at 50 ppm, acidified sodium chlorite at 50 ppm and alkyl dimethyl benzyl ammonium chloride at 175 ppm decreased thermotolerant coliform counts. However, Mead *et al.* (2000) reported that the addition of chlorine at 50 ppm during the pre-chilling stage did not prevent cross-contamination of carcasses by *Escherichia coli*. Buhr *et al.* (2005) also showed that the addition of chlorine at 20 ppm in pre-chilling tanks reduced the counts of coliforms, total aerobes, *Escherichia coli* and *Campylobacter* spp. in broiler carcasses, but did the number of carcasses positive for *Salmonella* spp was not decreased.

In the present study, acidified sodium chlorite decreased the population of coliforms. Oyarzabal *et al.* (2004) and Sexton *et al.* (2007), using that disinfectant in pre-chilling tanks also reported a reduction in *Escherichia coli* and *Salmonella* spp. counts in broiler carcasses. In contrast, in our study, this compound did not reduce *Salmonella* Enteritidis counts in the presence

of organic matter. It should be noted, however, that Oyarzabal *et al.* (2004) and Sexton *et al.* (2007) applied different methods to assess disinfectant action, which could explain the divergent results.

Li *et al.* (2002) evaluated the action of a chorine solution (50 ppm) spray on broiler carcasses and reported positive results in terms of bacterial count reduction. In the present study, acidified sodium chlorite showed better bacterial effect than other tested chlorine-based compounds (sodium hypochlorite and chlorine dioxide). The lower pH and the higher concentration (50 ppm) of sodium chlorite applied may have contributed for this result.

In agreement with the report of Bauermeister *et al.* (2008), in the present study, peracetic acid was more effective in reducing microbial counts than chlorine-based compounds. The weaker microbicidal effect of chlorine may be explained by the fact that it is easily neutralized by organic compounds (Northcutt & Lacy, 2000).

Kich *et al.* (2004) observed that sodium hypochlorite (1,000 ppm), quaternary ammonium (400 ppm), and peracetic acid (53.33 ppm) were all able to reduce *Salmonella* Typhimurium in the absence of organic matter. These authors also reported that, in the presence of organic matter, only peracetic acid reduced the counts of this pathogen. In the present study, none of the disinfectant tested, including peracetic acid, reduced *Salmonella* Enteritidis counts in the presence organic matter. Differences in amounts of organic matter and disinfectant concentrations could explain the differences between our results and those described by Kich *et al.* (2004).

According to Sidhu *et al.* (2002), the inefficiency of quaternary ammonium solutions against mesophyll and psychrotrophic present in products of animal origin could be a result of resistance induced by the wide utilization of such substance as disinfectant in animal production facilities. For instance, all *Pseudomonas aeruginosa* strains isolated of food of animal origin were resistant to that compound (Langsrud & Sundhein, 1997). In the present study, the ineffectiveness of alkyl dimethyl benzyl ammonium chloride in reducing mesophyll and psychrotrophic microorganisms could be also a result of neutralization by organic matter.

Taking into account the antagonist effect of organic matter on disinfectant activity, as demonstrated here and previously by other authors (Kich *et al.* 2004; Lopes *et al.* 2007), it is clear that the cleaning of pre-chilling tanks is essential to enhance disinfectant efficiency against contaminating bacteria. Chlorine-



based compounds, when applied at the concentration permitted by the Brazilian legislation, did not reduce the counts of meat quality indicator microorganisms nor of *Salmonella* Enteritidis. In the present study, acidified sodium chlorine, alkyl dimethyl benzyl ammonium chloride, and peracetic acid at 175, 50 and 50 ppm, respectively, were able to reduce total and thermotolerant coliform counts. In terms of microbial control, those three compounds presented better results than sodium hypochlorite at 5 ppm typically applied in Brazilian poultry processing plants.

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