Antibacterial activity of Timsen[®] (*n*-alkyl dimethyl benzyl ammonium chloride-40%) in scalding and precooling water in poultry slaughterhouses

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Abstract The objective of this study was to evaluate the efficacy of a product based on *n*-alkyl dimethyl benzyl ammonium chloride-40%, marketed as Timsen[®], during scalding and precooling of poultry carcasses in slaughterhouses. To this end, three poultry slaughterhouses (A, B and C) were evaluated. The product was added (200 ppm) to the scalding (58 °C) and precooling water (4 °C), and microbiological analyses were performed of the water and the poultry carcasses before and after Timsen[®] addition. The product controlled the multiplication of aerobic mesophilic microorganisms, both in the scalding as in the precooling water. In a comparison of carcasses soaked in Timsen[®]-treated scalding and precooling water with carcasses soaked in untreated water, the count of aerobic mesophilic microorganisms in the later was higher and thermotolerant coliform was not detected in samples of carcasses soaked in Timsen[®]-treated water. When the scalding and precooling water was not treated with the product, *Listeria* spp. was isolated from poultry carcasses of two slaughterhouses (A and C), while these microorganisms were not detected when Timsen[®] was applied. The use of Timsen[®] in the scalding and precooling water enhanced the safety and control microbial contamination of poultry carcasses.

Keywords *Listeria monocytogenes* · Quaternary ammonium · Processing · Poultry

Introduction

The poultry slaughter process consists basically of the stages stunning, bleeding, scalding, defeathering, evisceration, washing, and cooling (Escudero-Gilete et al. 2005). Although all steps are essential, the scalding and cooling are important steps that influence product safety and quality (Bowker et al. 2014).

The shelf life of poultry meat is influenced by initial microbial counts on carcasses, which also reflect cross contamination during processing (Meredith et al. 2013a). The counts of aerobic mesophilic microorganisms as well as of the bacteria family *Enterobacteriaceae* can be used in the poultry processing industry as indicators of microbiological quality and hygiene during slaughter (Alonso-Calleja et al. 2004).

Poultry meat can be a source of pathogenic microorganisms (Uyttendale et al. 1997). One such microorganism is *Listeria monocytogenes*, a bacterium that can cause listeriosis in humans and animals. Food contaminated with *L. monocytogenes* is the main transmission route of this pathogen to humans (Allerberger and Wagner 2010), and there are reports of cases of listeriosis associated with consumption of undercooked poultry (Schuchat et al. 1992). Due to its high mortality rate (20–30%), listeriosis is one of the most important causes of death due to foodborne illness in the EU and USA (Barton Behravesh et al. 2011; EFSA/ECDC 2015).

The use of chemicals in the water during some stages of poultry processing can reduce the initial microbial counts and subsequent microbial growth, increasing the shelf life

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of products. Currently, a range of chemical compounds is available for decontamination of animal carcasses, including chlorine-based compounds, organic acids and trisodium phosphate. These treatments are generally applied by soaking or spraying the carcasses (Loretz et al. 2010).

The *n*-alkyl dimethyl benzyl ammonium chloride-40% (Timsen[®]) is a quaternary ammonium compound used as a broad-spectrum biocide, for room and equipment disinfection, with algicidal, bactericidal, fungicidal, and virucidal properties, even in the presence of organic matter (Sanphar 2016). The nonspecific action of quaternary ammonium compounds renders improbable the development of resistance (Gilbert and McBain 2003). Besides that, these compounds are generally considered biodegradable under aerobic conditions (Tezel and Pavlostathis 2015).

In recent years, several studies tested ways of reducing bacterial contamination of poultry carcasses (Buncic and Sofos 2012; Meredith et al. 2013b; Nagel et al. 2013; Koolman et al. 2014), although few investigated the application of antimicrobials directly in the scalding and precooling water in slaughterhouses. Therefore, the objective of this study was to evaluate the efficacy of a product based on *n*-alkyl dimethyl benzyl ammonium chloride-40%, marketed as Timsen[®], during scalding and precooling of poultry carcasses in slaughterhouses.

Materials and methods

Three samplings were performed in three poultry slaughterhouses with different slaughter rates. Slaughterhouse A and B had the capacity to slaughter 22,000 and 400 poultry a day, respectively, and slaughterhouse C 500 poultry a week. The compound *n*-alkyl dimethyl benzyl ammonium chloride-40% (Timsen[®]) was added to the scalding (58 °C) and precooling water (4 °C) at a concentration of 200 ppm after 2 h of continuous process flow, with a 20-min interval between application and sampling.

Samples of 500 mL of scalding and precooling water were collected in sterile bottles before and after Timsen[®] addition, resulting in a total of 12 samples. Nine samples of poultry carcasses were also collected before and nine after scalding, with and without Timsen[®] application as well as nine before and nine after precooling, with and without Timsen[®] application, resulting in 54 samples. The carcasses were labelled with colored seals, to ensure the sampling of the same carcass at each stage. The carcasses were sampled by whole carcass rinse (WCR) method, where 450 mL of buffered peptone water (BPW Acumedia[®]) were used for feathered carcasses (± 3.0 kg) and 225 mL for picked carcasses (± 2.0 kg).

All samples were refrigerated and sent to the Food Microbiology Laboratory–DCTA-FAEM of the Federal University of Pelotas, for microbiological analyses.

Microbiological analysis

The water samples were assessed by standard counting of aerobic mesophilic microorganisms. For poultry carcasses, aside from the standard count of aerobic mesophilic microorganisms, thermotolerant coliforms were counted and *Listeria* spp. isolated and identified.

Standard count of aerobic mesophilic microorganisms

The standard count of aerobic mesophilic microorganisms was performed according to the protocol described by the APHA (2001). The samples were diluted tenfold (1/10) to 10^{-4} and immediately sown in deep Petri dishes containing Plate Count agar (PCA Acumedia[®]) and incubated at 37 °C for 48 h. For water samples, the result was expressed in CFU mL⁻¹, and for poultry carcass samples in CFU g⁻¹.

Thermotolerant coliform count

We used the officially recommended methodology, according to Instrução Normativa No. 62, of August 26, 2003 (BRASIL 2003). First, serial dilutions were performed until 10^{-4} and then deep seeded in Violet Red Bile agar (VRBA, Acumedia[®]) and incubated at 37 °C for 24 h. After counting the typical coliform bacteria colonies, 3–5 typical colonies were inoculated in *Escherichia coli* (EC, Acumedia[®]) broth and incubated at 45 °C in water bath for 24–48 h. The presence of thermotolerant coliforms was confirmed by gas formation in the Durhan tube. The result was expressed in CFU g⁻¹.

Isolation and identification of *Listeria* spp.

Listeria spp. was analyzed as described by Farber et al. (1994). First, the primary selective enrichment was performed in *Listeria* Enrichment Broth (LEB—Oxoid[®]), and incubated at 30 °C for 24 h. Thereafter, the differential selective enrichment was performed by inoculating an aliquot of 0.1 mL of LEB on Fraser broth (Oxoid[®]) and incubation at 37 °C for 48 h. Tubes in which darkening of Fraser broth was observed were used for seeding on Petri dishes containing the selective agars Oxford (Oxoid[®]) and Palcam (Oxoid[®]), which were incubated at 37 °C for 48 h. Then, 3–5 characteristic *Listeria* spp. colonies were selected, plated on Tryptone Soy agar supplemented with 0.6% yeast extract (Oxoid[®]) (TSA-YE) and incubated at 37 °C for 24 h for the following phenotypic tests:

fermentation of carbohydrates (dextrose, rhamnose, xylose, and mannitol), motility, determination of β -hemolysis, and catalase production capacity.

Results and discussion

Effectiveness of Timsen® in the scalding and precooling water

The application of Timsen® controlled the multiplication of aerobic mesophilic microorganisms both in the scalding and in the precooling water, in all three slaughterhouses, regardless of the slaughter rate, since after Timsen® application, this group of microorganisms were not detected in the samples (Fig. 1).

High levels of organic matter, which often occur in scalding and precooling stages, can reduce the effectiveness of antimicrobial agents (Loretz et al. 2010). However, in this study, Timsen® proved to be an effective antimicrobial agent maintaining its activity even in the presence of organic matter. These results suggested that the use of this compound in scalding and precooling water can be an effective alternative for the control of microbial contamination in these critical stages in poultry slaughterhouses.

Fig. 1 Counts of aerobic mesophilic microorganisms in scalding (a) and precooling water (b) in three poultry slaughterhouses, before and after Timsen® addition. SA slaughterhouse A, SB slaughterhouse B, SC slaughterhouse C. Results are expressed as mean $\pm$ standard deviation; significant differences ($p \leq 0.05$) are indicated by different letters

Microbiological evaluation of poultry carcasses before and after scalding and precooling

In addition to the scalding and precooling water, the poultry carcasses treated with Timsen® in these two stages were evaluated and compared with control carcasses subjected to the common treatment of the evaluated slaughterhouses. The carcasses were sampled by whole carcass rinse method. Rinse sampling is a common method for microbiological analysis during poultry processing (Williams et al. 2010). This method consists of placing carcass in a bag with a known volume of rinse fluid. The carcass is massaged (60–120 s) within the bag to aid in the removal of bacteria. Recently Panzenhagen et al. (2016), for example, used the rinse method for isolation of *Campylobacter* spp., and isolated the pathogen in 45% of the sample carcasses.

There was a significant difference ($p \leq 0.05$) in mesophilic microorganism counts between the carcasses to which the product was applied compared to the control carcasses (Fig. 2). It is worth mentioning that in slaughterhouse A, possibly, there was cross-contamination, since after Timsen® application, no aerobic mesophilic microorganisms could be detected in carcass samples after scalding, while in the subsequent step (after precooling), $1.6 \log \text{CFU g}^{-1}$ were observed. In slaughterhouse B, after scalding decreased the aerobic mesophilic microorganisms count by $4.4 \log \text{CFU g}^{-1}$ and after precooling, no mesophilic microorganisms were detectable, demonstrating the reduction of microbial contamination by the product. In slaughterhouse C, no mesophilic microorganisms were detected after Timsen® application in any of the evaluated stages of the slaughter process. Different from the current study, a decrease of $1.1 \log \text{CFU cm}^{-2}$ of aerobic bacteria in poultry carcasses was observed by the addition of 0.25% lactic acid to scalding water ($58^\circ\text{C}/2 \text{ min}$) (Sakhare et al. 1999). Koolman et al. (2014), using decontamination by immersion on solution containing 12% trisodium phosphate, observed a reduction of only $0.9 \log \text{CFU cm}^{-2}$ in counts of total viable microorganisms. Therefore, more satisfactory results were obtained with the application of Timsen®.

Carcasses can be contaminated by fecal material from the birds' intestines during evisceration. The count of thermotolerant coliforms is important to assess hygiene during the slaughter process, and also serves as an indicator of the probability of the presence of enteric pathogens in the end product. In this study, thermotolerant coliforms were detected at levels of 1.4, 3.5 and $1.8 \log \text{CFU g}^{-1}$ in the control samples after precooling in the slaughterhouses A, B and C, respectively. These microorganisms were not detected after scalding and precooling in the carcasses when the scalding and

Fig. 2 Counts of aerobic mesophilic microorganisms before (a) and after scalding (b) and after precooling (c) in three poultry slaughterhouses, before and after Timsen® addition. SA slaughterhouse A, SB slaughterhouse B, SC slaughterhouse C. Results are expressed as mean $\pm$ standard deviation; significant differences ($p \leq 0.05$) are indicate by different letters

precooling water contained Timsen®, in all evaluated slaughterhouses ($p \leq 0.05$) (Fig. 3). While a previous study reported a reduction of $0.7 \log \text{CFU cm}^{-2}$ in coliform counts when the chlorinated water (50 ppm) for decontamination of poultry carcasses was used (Sinhamahapatra et al. 2004). In fact, a low concentration of 50 ppm of chlorinated water was applied, while in our study a concentration of 200 ppm of Timsen® was used in the scalding and precooling water.

From the control samples without Timsen®, three *Listeria* species were isolated: *L. monocytogenes*, *L. seeligeri* and *L. innocua*. *L. monocytogenes* and *L. seeligeri* were isolated in slaughterhouse A, before scalding and after precooling. It is noteworthy that these two bacteria were not isolated after scalding, however,

Fig. 3 Counts of thermotolerant coliforms before (a) and after scalding (b) and after precooling (c) in three poultry slaughterhouses, before and after Timsen® addition. SA slaughterhouse A, SB slaughterhouse B, SC slaughterhouse C. Results are expressed as mean $\pm$ standard deviation; significant differences ($p \leq 0.05$) are indicate by different letters

after precooling, they were isolated again, which can be due to cross-contamination in the processing environment. The presence of *L. monocytogenes* after precooling is an alarming result, because to the ability of the bacteria to multiply at refrigeration temperature, their number may increase during the food storage period (Melero et al. 2012), reinforcing the importance of the elimination of this microorganism in the poultry processing environment.

In slaughterhouse C, *L. innocua* was isolated after scalding and precooling, but before scalding this microorganism was not detected. Although *L. innocua* is not pathogenic to humans, its presence as well as that of other *Listeria* species indicate the possibility of contamination by *L. monocytogenes*.

Poultry carcasses subjected to scalding and precooling using Timsen® did not show the presence of *Listeria* spp., demonstrating the effectiveness of the product in the elimination of this important pathogen. In tests of different Timsen® concentrations against *L. monocytogenes*, isolated in poultry slaughterhouses, Russell (2000) found that 25 ppm, within a contact time of 1 min with the sanitizer, was sufficient to eliminate the bacteria, demonstrating the sensitivity of this pathogen to the product. It is noteworthy that the author used a pure culture, i.e., without interference of organic matter or of other microorganisms. According to Lecompte et al. (2008), the use of lactic acid (10%) in chicken thighs artificially infected with *L. innocua* caused a reduction of 2.5 log CFU cm⁻² in the population. In another study, the same acid (1%), applied to poultry breast artificially contaminated with *L. monocytogenes*, reduced the bacterial count by 2.3 log CFU cm⁻², while trisodium phosphate (1%) decreased the count by 2.1 log CFU cm⁻² (Hwang and Beuchat 1995).

According to Bolton et al. (2014), to minimize microbial contamination of poultry meat, good agricultural practices and hygienic processing must be used. Analyzing the impact of the stages of the slaughter process on carcass contamination is fundamental for an adequate management of the procedures and to ensure the quality and safety of the end product. The sanitary quality of carcasses can be obtained by reducing the negative impact of the stages in which microbial contamination may increase and by improvements in the stages related to reducing microbial contamination (Belluco et al. 2016). Thus, the results obtained by Timsen® application in this study show the potential of this product for use in scalding and precooling water in poultry slaughterhouses.

Conclusion

The use of the product based on *n*-alkyl dimethyl benzyl ammonium chloride-40%, marketed as Timsen®, is an alternative for the elimination of microbial contamination of poultry carcasses during the scalding and precooling stages in poultry slaughterhouses, enhancing quality and safety of the final product.

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References

Allerberger F, Wagner M (2010) Listeriosis: a resurgent foodborne infection. Clin Microbiol Infect 16:16–23

- Alonso-Calleja C, Martínez-Fernández B, Prieto M, Capita R (2004) Microbiological quality of vacuum-packed retail ostrich meat in Spain. Food Microbiol 21:241–246
- APHA (2001) Compendium of Methods for Microbiological Examination of Foods, 4th edn. American Public Health Association, Washington, D.C.
- Barton Behravesh C, Jones TF, Vugia DJ, Long C, Marcus R, Smith K, Thomas S, Zansky S, Fullerton KE, Henao OL, Scallan E (2011) Deaths associated with bacterial pathogens transmitted commonly through food: foodborne diseases active surveillance network (FoodNet), 1996–2005. J Infect Dis 204:263–267
- Belluco S, Barco L, Roccato A, Ricci A (2016) *Escherichia coli* and *Enterobacteriaceae* counts on poultry carcasses along the slaughterline: a systematic review and meta-analysis. Food Control 60:269–280
- Bolton DJ, Meredith H, Walsh D, McDowell DA (2014) The effect of chemical treatments in laboratory and broiler plant studies on the microbial status and shelf-life of poultry. Food Control 36:230–237
- Bowker BC, Zhuang H, Buhr RJ (2014) Impact of carcass scalding and chilling on muscle proteins and meat quality of broiler breast fillets. Food Sci Technol LWT 59:156–162
- BRASIL (2003) Instrução Normativa nº62—Métodos analíticos oficiais para análises microbiológicas para controle de produtos de origem animal e água. Publicado no Diário Oficial da União. <http://sistemasweb.agricultura.gov.br/sislegis/action/detalhaAto.do?method=consultarLegislacaoFederal>. Accessed 25 Aug 2016
- Buncic S, Sofos J (2012) Interventions to control *Salmonella* contamination during poultry, cattle and pig slaughter. Food Res Int 45:641–655
- EFSA/ECDC (2015) The European union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014. EFSA J 13:1–191. http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/4329.pdf
- Escudero-Gilete ML, Gonzalez-Miret ML, Heredia FJ (2005) Multivariate study of the decontamination process as function of time, pressure and quantity of water used in washing stage after evisceration in poultry meat production. J Food Eng 69:245–251
- Farber JM, Warburton DW, Babiuk T (1994) Isolation of *Listeria monocytogenes* from all food and environmental samples. In: Government of Canada. HBP method. Polyscience Publications, Quebec
- Gilbert P, McBain AJ (2003) Potential impact of increased use of biocides in consumer products on prevalence of antibiotic resistance. Clin Microbiol Rev 16:189–208
- Hwang CA, Beuchat LR (1995) Efficacy of selected chemicals for killing pathogenic and spoilage microorganisms on chicken skin. J Food Protect 58:19–23
- Koolman L, Whyte P, Meade J, Lyng J, Bolton D (2014) Use of chemical treatments applied alone and in combination to reduce *Campylobacter* on raw poultry. Food Control 46:299–303
- Lecompte J-Y, Kondjoyan A, Sarter S, Portanguen S, Collignan A (2008) Effects of steam and lactic treatments on inactivation of *Listeria innocua* surface-inoculated on chicken skins. Int J Food Microbiol 127:155–161
- Loretz M, Stephan R, Zweifel C (2010) Antimicrobial activity of decontamination treatments for poultry carcasses: a literature survey. Food Control 21:791–804
- Melero B, Diez AM, Rajkovic A, Jaime I, Rovira J (2012) Behaviour of non-stressed and stressed *Listeria monocytogenes* and *Campylobacter jejuni* cells on fresh chicken burger meat packaged under modified atmosphere and inoculated with protective culture. Int J Food Microbiol 158:107–112
- Meredith H, Walsh D, McDowell D, Bolton DJ (2013a) An investigation of the immediate and storage effects of chemical treatments on *Campylobacter* and sensory characteristics of poultry. Int J Food Microbiol 166:309–315

- Meredith H, McDowell D, Bolton DJ (2013b) An evaluation of trisodium phosphate, citric acid and lactic acid cloacal wash treatments to reduce *Campylobacter*, total viable counts (TVC) and total enterobacteriaceae counts (TEC) on broiler carcasses during processing. *Food Control* 32:149–152
- Nagel GM, Bauermeister LJ, Bratcher CL, Singh M, McKee SR (2013) *Salmonella* and *Campylobacter* reduction and quality characteristics of poultry carcasses treated with various antimicrobials in a post-chill immersion tank. *Int J Food Microbiol* 165:281–286
- Panzenhagen PHN, Aguiar WS, Frasso BS, Pereira VLA, Abreu DLC, Rodrigues DP, Nascimento ER, Aquino MHC (2016) Prevalence and fluoroquinolones resistance of *Campylobacter* and *Salmonella* isolates from poultry carcasses in Rio de Janeiro, Brazil. *Food Control* 61:243–247
- Russel SM (2000) Effect of a novel sanitizer on pathogenic, spoilage, and indicator populations of bacteria from chicken carcasses. *Appl Poult Sci* 9:393–402
- Sakhare PZ, Sachindra NM, Yashoda KP, Narashima Rao D (1999) Efficacy of intermittent decontamination treatments during processing in reducing the microbial load on broiler chicken carcass. *Food Control* 10:189–194
- Sanphar (2016) Produtos. <http://www.sanphar.net/pt/solucoes/biosseguranca/>. Accessed 24 Aug 2016
- Schuchat A, Deaver K, Wenger JD, Swaminathan B, Broome CV (1992) Role of food in sporadic listeriosis: I. Case–Control-study of dietary risk factors. *J Am Med Assoc* 267:2041–2045
- Sinhamahapatra M, Biswas S, Das AK, Bhattacharyya D (2004) Comparative study of different surface decontaminants on chicken quality. *Br Poult Sci* 45:624–630
- Tezel U, Pavlostathis SG (2015) Quaternary ammonium disinfectants: microbial adaptation, degradation and ecology. *Curr Opin Biotechnol* 33:296–304
- Uyttendale MR, Neyts KD, Lips RM, Devere JM (1997) Incidence of *Listeria monocytogenes* in poultry products obtained from Belgian and French abattoirs. *Food Microbiol* 14:339–345
- Williams MS, Ebel ED, Golden NJ, Berrang ME, Bailey JS, Hartnett E (2010) Estimating removal rates of bacteria from poultry carcasses using two whole-carcass rinse volumes. *Int J Food Microbiol* 139:140–146