Food Control 30 (2013) 176–183

Contents lists available at SciVerse ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Synergistic effect of low concentration electrolyzed water and calcium lactate to ensure microbial safety, shelf life and sensory quality of fresh pork

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ARTICLE INFO

Article history:
Received 15 December 2011
Received in revised form
18 June 2012
Accepted 23 June 2012

Keywords:
Combination of LcEW + CaL
Microbial safety
Shelf life
Sensory quality
Pork meat

ABSTRACT

The objectives of this study were to evaluate the effectiveness of low concentration electrolyzed water (LcEW) and other carcass decontaminants against *Escherichia coli* O157:H7 and *Listeria monocytogenes* in fresh pork and to conduct the shelf life/sensory study of pork. Pork samples were inoculated with approximately 5 log cfu/g of afore mentioned pathogens and dip treated with distilled water (DW), aqueous ozone (AO), 3% lactic acid (LA), 3% calcium lactate (CaL), sodium hypochlorite solution (NaOCl), LcEW, strong acidic electrolyzed water (SAEW), and LcEW + CaL for 5 min at room temperature (23 ± 2 °C). The greatest reduction (3.0–3.2 log cfu/g) was achieved with LcEW + CaL against pathogens and significantly differed ($p < 0.05$) from other treatments. This combination also extended shelf life of pork up to 6 days at 4 °C storage.

1. Introduction

About 40 percent of all meat consumed in the world is pork, followed by poultry meat at 30 percent, and beef at 25 percent (FAO, 2006). Although the consumption of pork products is increasing, the microbial safety of pork during storage and marketing remains a concern. Meat products are highly perishable, and food poisoning can occur as a result of careless processing and storage (Aymerich, Picouet, & Monfort, 2008). The main flora responsible for spoilage in fresh meat products during aerobic storage is the Pseudomonas species and they are dominant in poultry meat, pork and beef and lamb (Coates, Beattie, Morgan, & Widders, 1995). Microbial contamination in meat is an important factor associated with meat quality. It has been found that bacterial contamination, such as *Salmonella* Typhimurium, *Escherichia coli* O157:H7 and *Listeria monocytogenes*, impacted meat safety (Cutter, 2000; Dorsa, Cutter, & Siragusa, 1998; Nissen, Alvseike, Bredholt, Holck, & Nesbakken, 2000). Therefore, to improve the microbial safety of pork during processing and storage, various processing techniques have been used for reduction of bacterial contaminants to extend shelf life (Latha, Sherikar, Waskar, Dubal, & Ahmed, 2009; Schirmer & Langsrud, 2010; Viana, Gomide, & Vanetti, 2005).

Constant efforts have been made to create effective and new technologies for the decontamination of carcasses and meat products (Huffman, 2002; Zhou, Xu, & Liu, 2010). Several intervention strategies have been developed to reduce the level of bacteria on pork or other animal carcass surfaces such as washing and sanitizing with hot or chilled water (Frederick, Miller, Thompson, & Ramsey, 1994; Özdemir et al., 2006), chlorinated and electrolyzed water (Ding, Rahman, Purev, & Oh, 2010; Fabrizio & Cutter, 2004; Park, Hung, & Brackett, 2002), food grade acids (Dubal et al., 2004; Pipek et al., 2006), salts (Jensen et al., 2003; Latha et al., 2009), ozone (Jaksch et al., 2004), chlorine dioxide (Pohlman, Stivarius, McElyea, Johnson, & Johnson, 2002), essential oil and nisin (Solomakos, Govaris, Koidis, & Botsoglou, 2008). All these sanitizers act differently on different types of organisms, but the information is limited on the action of these sanitizers on artificially inoculated specific organisms in meat. Moreover, most of these sanitizers are made from the dilution of condensed solutions, which in handling involves some risk and is troublesome. A sanitizer named low concentration electrolyzed water (LcEW) that is not produced from the dilution of a hazardous condensed solution has been reported in several previous researches as a safe and promising sanitizer (Ding, Rahman, & Oh, 2011; Rahman, Ding, & Oh, 2010a, 2010b). Thus, LcEW is being used in this study as an alternative non-thermal sanitizer, and furthermore, it has been

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http://dx.doi.org/10.1016/j.foodcont.2012.06.041

reported from our previous research that LcEW treatment could also help to maintain the physicochemical and sensory quality of fresh chicken breast meat (Rahman, Park, Song, Al-Harbi, & Oh, 2012). Combinations of LcEW and other measures are also possible. Organic acid salts such as calcium lactate have been used in the meat industry because of their ability to increase flavor, prolong shelf life, and improve the microbiological safety of products (Lawrence, Dikeman, Hunt, Kastner, & Johnson, 2003; Naveena, Sen, Muthukumar, Vaithiyanathan, & Babji, 2006; Selgas, Salazar, & García, 2009). Meat and meat products are considered to be a relatively minor source of calcium (Fennema, 1996), so CaL dipping is useful for enriching these meat products with calcium. In addition, CaL helps to maintain tenderness and palatability of meat products (Lawrence et al., 2003). Therefore, we combined LcEW + CaL and applied in our study to find any synergistic or hurdle effect. Accordingly, the present work was undertaken to study and compare the antimicrobial effect of LcEW alone and its combination with CaL as a safe and natural sanitizer in handling or food application comparison with other commercial sanitizers against background flora and inoculated pathogens associated with fresh pork. Sensory quality and shelf life of pork at refrigeration temperature (4 °C) was also studied.

2. Materials and methods

2.1. Bacterial cultures

The three strains each of *E. coli* O157:H7 (B0259, B0297 and B0299) and *L. monocytogenes* (ATCC 19115, ATCC 19111 and Scott A) used in this experiment were obtained from Department of Food Science, University of Georgia (Griffin, GA, USA), and Health Research Department (Gyeonggi-do, Republic of Korea), respectively. Stock cultures of each pathogen were transferred into tryptic soy broth (TSB; Difco, NJ, USA) and incubated for 24 h at 35 °C. Following incubation, 10 mL of each culture was sedimented by centrifugation (4000× g for 10 min at 4 °C), washed and resuspended in 10 mL of 0.1% peptone water (pH 7.1) to obtain a final cell concentration of 10^9 cfu/mL. Subsequently, resulting suspensions of each strain of the 2 pathogens were combined to construct culture cocktails. These culture cocktails were used in the following experiments. The bacterial population in each cocktail culture was confirmed by plating 0.1 mL portions of appropriately diluted culture on tryptic soy agar (TSA) plates and incubating the plates at 35 °C for 24 h.

2.2. Sample preparation

Boneless pork loins (48 h post-slaughter) were obtained from a retail store in Chuncheon, Korea. External fats and fascia were removed and then stored in a refrigerator at 4 °C prior to use for the experiment within 3 h. Pork samples were cut into pieces of similar size (2.5 × 2.5 cm) using a sterile knife. Before inoculation, each sample weighed 10 ± 0.2 g.

2.3. Inoculation

To destroy the background microflora, pork samples were surface treated using UV light in a biological safety hood. Surfaces were evenly exposed to UV light by turning sections every 10 min for a total time of no more than 30 min (Cutter & Siragusa, 1994). After applying this treatment, the naturally existing bacterial population was reduced to an undetectable level (with 10 cfu/g detection limit). Accordingly, mixed inocula (0.1 mL, more than 10^9 cfu/mL) of *E. coli* O157:H7 and *L. monocytogenes* were spread separately on the top and bottom surface of each piece of fresh pork using a sterile glass rod to obtain an inoculated level of 10^5 log cfu/g (Zhang, Kong, Xiong, & Sun, 2009). Then the samples were kept in a laminar flow hood for 20–30 min at room temperature (23 ± 2 °C) to allow for bacterial attachment. Inoculated samples without LcEW, SAEW, and LcEW + CaL treatments were used as control.

2.4. Preparation of treatment solutions

Low concentration electrolyzed water (LcEW), with a pH of 6.8,oxidation reduction potential (ORP) of 700–720 mV and available chlorine concentration (ACC) of 10 mg/L used in this study was produced by electrolysis of a dilute NaCl solution (0.9%) in a chamber without a membrane using an electrolysis device (model D-7, Dolki Co. Ltd., Wonju, Korea) at a setting of 3 V and 1.47 A. Strong acid electrolyzed water (SAEW) with a pH of 2.54, ORP of 1100–1120 mV was generated using electrolyzed water (EW) generator (A2-1000, Korean E& S Fist Inc, Seoul, Korea) including a small amount of salt solution (0.1%) and tap water at a setting of 12 A. with a residual chlorine concentration of about 50 mg/L. As reported, SAEW was made by EW generator with a membrane to separate the positive pole and negative pole, which had an acidic pH, higher ORP value and always initially had a higher residual chlorine concentration, compared to LcEW (Rahman et al., 2010a). The sodium hypochlorite solution (NaOCl: pH 9.8, 100 mg/L available chlorine) was prepared with the addition of 0.1 g of NaOCl (DC Chemical Co., Seoul, Korea) in 1 L of sterile DW. 3% (v/v) lactic acid (pH 2.35) solutions were prepared with DW by using LA (90%, Merck). 3% (w/v) calcium lactate (pH 6.5) solutions were prepared with DW by using CaL (98%, Yakuri pure chemicals co. ltd., Kyoto, Japan). Aqueous ozone (5 ppm) was produced on site by an electrochemical process using a green water ozone generator (GW-1000, Youl chon, Korea). Distilled water was used as control. The pH, ORP and available chlorine concentration of treatment solutions (LcEW and SAEW) were measured immediately before treatment with a dual-scale pH meter (Accumet model 15, Fisher Scientific Co., Fair Lawn, NJ) with pH and ORP electrodes. The residual chlorine was determined by a colorimetric method using a digital chlorine test kit (RC-3F, Kasahara Chemical Instruments Corp., Saitama, Japan). The detection limit is 0–300 mg/L.

2.5. Dip wash treatments and microbiological analysis of meat samples

Inoculated and uninoculated pork samples (10 g) were placed in sterile containers and immersed in treatment solutions (DW, AO, 3% LA, 3% CaL, NaOCl, LcEW, SAEW, and LcEW + CaL) at room temperature (23 ± 2 °C). Unwashed meat samples were used as control. To evaluate the effect of dipping time on the reduction of microorganisms, each 10 g piece of uninoculated pork was dipped for 0, 1, 3, 5, 7, and 10 min, respectively and finally 5 min dipping was chosen for subsequent experiment. Following treatments, all samples were aseptically excised and immediately placed in a stomacher bag (Nasco Whirl-Pak, Janesville, WI, USA) containing 90 mL of BPW and homogenized for 2 min with a Seward stomacher (400 Circulator, Seward, London, UK). After homogenization, 1 mL aliquots of the sample were serially diluted in 9 mL of sterile buffered peptone water and 0.1 mL of sample or diluents was spread-plated onto each selective medium. Total bacterial counts were determined by plating appropriately diluted samples onto Tryptic Soy Agar (TSA). Yeasts and molds were plated on Potato Dextrose Agar (PDA; Difco). Two selective media of Sorbitol MacConkey agar (SMAC; Difco) and Oxford Agar Base (OAB; Difco) were used for the enumeration of *E. coli* O157:H7 and *L. monocytogenes*, respectively. All plates were incubated at 37 °C for 24 h, except for