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Combined effects of slightly acidic electrolyzed water and fumaric acid on the reduction of foodborne pathogens and shelf life extension of fresh pork

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ABSTRACT

This study evaluated the efficacy of the individual treatments (slightly acidic electrolyzed water [SAcEW] or fumaric acid [FA]) and their combination to reduce *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella* Typhimurium in fresh pork as well as to study the shelf life and sensory quality (color, odor, and texture) of pork during storage at 4 and 10 °C. The inoculated pork samples (10 g) were dipped for 3 min in each treatment (tap water [TW], SAcEW, strong acidic electrolyzed water [StAEW], 0.5% FA, or SAcEW + 0.5% FA) with or without mild heat (40 °C). Decontamination of fresh pork with SAcEW +0.5% FA at 40 °C for 3 min showed greater bactericidal effect compared to other treatments, which significantly ($P < 0.05$) reduced *E. coli* O157:H7, *L. monocytogenes*, *S. aureus*, and *S.* Typhimurium by 2.59, 2.69, 2.38, and 2.99 log CFU/g, respectively. This combined treatment significantly ($P < 0.05$) yielded in a longer lag time of naturally occurring bacteria (TBC) on pork stored at 4 °C. This combined treatment also prolonged the shelf life of pork up to 6 days and 4–5 days when stored at 4 °C and 10 °C, respectively, compared to those of the untreated pork. The results suggest that the combined treatment of SAcEW + 0.5% FA has potential as a novel method to enhance the microbial safety and quality of fresh pork.

1. Introduction

Pork is the most consumed meat in the world, but pathogen contaminations such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella* Typhimurium have impacted its safety and quality (Baer, Miller, & Dilger, 2013; Mataragas, Skandamis, & Drosinos, 2008). *Salmonella* spp., *L. monocytogenes*, and *S. aureus* are among the top pathogens associated with pork and pork products, which cause foodborne illnesses and deaths annually. The Centers for Disease Control and Prevention (CDC) reported that these pathogens have caused 47.8 million illnesses and 3000 deaths each year (CDC, 2011). Moreover, product recalls, decreased sales from damaged organizational reputation, and spoilage of meat can lead to food waste and economic losses as well as the loss of consumer confidence (Nychas, Marshall, & Sofos, 2007; Scharff, 2010). Therefore, to improve the microbial safety and quality of pork during processing and storage, various chemical sanitizers have been investigated to reduce its microbial contaminations and extend shelf life. In recent years, antimicrobial agents from chemicals such as chlorinated solutions, electrolyzed water (EW), organic acids, and salts, alone or in combination have been investigated and shown to reduce microbial contaminations of pork (Chen et al., 2012; Smulders & Greer, 1998).

Slightly acidic electrolyzed water (SAcEW) is a type of EW and promising sanitizer for food products (Huang, Hung, Hsu, Huang, & Hwang, 2008). However, the use of SAcEW as an individual treatment has not effectively reduced bacterial contaminations in fresh meat (Ding, Rahman, Purev, & Oh, 2010; Rahman, Wang, & Oh, 2013). Therefore, it has frequently been combined with other chemical sanitizers to enhance its bactericidal efficacy. Organic acids are GRAS (Generally Recognized as Safe) compounds, which have ability to inactivate foodborne pathogens (Chen et al., 2012). Among the organic acids used for antimicrobial agent on meat, fumaric acid (FA) has shown stronger bactericidal effect compared to acetic and lactic acid (Podolak, Zayas, Kastner, & Fung, 1995, 1996). Even though the SAcEW and FA seem to be promising sanitizers for meat

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and meat products, their application on meat and meat products remains limited. Moreover, there is no research on the application of the combined treatment (SAcEW and FA) for decontamination and extending the shelf life of meat and meat products published to date. Therefore, the current study was conducted to evaluate the efficacy of the individual treatments (SAcEW or FA) and their combination on reducing *E. coli* O157:H7, *L. monocytogenes*, *S. aureus*, and *S.* Typhimurium in fresh pork. The shelf life and sensory quality of pork during storage at 4 and 10 °C were also studied.

2. Materials and methods

2.1. Preparation of bacterial cultures

The two strains of *E. coli* O157:H7 (ATCC 43894 and ATCC 43895), *L. monocytogenes* (ATCC 19114 and ATCC 19115), *S. aureus* (ATCC 12598 and ATCC 14458), and *S.* Typhimurium (ATCC 13311 and ATCC 14028) were obtained from the Department of Food Science, University of Georgia (Griffin, GA, USA), the Korean National Institute of Health (Seoul, Korea), and the Health Research Department (Gyeonggi-do, Republic of Korea), respectively. Prior to use, each strain of pathogens was separately grown in tryptic soy broth (TSB; Difco, Becton, Dickinson and Company Sparks, MD, USA) at 37 °C with two consecutive transfers after a 24 h period for a total 48 h of incubation. All working cultures grown in TSB were separately centrifuged at 4000 × *g* for 10 min at 4 °C, and the supernatants were discarded. The cell pellets were washed twice with 0.1% sterile buffered peptone water (BPW; Difco, Becton, Dickinson and Company Sparks, MD, USA) pH 7.1, and resuspended in 10 mL of the same solution to obtain a final cell concentration of approximately 8 log CFU/mL.

The two strains each of *E. coli* O157:H7, *L. monocytogenes*, *S. aureus*, or *S.* Typhimurium was combined in a cocktail with approximately equal numbers in the final population (8 log CFU/mL). The bacterial population in each culture cocktail was confirmed by plating 0.1 mL portions of appropriately diluted culture on tryptic soy agar (TSA; Difco, Becton, Dickinson and Company Sparks, MD, USA) plates, and then incubating the plates at 37 °C for 24 h. The prepared culture cocktails were then used in subsequent experiments.

2.2. Preparation of pork samples

Boneless pork loin was transported from a local slaughterhouse (Kangwon LPC, Wonju, Kangwon, Korea) to the laboratory under temperature-controlled conditions in a sterile plastic container. All pork samples were then separately cut into pieces using a sterile knife. Aliquots with a weight of 10 ± 0.3 g were used for the decontamination and storage tests, and those of 25 ± 0.3 g were used for the sensory analysis and measurement of the pH value.

2.3. Inoculation of pathogens to pork samples

Pork samples were placed on sterile petri dishes in a laminar flow hood, then spot inoculated by pipetting 0.1 mL of each culture cocktail (approximately 8 log CFU/mL) onto the surface to obtain an initial level of approximately 6 log CFU/g. The inoculated pork samples were air-dried in a laminar flow hood for 1 h at room temperature to allow attachment of bacteria, and then immediately exposed to the treatments.

2.4. Preparation of sanitizing solutions

The commercially available slightly acidic electrolyzed water (SAcEW; 30 mg/L) and strong acidic electrolyzed water (StAEW; 30 mg/L) used in this study were obtained from the Korea Advanced Institute of Science and Technology (KAIST, Daejeon, Korea). SAcEW and StAEW were produced by electrolysis of 6% and 9% HCl solutions, respectively, using an electrolysis device. The crystalline fumaric acid (FA) (Daejung Chemicals and Metals Co., Siheung, Gyeonggi, Korea) was dissolved in 1 L of deionized water (DW) to give a final concentration of 0.5% FA solutions (w/v). The pH, oxidation reduction potential (ORP), and available chlorine concentration (ACC) of the sanitizers were measured with a dual-scale pH meter (Accumet model 15, Fisher Scientific Co., Fair Lawn, NJ, USA) bearing pH and ORP electrodes. The ACC was determined by a colorimetric method using a digital chlorine test kit (RC-3F, Kasahara Chemical Instruments Corp., Saitama, Japan). The detection limit was 1–300 mg/L. The physicochemical properties of the tested sanitizing solutions are summarized in Table 1.

2.5. Decontamination treatment and microbiological analysis

The preliminary *in vitro* experiments were carried out to determine the optimal condition for each individual treatment of SAcEW, StAEW, and organic acids (lactic acid [LA], citric acid [CA], acetic acid [AA], and fumaric acid [FA]). The experiments were conducted at different temperatures (25, 30, 40, 50, and 60 °C), different ACCs for EW (5, 10, 20, and 30 mg/L), different concentrations for organic acids (0.125, 0.25, and 0.5%), and different exposure times (1, 3, and 5 min). The results suggested that dipping with EW (30 mg/L) or 0.5% FA at 40 °C for 3 min was the most satisfactory method (data not shown). These methods were then employed in the *in vivo* study.

For the *in vivo* study, inoculated pork samples (10 g) were placed in a sterile container and dipped for 3 min in each treatment (tap water [TW], SAcEW, StAEW, 0.5% FA, SAcEW + 0.5% FA) with or without mild heat (40 °C). Unwashed pork samples were used as control. The excess solution was removed from pork samples with sterile paper towels. Each 10 g pork sample was then mixed with 90 mL of 0.1% sterile BPW and homogenized for 2 min in a Seward stomacher (400 Circulator, Seward, London, UK). After homogenization, 1 mL aliquot of each sample was serially diluted in 9 mL of 0.1% sterile BPW, and 0.1 mL of diluents was spread-plated onto sorbitol MacConkey's agar (Difco, Becton, Dickinson and Company Sparks, MD, USA), modified Oxford agar base with the addition of the Oxford antimicrobic supplement (Oxoid, Basingstoke, UK), Baird Parker agar (Difco, Becton, Dickinson and Company Sparks, MD, USA) supplemented with 50 mL of the egg yolk Tellurite, and Brilliant Green agar (Oxoid, Basingstoke, UK) to enumerate *E. coli* O157:H7, *L. monocytogenes*, *S. aureus*, and *S.* Typhimurium,

Table 1
Physicochemical properties of the tested sanitizing solutions.[a]

Tested solutions	pH	ORP (mV)[b]	ACC (mg/L)[c]
TW	7.17 ± 0.12^{A}	358 ± 10^{A}	ND[h]
SAcEW[d]	6.29 ± 0.17^{B}	826 ± 16^{C}	30 ± 0.2
StAEW[e]	2.31 ± 0.08^{C}	1159 ± 12^{D}	30 ± 0.6
0.5% FA[f]	2.34 ± 0.11^{C}	570 ± 9^{B}	ND
SAcEW + 0.5% FA[g]	2.95 ± 0.13^{C}	1091 ± 19^{D}	15 ± 0.09

[A–D] Numbers within each column followed by different capital letters are significantly different ($P < 0.05$).
[a] Values are mean ± standard deviation, $n = 6$.
[b] Oxidation reduction potential.
[c] Available chlorine concentration.
[d] Slightly acidic electrolyzed water.
[e] Strong acidic electrolyzed water.
[f] 0.5% fumaric acid.
[g] Slightly acidic electrolyzed water (30 mg/L) + 0.5% fumaric acid.
[h] Not detected.