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Short communication

Evaluation of a novel antimicrobial solution and its potential for control *Escherichia coli* O157:H7, non-O157:H7 shiga toxin-producing *E. coli*, *Salmonella* spp., and *Listeria monocytogenes* on beef

Kimberly D. Fisher [a], Christy L. Bratcher [a], Tony Z. Jin [b], Sacit F. Bilgili [c], Walter F. Owsley [a], Luxin Wang [a, *]

[a] Department of Animal Sciences, Auburn University, Auburn, AL 36849, USA
[b] Eastern Regional Research Center, Agricultural Research Service, USDA, Wyndmoor, PA 19038-8598, USA
[c] Department of Poultry Science, Auburn University, Auburn, AL 36849, USA

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ABSTRACT

The goal of this study was to evaluate the efficacy of a novel antimicrobial solution made with chitosan, lauric arginate ester, and organic acids on *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*, and non-O157 shiga toxin-producing *E. coli* cocktails and to test its potential to be used as a marinade for raw beef. Fresh beef top round steaks were surface-inoculated with the pathogen cocktails at approximately 2.5 or 4.5 Log CFU/cm^2, marinated with the antimicrobial solution (AMS), and then stored at 4 °C for 6, 24, and 48 h. Three commercially available marinades were used for comparison. Results revealed that AMS had the most antimicrobial effect regardless of the type or inoculation level of pathogens ($P < 0.05$). After 6 h, the AMS marination reduced all pathogens to levels below the limit of detection (<1 Log CFU/cm^2), resulting in a 3.5 Log CFU/cm^2 reduction. When AMS was diluted with autoclaved distilled water by 5 times (AMS 1:5) or 10 times (AMS 1:10), its antimicrobial efficacy was impacted by marination time, the inoculated pathogens, and the inoculation levels. This study demonstrates that the developed antimicrobial solution has a great potential to be used during marination by consumers to ensure better food safety.

1. Introduction

Foodborne pathogen involved outbreaks have been of public concern. *Listeria* and *Salmonella* (nontyphoidal) are pathogens that cause the most death according to the CDC (CDC., 2011). *Escherichia coli* O157:H7 has been one of the top five pathogens that contribute to domestically acquired foodborne illness resulting in hospitalization (CDC., 2011). In 2012, six additional shiga toxin producing *E. coli* serovars, including *E. coli* O26, O111, O103, O121, O45, and O145, were placed on the zero tolerance adulterant list (USDA., 2011).

Since 1993, the beef industry spent more than $420 million on beef safety research which generated a significant amount of information and usable best practices (Muras, Lucia, Hardin, Savell, & Harris, 2009). The development of novel antimicrobial solutions is one of the major achievements. Among those solutions, a novel antimicrobial solution made with generally recognized as safe (GRAS) antimicrobial compounds was first proposed to be used as a ready-to-eat turkey breast surface treatment in 2014 (Guo, Jin, Wang, Scullen, & Sommers, 2014). In 2015, Wang, Zhao, Yuan, and Jin (2015) evaluated its application on roast beef and their results showed that this antimicrobial solution made with chitosan, lauric arginate ester (LAE), and organic acids inhibited the growth of inoculated *Listeria monocytogenes*. The sensory evaluation conducted (Wang et al., 2015) showed that although a slightly bitter taste was noticed by panelists immediately after the application of the solution, panelists were not able to tell the difference between the treated and untreated samples after 15 days of storage. The proposed antimicrobial also had a color protective effect on roast beef. The treated samples had a fresher looking color than the untreated samples after 30 days of storage at 4 °C.

The goal of this study was to investigate the efficacy of the antimicrobial solution against foodborne pathogens, including

* Corresponding author. 210 Upchurch Hall, Auburn University, Auburn, AL, 36849, USA.
E-mail address: lzw0022@auburn.edu (L. Wang).

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E. coli O157:H7, *Salmonella*, *L. monocytogenes*, and non-O157 Shiga toxin-producing *E. coli*, and to evaluate its efficacy on fresh meat when using it as a meat marinade at 4 °C.

Marination of meat is an emerging industrial technique for improving meat tenderness, flavor and extending meat shelf life (Pathania, McKee, Bilgili, & Singh, 2010). Marination provides the opportunity to allow potential antimicrobial components to be in contact with pathogenic bacteria. Thus, marination becomes one food preparation step which not only adds product value but also provides an opportunity to achieve better food safety (Pathania et al., 2010). This study also used three commercially available marinades (a balsamic & roasted onion classic marinade, a lemon & cracked pepper marinade, and a classic steakhouse marinade) for comparison. The specific objectives of this study were to 1) determine the effectiveness of three concentrations of an antimicrobial solution used during marination at 4 °C, and 2) compare its antimicrobial effects with three retail marinades.

2. Materials and methods

2.1. Bacteria culture

Four bacterial cocktails were used in the study: an *E. coli* O157:H7 cocktail (ECO157), a non-O157 shiga toxin-producing *E. coli* cocktail (STEC), a *Salmonella* spp. cocktail (SAL), and a *L. monocytogenes* cocktail (LM). The strains included in each cocktail are listed in Table 1. All strains were obtained from the Food Microbiology and Safety lab at Auburn University. To prepare each cocktail, individual strains were grown in 9 mL of sterile tryptic soy broth (TSB) at 37 °C for 24 h, they were then washed by centrifugation (3650 rpm) for 20 min (5810R Eppendorf, Hauppauge, New York, USA) and resuspended in 9 mL of autoclaved distilled water. Equal volumes of washed strains were transferred and mixed in a new tube. The initial cell counts of the cocktails were checked by diluting and plating cocktail cultures on selective MacConkey Agar with Sorbitol (for ECO157), CHROMagar™ STEC (for STEC), XLT4 (for SAL) and Modified Oxford Medium (for LM). Plates were enumerated after 24 h incubation at 35 °C. The original cocktails were then diluted in 0.1% buffered peptone water (BPW) before they were used for meat inoculation. All of the media used in this study was purchased from Neogen Corporation (Lansing, Michigan, USA).

2.2. Antimicrobial solution preparation

The stock antimicrobial solution (AMS) containing 5% chitosan (low molecular weight, 150 kDa, 75–85% deacetylation) (w/v), 2% each of acetic, lactic and levulinic acids and 4% lauric arginate acid (LAE) (v/v) was made followed the protocols described by Wang et al. (2015). Chitosan, acetic acid, lactic acid and levulinic acid were purchased from Sigma–Aldrich (St. Louis, Missouri, USA); lauric arginate ester (LAE) solution (CytoGuard®) containing 20% LAE was received from A&B Ingredients (Farefield, New Jersey, USA). In addition to AMS, the 1:5 dilution (AMS 1:5) and the 1:10 dilution (AMS 1:10) were also prepared by adding distilled water. The pH values of AMS, AMS 1:5, and AMS 1:10 were 3.0 ± 0.2, 4.2 ± 0.1 and 5.6 ± 0.2, respectively.

2.3. Meat sample inoculation and marination

Beef top round steaks were fabricated at the Lambert Powell Meats Laboratory at Auburn University. Lean meat samples were cut into 100 cm^2 pieces. Three 25-g subsamples were taken from the beef top round steaks and weighed. Those samples were enriched and plated to check for the presence or absence of pathogens following the FDA Bacteriological Analytical Manual (8th edition). Beef samples were shown to be negative for *E. coli* O157:H7, STEC, *Salmonella* spp., and *L. monocytogenes* before they were used for inoculation.

To inoculate the beef, one side of each piece was spread with 1 mL of the assigned inoculum cocktail using a disposable L-shaped cell spreader (VWR International, LLC, Radnor, Pennsylvania, USA). After inoculation, 30 min of contact time was allowed to let the cells attach to the meat surface. After the contact time, each piece of the inoculated beef sample was placed in a stomacher bag (Nasco Whirl-Pak®, Fort Atkinson, Wisconsin, USA) and marinated by adding 30 mL of the assigned antimicrobial solution or water control. The marinated beef samples were then stored at 4 °C and sampled immediately at time 0 and after 6, 24, and 48 h of marination.

Table 1
Strains used in cocktails.

Cocktails	Strain sources ATCC number or ID code
Escherichia coli O157:H7	*E. coli* O157:H7 ATCC 35150
	E. coli O157:H7 ATCC 43894
	E. coli O157:H7 AU − 1
	E. coli O157:H7 505B
	E. coli O157:H7 AU − 3
Non-O157 Shiga toxin-producing *E. coli*	*E. coli* O145 TWO9356
	E. coli O26 TWO7814
	E. coli O121 TWO8039
	E. coli O45 TWO14003
	E. coli O111 TWO7926
	E. coli O103 TWO8101
Salmonella spp.	*Salmonella* Enteritidis
	Salmonella Kentucky
	Salmonella Montevideo
	Salmonella Thompson
	Salmonella Stanley
Listeria monocytogenes	*L. monocytogenes* Scott A ATCC 49594
	L. monocytogenes ATCC 19115, 4b
	L. monocytogenes ATCC 7644
	L. monocytogenes 101 M, 4b
	L. monocytogenes 108M, 1/2 b