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Lactic acid, hot water, and microwave treatment to reduce natural microflora and pathogens in vacuum-packaged beef

Abstract

Combined lactic acid (2%), hot water, and microwave treatments were used to reduce natural microflora and the pathogens Escherichia coli O157:H7, Salmonella typhimurium, and Listeria monocytogenes in vacuum-packaged beef. Hot water at 158EF followed by vacuum packaging and 5 sec. of microwave were acceptable for microbial reduction. Dipping inoculated meat for 20 sec. into 2% room temperature lactic acid prior to that treatment at 158EF reduced E. coli O157:H7 by 1.05 log CFU/cm2, S. typhimurium by .7 log CFU/cm2, and L. monocytogenes by .85 log CFU/cm2 (CFU is colony forming unit). One log equals a 90% reduction, and 2 log a 99% reduction. With this treatment, meat color reverted to an acceptable value after 14 hr of storage at 39EF. Part 3 of the experiment combined 2% lactic acid and hot water treatments into one step. Dipping for 20 sec. in 176EF, 2% lactic acid then vacuum packaging and microwaving for 5 sec. reduced natural microflora by 1.8 log CFU/cm2, E. coli O157:H7 by 1.18 log CFU/cm2, S. typhimurium by 1.5 log CFU/cm2, and L. monocytogenes by 1.5 log CFU/cm2, with acceptable color values after 14 hr storage at 40EF. This combination was the most effective in reducing both natural and inoculated microorganisms and provides a low-cost alternative for decontamination of meat surfaces.

Keywords

Cattlemen's Day, 1999; Kansas Agricultural Experiment Station contribution; no. 99-339-S; Report of progress (Kansas State University. Agricultural Experiment Station and Cooperative Extension Service); 831; Beef; Pathogens; E. coli O157:H7; S. typhimurium; L. monocytogenes

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LACTIC ACID, HOT WATER, AND MICROWAVE TREATMENT TO REDUCE NATURAL MICROFLORA AND PATHOGENS IN VACUUM-PACKAGED BEEF

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Summary

Combined lactic acid (2%), hot water, and microwave treatments were used to reduce natural microflora and the pathogens *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* in vacuum-packaged beef. Hot water at 158EF followed by vacuum packaging and 5 sec. of microwave were acceptable for microbial reduction. Dipping inoculated meat for 20 sec. into 2% room temperature lactic acid prior to that treatment at 158EF reduced *E. coli* O157:H7 by 1.05 log CFU/cm^2, *S. typhimurium* by .7 log CFU/cm^2, and *L. monocytogenes* by .85 log CFU/cm^2 (CFU is colony forming unit). One log equals a 90% reduction, and 2 log a 99% reduction. With this treatment, meat color reverted to an acceptable value after 14 hr of storage at 39EF. Part 3 of the experiment combined 2% lactic acid and hot water treatments into one step. Dipping for 20 sec. in 176EF, 2% lactic acid then vacuum packaging and microwaving for 5 sec. reduced natural microflora by 1.8 log CFU/cm^2, *E. coli* O157:H7 by 1.18 log CFU/cm^2, *S. typhimurium* by 1.5 log CFU/cm^2, and *L. monocytogenes* by 1.5 log CFU/cm^2, with acceptable color values after 14 hr storage at 40EF. This combination was the most effective in reducing both natural and inoculated microorganisms and provides a low-cost alternative for decontamination of meat surfaces.

(Key Words: Beef, Pathogens, *E. coli* O157:H7, *S. typhimurium*, *L. monocytogenes*.)

Introduction

Much carcass decontamination research has been done using hot water, lactic acid, and microwave treatments. A combined carcass washing followed by a hot water spray (203EF) reduced inoculated pathogens on the surface of the carcasses. Using hot water decontamination cabinets (176EF, 10 and 20 sec.) significantly reduced pathogens of the surface of beef briskets. Steam Pasteurization™ reduces surface microorganisms, but it is not cost effective for small plants. Organic acids such as lactic acid may injure bacterial cells and make them more susceptible to hot water and microwave treatments. Use of microwaves also may reduce natural microflora and pathogens on meat surfaces. This experiment was performed to achieve the optimal combination of heat, lactic acid, and microwave treatment for microbial reduction. The goal was to reduce natural microflora, *E. coli* O157:H7, *S. typhimurium*, and *L. monocytogenes*, while maintaining fresh meat color.

Experimental Procedures

Escherichia coli O157:H7, *S. typhimurium*, and *L. monocytogenes* from the Food Microbiology Culture Collection at Kansas State University were inoculated into Brain Heart Infusion (BHI, Difco, Detroit, MI) broth and incubated at 100EF for 24 hr.

Beef sirloin steak from local grocery stores was cut into uniform pieces (2 3/4 × 2 3/4 in.) using a knife sterilized in an alcohol flame for 2 sec. In the first experiment, meat samples were not inoculated, and only natural microflora was enumerated. In experiments 2 and 3, meat samples were inoculated

with *E. coli* O157:H7, *S. typhimurium*, or *L. monocytogenes*, then vacuum packaged, dipped into hot solution (water or 2% lactic acid), and microwaved. Each pathogen was tested separately. The meat was immersed into a 2.0 liter beaker containing 1.0 liter of the appropriate inoculum (ca. 8.0-9.0 log CFU/ml). For experiment 2, meat was soaked in the inoculum for 10 sec. and held for 10 min at room temperature (77EF). Meat samples then were dipped into 2% lactic acid; placed into a nylon film bag (7 × 12 in.); and vacuum packaged with 100 mbar pressure. All vacuum packaging was performed in a bacteriological safety hood.

Experiment 1: Hot Water and Microwave Treatments. This experiment tested 140, 158, 176, and 194EF hot water treatments, followed by vacuum packaging and microwave treatment of 5, 7, and 10 sec. Meat samples were exposed to 140EF hot water for 10, 25, 40, or 55 sec.; to 158EF water for 5, 10, 15, or 20 sec.; to 176EF water for 3, 5, and 7 sec. or to 194EF hot water for 1, 2, or 3 sec. Each water treatment then was followed by 5, 7, or 10 sec. of microwave treatment. During each treatment, the immediate meat color change was monitored visually. If it changed to a cooked color during treatment, the sample was removed immediately, and a viable cell count (VCC) for natural microflora was performed. After treatment, color was assessed immediately using a scale of 1 to 5: 1=bright red fresh meat color, 2=red fresh meat color, 3= intermediate color, 4= cooked color, 5=very cooked color. Any treatment that caused an irrecoverable cooked color was considered unacceptable. Controls were used to monitor actual microbial reduction. To check the VCC, the center of each treated sample was cored with a sterile coring device (1.75 in^2). Then the core surfaces were removed aseptically and transferred to a sterile stomacher bag containing 56 ml of .1% sterile peptone diluent. The samples were stomached for 2 min, and 9 ml dilutions were prepared in .1% sterile peptone diluent. Natural microflora was enumerated by plating onto Plate Count Agar (PCA, Difco), and incubating at 90EF for 25 hr. Water temperatures of 158 and 176EF were chosen for further research.

Experiment 2: 2% Lactic Acid, Hot Water, and Microwave Treatments. After inoculation, samples were dipped into 2% room temperature lactic acid solution for 20 sec., then vacuum packaged and dipped into 158EF water for 30 sec., followed by 5 sec. of microwave treatment. Color was evaluated visually during and after treatment and after 15-18 hr of refrigerator storage. The vacuum bags were opened, and color recovery was examined every 2 hr up to 14 hr and also at 24 hr. VCCs were performed using procedures from experiment 1. *E. coli* O157:H7 was enumerated using MacConkey Sorbitol Agar (MSA, Difco), *S. typhimurium* was plated on Brilliant Green Agar (BGA, Difco), and *L. monocytogenes* was enumerated using Modified Oxford agar media (MOX, Difco).

Experiment 3: 2% Lactic Acid (176EF) Followedby Microwave Treatments. Based on antimicrobial activity and acceptable color values, meat samples were inoculated, dipped into 2% lactic acid solution (176EF) for 20 sec., then vacuum packaged and microwaved for 5 sec. After treatment, samples were stored at 40EF for 24 hr. After the bags were opened, color was evaluated every 2 hr up to 14 hr and at 24 hr. This combined the hot water and 2% lactic acid treatments into one step. Microbial counts were performed as in experiment 2.

Results and Discussion

Experiment 1: Hot Water and Microwave Treatments. Table 1 shows a typical data set for time, temperature, and microwave treatments with one hot water temperature (158EF). After treatment, the vacuum bags were opened, color changes were evaluated, and VCCs were taken. For 140EF hot water, 40 sec. was the maximum time with acceptable meat color, but this temperature/time did not reduce microbial numbers. At 158EF, 15 sec. was optimal with acceptable color changes. Natural microorganisms were reduced from 8.0 × 10^2 to 4.5 × 10^2 CFU/cm^2 (.3 log reduction). When the time was increased to 20 sec. in 158EF water, natural microorganisms were reduced by .6 log CFU/cm^2, but this 5 sec.

increase caused immediate cooked color. With 176EF hot water, 5 sec. was the maximum time of exposure with acceptable color, but this treatment did not reduce microorganisms (data not shown). For 194EF water, maximum dip time with acceptable color changes was 2 sec. Natural organisms were reduced from 1.6×10^2 to 7.0×10^1 (.4 log reduction). For microwave treatment of 7 sec., optimal times for each temperature were as follows: 40 sec. for 140EF, 15 sec. for 158EF, 5 sec. for 176EF, and 2 sec. for 194EF. Of these, the most microbial reduction (.3 to .5 logs) was seen with 176EF for 5 sec.

Temperatures of 140, 158, 176, and 194EF were tested with a microwave time of 10 sec. Maximum times for each hot water submersion were 40 sec., 10 sec., 3 sec., and 1 sec. for those temperatures. When 10 sec. or less of microwave treatment was used, microbial reduction was not significant.

Based on preliminary experiments, 158EF was chosen as the best temperature to use in further studies.

Experiment 2: 2% Lactic Acid, Hot Water, and Microwave Treatments. Samples exposed to 158EF water for 15 sec recovered good color after 6 hr of storage (Table 2), but samples exposed to 158EF for 30 sec. recovered only barely acceptable color values after 24 hr of storage. However, dipping those samples in 2% lactic acid before packaging reduced natural microflora (1.8 log CFU/cm^2) with recoverable color values (data not shown).

With the combination of 2% lactic acid dip for 20 sec., 158EF water for 15 sec., and microwave for 5 sec., pathogen reductions were as follows: .8 log CFU/cm^2 for *E. coli* O157:H7, .7 for *S. typhimurium*, and .7 for *L. monocytogenes*. For 158EF water for 30 sec., pathogen reductions were similar: 1 log CFU/cm^2 for *E. coli*, 12 for *S. typhimurium*, and .9 to 3.50 log for *L. monocytogenes*. Using these three treatments of lactic acid, hot water, and microwave killed 90% of foodborne pathogens (data not shown).

Experiment 3: 2% Lactic Acid (176EF) Followed by Microwave Treatments. The color of meat subjected to 2% lactic acid (176EF) for 15 and 20 sec. immediately changed to 5 (very cooked color, Table 3). When the opened meat samples were stored at 40EF, the color recovered to 3 (intermediate), which was acceptable. For 15 and 20 sec. treatments, color recovered in 8 hr and 14 hr, respectively. Natural microorganisms were reduced by 1.8, *E. coli* O157:H7 by 1.6, and *S. typhimurium* and *L. monocytogenes* by 1.5 log CFU/cm^2 (data not shown).

In conclusion, dipping meat in 2% lactic acid (176EF) for 15 sec., vacuum packaging the meat, and then treating the meat in a microwave for 5 sec. gave 90 to 99% reduction of pathogens and still allowed the meat to have acceptable color.

Table 1. Evaluation of Meat Surface Color and Microbial Counts after Hot Water (158EF) and Microwave (MW) Treatments

Item	Meat Color Score	Before Trt CFU/cm^2	After Trt CFU/cm^2
Control	1.0	8.0×10^2	8.0×10^2
5 sec. hot water, 5 sec. MW	1.3	8.0×10^2	1.6×10^3
10 sec. hot water, 5 sec. MW	2.3	8.0×10^2	6.6×10^2
15 sec. hot water, 5 sec. MW	3.0	8.0×10^2	4.5×10^2
20 sec. hot water, 5 sec. MW	5.0	8.0×10^2	2.0×10^2

Color scores: 1=bright red fresh meat color, 2=red fresh meat color, 3=intermediate, 4=cooked color, and 5=very cooked color.

Table 2. Mean Color Scores[a] during Storage at 40EF after Combined Treatment of Lactic Acid, Hot Water (158EF), and Microwave (5 sec.)

	Treatments[b]		
Conditions	Control	L,15H,5M	L,30H,5M
After treatment			
0 hr	1	3.2	5
Open			
0 hr	2.6	5	5
2 hr	2	4.2	4.8
4 hr	1	3.2	4.2
6 hr	1	2.2	4
8 hr	1	2.2	3.6
10 hr	1	2	3.2
12 hr	1	2	3.2
14 hr	1	1.8	3.2
24 hr	1	1.4	3

[a]Color scores: 1 = bright red fresh meat color, 2 = red fresh meat color, 3 = intermediate, 4 = cooked color, and 5 = very cooked color.
[b]L = 2% lactic acid spray, 15 H and 30 H = 15 and 30 sec. dip in 158EF water, 5M = 5 sec. in microwave.

Table 3. Color Changes[a] during Storage at 40EF after Combined Treatment of Hot 2% Lactic Acid (176EF) Solution and Microwave (5 Sec.)

	Treatments[b]				
Conditions	Control	5L, 5M	10L,5M	15L,5M	20L,5M
After treatment					
0 hr	1	2.2	3.6	5	5
Open					
0 hr	1	2.2	3.6	4.8	4.6
2 hr	1	1.6	3.0	4.8	4.6
4 hr	1	1.6	2.6	4.6	4.4
6 hr	1	1.6	2.2	3.6	3.6
8 hr	1	1.8	2.2	3.0	3.8
10 hr	1	1.4	1.6	2.8	3.4
12 hr	1	1.4	1.6	2.0	3.2
14 hr	1	1.4	1.8	1.8	3.0
24 hr	1	1.2	1.4	1.6	2.8

[a]Color scores: 1 = bright red fresh meat color, 2 = red fresh meat color, 3 = intermediate, 4 = cooked color, and 5 = very cooked color.
[b]L = 2% lactic acid; 5H, 10H, 15 H, and 20H are sec. application in 176EF; 5M = 5 sec. in microwave.