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Research Note

Combination Spray Washes of Saponin with Water or Acetic Acid to Reduce Aerobic and Pathogenic Bacteria on Lean Beef Surfaces†

CATHERINE N. CUTTER*

U.S. Department of Agriculture, Agricultural Research Service, Roman L. Hruska U.S. Meat Animal Research Center, P.O. Box 166, Clay Center, Nebraska 68933, USA

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ABSTRACT

Saponins are naturally occurring compounds known as triterpenoid glycosides found in a variety of plant species. Saponins are approved for use in the food industry as foaming agents. When combined with water or organic acid in spray treatments, saponins' foaming property may improve carcass decontamination. In the first experiment of this study, lean beef carcass surfaces were experimentally inoculated with a fecal slurry containing antibiotic-resistant *Escherichia coli* O157:H7 and *Salmonella* Typhimurium. Spray-washing treatments with 1% saponin followed by a water wash, or 1% saponin followed by 2% acetic acid, were more effective for reducing aerobic bacteria than saponin, water, or 2% acetic acid washes alone. However, 1% saponin followed by a either a water or 2% acetic acid wash was no more effective than a 2% acetic acid wash for reducing populations of *E. coli* O157:H7 or *Salmonella* Typhimurium. In the second experiment, experimentally inoculated beef surfaces were subjected to spray treatments with water followed by another water wash, water followed by a 2% acetic acid wash, 1% saponin followed by a water wash, or 1% saponin followed by a 2% acetic acid wash. When examined for effectiveness against all bacterial populations, 1% saponin followed by a water wash and 1% saponin followed by a 2% acetic acid wash were as effective as two water washes or a water wash followed by 2% acetic acid for reducing aerobic bacteria, *E. coli* O157:H7, and *Salmonella* Typhimurium from beef surfaces. Under the conditions described, reductions associated with combination spray washes may be attributed to the physical removal of bacteria during the spraying process, not to any specific action of saponin.

Saponins are a class of naturally occurring compounds known as triterpenoid glycosides. They are found in roots, shoots, seeds, and flowers of many plant species, including chickpeas, navy and kidney beans, soybeans, alfalfa, cereals, peanuts, and asparagus *(7, 15)*. These compounds are stable in basic solutions but hydrolyzed readily in acid solutions or by glycosidases (pectinases, amylases *(7)*). Saponins have demonstrated antifungal and antibacterial activity against a wide variety of microbes, including rhizosphere bacteria *(15)*, *Pseudomonas* spp., *Streptococcus faecalis, Staphylococcus aureus (1), E. coli, Klebsiella* spp., and *Salmonella* Typhimurium *(10)*. Saponins have also been found to exhibit growth-modulating effects on certain bacteria, including *E. coli (11)*. Saponins also have been used as emulsifiers or foaming agents in soft drinks, baked goods, confectionery, and dairy desserts *(7, 15)*. These compounds are capable of lysing red blood cells, are used as adjuvants for vaccines, are known to lower blood cholesterol, and are highly toxic to fish *(7, 15)*.

Saponin No. 374 (Technology Management and Funding, L. P., Princeton, N.J.) is produced by *Quillaja saponaria,* or soapbark, an evergreen tree found in South America *(14)*. In general, saponins are water-soluble, colorless compounds commonly used as food additives *(3)*. Rodrick *(10)* demonstrated that a 10,000-ppm concentration of saponin No. 374 could be used as an antimicrobial to reduce total plate counts (TPC) and fecal coliforms by approximately 1 $\log_{10}$ CFU/g when it was directly added to oyster meat.

Researchers have demonstrated that combination spray treatments of hydrogen peroxide followed by acetic acid or sodium bicarbonate were more effective than single washes of the individual compounds for reducing bacterial populations on meat surfaces *(2)*. Other researchers have indicated that ambient water followed by a hot water wash reduced contamination more effectively than single washes of either ambient-temperature or hot water *(6)*. Combination spray treatments of high-pressure ambient water followed by a low-pressure antimicrobial rinse or hot-water spray are currently used by processors to reduce

* Author for correspondence. Tel: 402-762-4386; Fax: 402-762-4149; E-mail: cutter@email.marc.usda.gov.

† Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product. Use of a name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

TABLE 1. *Experiment 1: remaining bacterial populations following treatments of experimentally inoculated beef carcass tissue with water, 1% saponin, 2% acetic acid, 1% saponin followed by a water wash, or 1% saponin followed by a 2% acetic acid wash*[a]

Organism	Untreated	Water	Saponin	Acetic acid	Saponin–Water	Saponin–Acetic acid
Aerobic bacteria	6.59 A[b]	4.49 C	4.74 B	4.06 D	3.36 E	3.23 E
E. coli O157:H7	6.19 A	3.92 B	4.23 B	2.99 C	3.08 C	2.79 C
Salmonella Typhimurium	5.63 A	3.40 B	3.78 B	0.69 E	2.55 C	1.19 D

[a] All sprays were applied at 125 psi, for 15 s at 40°C ± 2°C; $n = 6$.
[b] Data with the same letter in the same row are not significantly different.

both bacterial and visible contamination on meat animal carcasses *(8)*.

When combined with other established treatments, saponins' foaming property may improve carcass decontamination. An experimental protocol and findings are presented to address the effects of saponin alone or in combination with water or acetic acid spray washes for reducing mesophilic aerobic bacteria, *Salmonella* Typhimurium, and *E. coli* O157:H7 attached to beef surfaces.

MATERIALS AND METHODS

Bacterial cultures. A streptomycin-resistant strain of *E. coli* O157:H7 and a nalidixic acid–resistant strain of *Salmonella* Typhimurium were obtained from the Roman L. Hruska U.S. Meat Animal Research Center (MARC) culture collection. The strains were maintained in 75% glycerol at −20°C *(5)*. *E. coli* O157:H7 and *Salmonella* Typhimurium were propagated for 18 h in trypticase soy broth (TSB; Difco, Detroit, Mich.) containing 500 μg/ml of streptomycin sulfate (Sigma, St. Louis, Mo.) or 250 μg/ml of nalidixic acid (Sigma), respectively, at 37°C for 18 h.

Beef shortplates, bovine feces, and inoculation procedures. The cutaneous trunci muscle (consisting of lean tissue) was obtained within 15 min after exsanguination from beef carcass sides processed at a local slaughterhouse. Tissues were placed in plastic bags, stored in insulated carriers to prevent rapid cooling, transported to MARC, and used within 2 h of slaughter. Tissues were cut into 12-cm × 12-cm × 0.5-cm sections before spray washing in order to fit on stainless steel boards used in the insertable hood.

On each experiment day, feces were obtained from three cows fed a corn silage ration. One hundred grams of each feces sample were obtained and mixed together with 100 ml of sterile physiological saline containing select bacterial organisms (approximately 6 $\log_{10}$ CFU/g feces). Individual pieces of 12-cm × 12-cm beef carcass tissue were inoculated with the fecal slurry using a paintbrush and left undisturbed for 15 min. The final concentrations of *E. coli* O157:H7 and *Salmonella* Typhimurium on meat surfaces were approximately 6 and 5 $\log_{10}$ CFU/cm^2, respectively.

Spray washing procedures. All spray washes were conducted in the insertable pod in a laminar airflow hood *(6)*. Saponin No. 374 was mixed in tap water to obtain a final concentration of 1% (wt/vol). The operation parameters for the washer were as follows: spray nozzle oscillation speed, 60 cycles/min; exposure time of inoculated tissue to spray, 15 s; line pressure, 125 psi; flow rate, 4.8 liters/min; nozzle, 25/10 Spraying Systems (Wheaton, Ill.); and spray temperature, 40 ± 2°C (Model 40605 automatic 10-point temperature scanner, Davis Instruments, Inc., Baltimore, Md.) The spray treatments used in experiment 1 were water, 1% saponin, 2% acetic acid, 1% saponin followed by a water wash, or 1% saponin followed by a 2% acetic acid wash. Because of the time required to change over the compounds and flush the lines during sequential spray washes, the time between the first and second wash was approximately 4 min. The spray treatments used in experiment 2 were water followed by another water wash, water followed by a 2% acetic acid wash, 1% saponin followed by a water wash, and 1% saponin followed by a 2% acetic acid wash. For sequential sprays, the time between the first and second wash was approximately 3 min. Immediately after spray washing, 25-cm^2 samples were aseptically excised from the spray-treated or untreated muscle.

Bacterial enumeration. After samples had been excised, individual 25-cm^2 pieces of beef tissue were pummeled for 2 min (Stomacher 400, Tekmar, Cincinnati, Ohio) in a Sterefil stomacher bag (Spiral Biotech, Bethesda, Md.) with 25 ml of buffered peptone water (BPW, pH 7.0; BBL Microbiology Systems, Cockeysville, Md.) containing 0.1% Tween 20 (Fisher, St. Louis, Mo.). Each stomachate was serially diluted in BPW and either spiral-plated (Model D Spiral Plater, Spiral Biotech) in duplicate or spread-plated in quadruplicate on its agar. For the detection of *E. coli* O157:H7 or *Salmonella* Typhimurium, stomachates were spiral-plated in duplicate onto sorbitol McConkey agar (SMAC; Difco) containing 500 μg/ml of streptomycin (Sigma) or Rambach agar (Merck, Darmstadt, Germany) containing 250 μg/ml of nalidixic acid (Sigma), respectively. Trypticase soy agar (Difco) was used for enumerating aerobic bacteria. All plates were enumerated manually or with the CASBA IV image analyzer (Spiral Biotech) after being incubated for 48 h at 37°C. The lowest detection level was 1.30 $\log_{10}$ CFU/cm^2 using spiral-plating procedures. Samples that were spread-plated in quadruplicate were used to detect the total number of CFU/cm^2.

Plate overlay assay. Saponin No. 374's effectiveness against bacteria was determined using a plate overlay assay *(13)*. Briefly, TSA plates were overlaid with 8 ml of semisoft TSA (0.5% wt/vol agar) seeded with 80 μl of an overnight broth culture of antibiotic-resistant *E. coli* O157:H7 or *Salmonella* Typhimurium. The seed density was approximately 1×10^6 CFU/ml of overlay. Twenty microliters of 1% saponin were placed directly on the seeded plate. Duplicate plates were scored (+ or −) for zones of inhibition after 24 h of incubation at respective temperature.

Statistical analyses. After enumeration, bacterial populations from duplicate plates were averaged and converted to $\log_{10}$ CFU/cm^2. Least squared means of bacterial populations from each treatment were calculated from six samples. Analysis of variance was performed using the General Linear Models pro-

TABLE 2. *Experiment 2; remaining bacterial populations following treatments of experimentally inoculated beef carcass tissue with water followed by a water wash, water followed by a 2% acetic acid wash, 1% saponin followed by a water wash, or 1% saponin followed by a 2% acetic acid wash*[a]

Organism	Untreated	Water–Water	Water–Acetic acid	Saponin–Water	Saponin–Acetic acid
Aerobic bacteria	6.41 A[b]	3.89 BC	3.61 C	3.63 C	4.08 B
E. coli O157:H7	6.17 A	3.29 C	3.09 C	3.22 C	3.87 B
Salmonella Typhimurium	5.60 A	2.78 BC	2.74 BC	2.67 C	3.25 B

[a] All sprays were applied at 125 psi for 15 s at 40°C ± 2°C; $n = 6$.
[b] Data with the same letter in the same row are not significantly different.

cedure of SAS (SAS for Windows 6.12, SAS Institute, Cary, N.C.). Inoculum counts were used as a covariant to normalize data between treatment replications. Statistical significance was defined as $P \leq 0.05$ unless otherwise noted.

RESULTS AND DISCUSSION

To determine if saponin exhibited any antimicrobial activity against *E. coli* O157:H7 and *Salmonella* Typhimurium, plate overlay assays were conducted. No zones of inhibition were detected using this assay. Previously, Rodrick *(10)* reported that adding 1% saponin to oyster meat reduced TPC and fecal coliforms by approximately 1.3 and 0.9 $\log_{10}$/g, respectively. There were no sustained reductions of TPC or fecal coliforms after 1% saponin treatment and 15 days of refrigerated storage. Additionally, saponin did not exhibit any immediate or sustained antimicrobial activity against *Vibrio* spp. inoculated on oyster meat *(10)*.

In experiment 1 of this study, beef surfaces inoculated with a fecal slurry containing *E. coli* O157:H7 and *Salmonella* Typhimurium were spray-treated with water, 1% saponin, 2% acetic acid, 1% saponin followed by a water wash, and 1% saponin followed by a 2% acetic acid wash. Remaining bacterial populations were then determined. Populations of mesophilic aerobic bacteria (APC) remaining after spray-washing with combinations of 1% saponin followed by a water wash and 1% saponin followed by a 2% acetic acid wash were lower than APC populations remaining after spray washing with water, 1% saponin, or 2% acetic acid (Table 1). However, the numbers of *E. coli* O157:H7 remaining after 1% saponin followed by a water wash or 1% saponin followed by a 2% acetic acid wash were not statistically different than populations remaining after 2% acetic acid washes. A single wash with 2% acetic acid was more effective against *Salmonella* Typhimurium than saponin followed by either water or acetic acid. Other studies have demonstrated that washes with organic acids are effective for reducing populations of pathogens *(4, 12)*.

To determine whether the efficacy of 1% saponin followed by a water wash or 1% saponin followed by a 2% acetic acid wash was due to saponin's foaming properties or to physical removal by the spray, another experiment was devised. In experiment 2, a water wash followed by another water wash, water followed by 2% acetic acid, 1% saponin followed by water, or 1% saponin followed by 2% acetic acid were applied to beef surfaces inoculated with the fecal slurry containing antibiotic-resistant pathogens. Remaining populations of aerobic bacteria, *Salmonella* Typhimurium, or *E. coli* O157:H7 after a water wash followed by another water wash, a water wash followed by 2% acetic acid wash, or 1% saponin followed by a water wash were not significantly or numerically different from each other (Table 2). Based on these results, it appears that treatments incorporating saponin as an initial wash do not offer any advantage over two water washes. Interestingly, a treatment consisting of 1% saponin followed by a 2% acetic acid wash resulted in higher levels of all the bacterial populations examined in this experiment. Because saponin is hydrolyzed under acidic conditions *(7)*, it was inactivated during the organic acid wash. This observation may explain why higher populations were observed after treatments with 1% saponin followed by a 2% acetic acid wash in experiment 2. Slight differences between contact times (3 min versus 4 min) also may influence the efficacy of saponin when followed by an organic acid wash. This study did not investigate the effect of the combination washes over long-term refrigerated storage; therefore, we do not know the treatments' effects on remaining bacterial populations over a prolonged holding time.

This study demonstrates that, under the conditions described, spray treatments with saponin, alone or in combination with water or acetic acid washes, were no more effective than combination water washes for reducing aerobic and pathogenic populations associated with fecal contamination on beef tissue. Any effect associated with saponin in sequential washes may be attributable to the physical removal of bacteria from the beef surface during the two-step spraying process, rather than to any specific foaming or removal effect of saponin.

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