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Inactivation of *Salmonella* and *Escherichia coli* O157:H7 on Lettuce and Poultry Skin by Combinations of Levulinic Acid and Sodium Dodecyl Sulfate

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ABSTRACT

Four organic acids (lactic acid, acetic acid, caprylic acid, and levulinic acid) and sodium dodecyl sulfate (SDS) were evaluated individually or in combination for their ability to inactivate *Salmonella* and *Escherichia coli* O157:H7. Results from pure culture assays in water with the treatment chemical revealed that 0.5% organic acid and 0.05 to 1% SDS, when used individually, reduced pathogen cell numbers by ≤2 log CFU/ml within 20 min at 21°C. The combination of any of these organic acids at 0.5% with 0.05% SDS resulted in >7 log CFU/ml inactivation of *Salmonella* and *E. coli* O157:H7 within 10 s at 21°C. A combination of levulinic acid and SDS was evaluated at different concentrations for pathogen reduction on lettuce at 21°C, on poultry (wings and skin) at 8°C, and in water containing chicken feces or feathers at 21°C. Results revealed that treatment of lettuce with a combination of 3% levulinic acid plus 1% SDS for <20 s reduced both *Salmonella* and *E. coli* O157:H7 populations by >6.7 log CFU/g on lettuce. *Salmonella* and aerobic bacterial populations on chicken wings were reduced by >5 log CFU/g by treatment with 3% levulinic acid plus 2% SDS for 1 min. Treating water heavily contaminated with chicken feces with 3% levulinic acid plus 2% SDS reduced *Salmonella* populations by >7 log CFU/ml within 20 s. The use of levulinic acid plus SDS as a wash solution may have practical application for killing foodborne enteric pathogens on fresh produce and uncooked poultry.

Escherichia coli O157:H7 and *Salmonella* are major causes of foodborne disease in the United States. Leafy vegetables, including lettuce and spinach, have been implicated in several large outbreaks of foodborne disease caused by *E. coli* O157:H7, a pathogen with increasing public health significance because of the severity of the gastrointestinal illness and the long-term, chronic sequelae that can result from infection. Contamination of raw produce with these pathogenic microorganisms can occur at many points in the food continuum, from the field through the time of consumption. Given sufficient time at a suitable temperature, growth of *E. coli* O157:H7 on some types of produce can exceed 10^7 CFU/g *(5).*

Salmonella is one of the most frequent causes of foodborne illness worldwide. Estimates in the United States suggest *Salmonella* causes 1.4 million cases of illness, approximately 20,000 hospitalizations, and more than 500 deaths annually *(16).* Live birds are an important reservoir of *Salmonella,* and eggs and poultry are the foods that have been implicated most commonly in such infections. The U.S. Department of Agriculture Food Safety and Inspection Service data indicate that *Salmonella*-positive broiler establishments increased from 11.5% in 2002 to 12.8% in 2003, 13.5% in 2004, and 16.3% in 2005 *(2).* Data from the Centers for Disease Control and Prevention revealed that human cases of salmonellosis did not change significantly in 2007 compared with 2004 through 2006 *(26).* Eating chicken is a major factor contributing to sporadic cases of *Salmonella* Enteritidis infections in the United States *(13).*

Many of the pathogen intervention strategies for produce and poultry involve the use of antimicrobial chemicals in rinses or washes; however, the efficacy of most chemical intervention treatments is reduced by the presence of organic matter. In one study, 1.4% sodium levulinate was as effective as 2.7% sodium lactate as an antimicrobial in both pork and turkey sausages *(3).* More effective antimicrobial treatments are needed that are practical, cost-effective, and safe to use.

Previously we identified and validated the efficacy of some chemical treatments that can kill large cell numbers of *E. coli* O157:H7 cells in drinking water for cattle *(30).* However, sensory and cost considerations limited their application. The objective of this study was to develop a chemical treatment that would be practical to use and effective for inactivating *E. coli* O157:H7 and *Salmonella* by at least 5 log CFU/g within seconds to a few minutes on produce or poultry when used in processing water.

MATERIALS AND METHODS

Bacterial strains. Strains of three different bacteria were used in this study. The five isolates of *E. coli* O157:H7 were 932 (human isolate), E009 (beef isolate), E0018 (cattle isolate), E0122 (cattle isolate), and E0139 (deer jerky isolate); the five isolates of *Salmonella* Typhimurium DT104 were H2662 (cattle isolate), 11942A (cattle isolate), 13068A (cattle isolate), 152N17-1 (dairy isolate), and H3279 (human isolate); and the five isolates of *Salmonella* Enteritidis were 564-88 (food isolate), 193-88 (human

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isolate), E39 (egg isolate), 460-88 (egg isolate), and 457-88 (poultry isolate). Each *Salmonella* and *E. coli* O157:H7 strain was grown in tryptic soy broth (Becton Dickinson, Sparks, MD) at 37°C for 18 h and then washed in 0.1 M phosphate-buffered saline, pH 7.2 (PBS). The cultures were used to prepare a five-strain cell suspension that was adjusted to the desired density using a spectrophotometric method (Spectronic Instruments, Rochester, NY) described previously *(30).* Viable cell populations were determined using the spread plate method. Serial dilutions were spread onto plates of tryptic soy agar (TSA; Becton Dickinson), xylose lysine deoxycholate agar (XLD; Becton Dickinson) (*Salmonella* only), and sorbitol MacConkey agar (SMA; Oxoid, Basingstoke, UK) (*E. coli* O157:H7 only) and incubated at 37°C for 24 h, after which colonies were counted.

Chemicals and chemical treatments. Acetic acid, caprylic acid, lactic acid, levulinic acid, and sodium dodecyl sulfate (SDS) (all from Sigma-Aldrich, St. Louis, MO) were tested individually or in combination at different concentrations and temperatures (8 or 21°C) for their killing effect on *Salmonella* Enteritidis, *Salmonella* Typhimurium, and *E. coli* O157:H7 in water, in water contaminated with or without chicken feces or feathers, on chicken skin with and without chicken feces, and on lettuce as a wash treatment.

Water. Deionized unchlorinated water was filter sterilized through a 0.2-μm-pore-size regenerated cellulose filter (Corning Inc., Corning, NY).

Fresh produce. Romaine lettuce was purchased from a local retail store. Before each study, the lettuce was tested for *Salmonella* and *E. coli* O157:H7. A 25-ml volume of sterile water and 25 g of cut lettuce were added to a Whirl-Pak bag (Nasco, Fort Atkinson, WI). The sample bag was pummeled in a stomacher blender at 150 beats for 1 min, and 0.1 ml of the contents was plated in duplicate on XLD or SMA plates for determination of *Salmonella* and *E. coli* O157:H7 populations, respectively, as described below. Only lettuce that was negative for *Salmonella* and *E. coli* O157:H7 was used for the study.

Chicken feces, feathers, skin, and wings. Feces from a poultry farm were collected from five different chickens and used as a mixture. Feathers were obtained from a slaughterhouse. Chicken with skin and chicken wings were purchased from a slaughter plant or a local retail store, and skin was separated immediately before use. A 10-ml volume of deionized water and 1.0 g of feces or feathers or a piece of skin (5 by 5 cm) was added to a Whirl-Pak bag. Each bag with feces, feather, or skin was pummeled in a stomacher blender at 150 beats for 1 min. Each bag with a chicken wing was massaged by hand for 1 min. The fluid was serially diluted (1:10) in 0.1% peptone, and 0.1 ml of each dilution was plated in duplicate on TSA and XLD plates to determine total aerobic plate counts (APCs) and whether these samples were contaminated with *Salmonella.* Only *Salmonella*-free chicken feces, feather, skin, or wing samples were used for the experiments.

Enumeration of *Salmonella* Enteritidis, *Salmonella* Typhimurium DT104, and *E. coli* O157:H7. At each sampling time (0, 1, 2, 5, 10, 20, 30, and 60 min), 1.0 ml of the treated bacterial suspension was mixed with 9.0 ml of PBS. The suspension was serially diluted (1:10) in 0.1% peptone water, and 0.1 ml of each dilution was surface plated in duplicate onto TSA and XLD for *Salmonella* Enteritidis, onto TSA and XLD containing ampicillin (32 μg/ml), tetracycline (16 μg/ml), and streptomycin (64 μg/ml) (TSA+ and XLD+) for *Salmonella* Typhimurium DT104, or onto TSA and SMA for *E. coli* O157:H7. Plates were incubated at 37°C for 48 h. Colonies typical of *Salmonella* (black) or *E. coli* O157:H7 (colorless) were randomly picked from plates with the highest dilution for confirmation of *Salmonella* or *E. coli* by biochemical tests (API 20E assay, bioMérieux, Hazelwood, MO) and latex agglutination assay (Oxoid). When *Salmonella* or *E. coli* O157:H7 were not detected by direct plating, a selective enrichment in universal preenrichment broth (UPB; Becton Dickinson) was performed by incubating 25 ml of treated bacterial suspension in a 500-ml flask containing 225 ml of UPB for 24 h at 37°C. After preenrichment, 1 ml of the culture was transferred to 10 ml of selenite cystine broth (Becton Dickinson) and incubated for 24 h at 37°C. After incubation, 10 μl of enrichment broth was spread on the surface of XLD plates with a bacteriological loop, and the plates were incubated for 24 h at 37°C. Typical *Salmonella* colonies were transferred to fresh XLD plates, which were incubated under similar conditions. All presumptive *Salmonella* isolates were tested with the *Salmonella* latex agglutination assay. Isolates positive for *Salmonella* by the latex agglutination assay were tested with the API 20E assay for biochemical characteristics indicative of *Salmonella.* Selective enrichment for *E. coli* O157:H7 was done according to the protocol reported previously *(30).* Studies with all chemical treatments were done in duplicate, two replicates were plated per sample, and results were reported as means and standard deviations.

Determination of *Salmonella* and *E. coli* O157:H7 inactivation on lettuce. Romaine lettuce (25-g samples, including outer and inner leaves and both vascular and photosynthetic tissues) was cut into ca. 5-cm-long pieces in a laminar flow hood. The samples were submerged in *Salmonella* or *E. coli* O157:H7 suspension (10^8 to 10^9 CFU/ml, 50 ml of bacterial solution in 950 ml of water) for 60 s and then air dried for 20 min in a laminar flow hood. The samples were then suspended in 500 ml of test solution in 1,000-ml beakers. The solutions were agitated on a magnetic stirrer at 100 rpm and 21°C for 0, 1, 2, and 5 min. Following treatment, the sample was placed in a stomacher bag containing 25 ml of PBS and pummeled for 1 min at 150 beats per min in a stomacher. The suspension was serially diluted (1:10) in 0.1% peptone and enumerated for *Salmonella* Enteritidis, *Salmonella* Typhimurium DT104, and *E. coli* O157:H7 according to the procedures described above. Samples treated with PBS only were used as the negative control.

Determination of *Salmonella* inactivation in water contaminated with chicken feces or feathers. The protocols used for contaminated water were the same as described previously *(29, 30)* with minor modifications. Chicken feces or feathers were weighed (wt/vol) and added to a glass beaker containing chemicals at predetermined concentrations ranging from 0.3 to 3% for organic acids and 0.05 to 2% for SDS and mixed with a magnetic stir bar with agitation at 150 rpm. A five-strain mixture of *Salmonella* Enteritidis (10^8 to 10^9 CFU in 1 ml) was added. A 1-ml sample was removed at 0, 2, 5, 10, 20, and 30 min and serially diluted (1:10) in PBS at room temperature. The counts of aerobic bacteria and *Salmonella* were determined according to the procedures described above. Samples treated with PBS only were used as the negative control.

Determination of *Salmonella* inactivation on chicken wings. Chicken wings (each ca. 12 cm long, 7 cm wide, and 85 to 90 g) were submerged in a glass beaker containing 500 ml of *Salmonella* Enteritidis (ca. 10^8 CFU/ml) for 60 s. Inoculated wings were air dried for 20 min in a laminar flow hood and then individually placed in a Whirl-Pak bag containing 200 ml of

TABLE 1. *Counts of* Salmonella *Enteritidis in pure culture and after treatment with different concentrations of organic acids and SDS individually and in combination at 21°C*

Chemical treatment	Mean ± SD *Salmonella* Enteritidis counts (log CFU/ml) at minute[a]:					
	0[b]	2	5	10	20	30
Salmonella Enteritidis only (pH 6.7) (control)	7.2 ± 0.1	7.0 ± 0.3	7.1 ± 0.2	7.2 ± 0.1	7.0 ± 0.5	7.2 ± 0.1
0.1% levulinic acid (pH 2.5)	7.1 ± 0.4[c]	7.1 ± 0.2[c]	6.9 ± 0.3[c]	7.0 ± 0.1[c]	6.9 ± 0.5[c]	6.9 ± 0.3[c]
0.5% levulinic acid (pH 2.6)	7.1 ± 0.1[c]	6.8 ± 0.3[c]	6.9 ± 0.5[c]	6.9 ± 0.3[c]	6.6 ± 0.5[c]	6.7 ± 0.7[c]
1.0% levulinic acid (pH 2.9)	6.9 ± 0.3[c]	6.7 ± 0.8[c]	6.8 ± 0.4[c]	6.9 ± 0.2[c]	6.9 ± 0.3[c]	6.7 ± 0.5[c]
1.5% levulinic acid (pH 2.8)	6.7 ± 0.3[c]	6.7 ± 0.3[c]	6.8 ± 0.3[c]	6.7 ± 0.3[c]	6.4 ± 0.3[c]	6.5 ± 0.3[c]
2.0% levulinic acid (pH 2.8)	6.7 ± 0.7[c]	6.7 ± 0.3[c]	6.7 ± 0.3[c]	6.8 ± 0.5[c]	6.5 ± 0.1[c]	6.0 ± 0.5[c]
2.5% levulinic acid (pH 2.6)	6.9 ± 0.1[c]	6.8 ± 0.7[c]	6.9 ± 0.3[c]	6.4 ± 0.6[c]	5.8 ± 0.5[c]	4.8 ± 0.1[d]
3.0% levulinic acid (pH 2.7)	6.6 ± 0.5[c]	6.8 ± 0.2[c]	6.5 ± 0.5[c]	6.2 ± 0.1[c]	5.1 ± 0.7[d]	3.8 ± 0.9[d]
0.5% acetic acid (pH 3.1)	7.1 ± 0.1[c]	7.0 ± 0.2[c]	6.8 ± 0.1[c]	6.7 ± 0.2[c]	6.6 ± 0.2[c]	6.5 ± 0.0[c]
0.5% lactic acid (pH 2.6)	6.5 ± 0.1[c]	6.1 ± 0.1[c]	5.9 ± 0.2[d]	5.8 ± 0.6[c]	5.5 ± 0.1[d]	5.2 ± 0.4[d]
0.05% SDS (pH 6.1)	7.1 ± 0.0[c]	7.0 ± 0.2[c]	7.2 ± 0.1[c]	7.1 ± 0.1[c]	7.2 ± 0.2[c]	7.1 ± 0.2[c]
0.5% SDS (pH 6.0)	6.6 ± 0.1[c]	6.3 ± 0.3[c]	6.3 ± 0.0[c]	6.1 ± 0.0[d]	6.2 ± 0.2[c]	6.2 ± 0.2[c]
1.0% SDS (pH 6.0)	6.2 ± 0.3[c]	4.6 ± 0.2[d]	4.9 ± 0.0[d]	4.5 ± 0.1[d]	4.2 ± 0.2[d]	3.9 ± 0.4[d]
1.5% SDS (pH 6.0)	3.4 ± 0.4[d]	2.1 ± 0.3[d]	1.5 ± 0.5[d]	0.8 ± 0.2[d]	+[d]	+[d]
2.0% SDS (pH 6.0)	2.8 ± 0.6[d]	+[d]	+[d]	+[d]	+[d]	+[d]
0.3% levulinic acid + 0.05% SDS (pH 3.1)	−[d]	−[d]	−[d]	−[d]	−[d]	−[d]
0.4% levulinic acid + 0.05% SDS (pH 2.9)	−[d]	−[d]	−[d]	−[d]	−[d]	−[d]
0.5% levulinic acid + 0.05% SDS (pH 3.0)	−[d]	−[d]	−[d]	−[d]	−[d]	−[d]
0.5% levulinic acid + 0.03% SDS (pH 3.0)	−[d]	−[d]	−[d]	−[d]	−[d]	−[d]
0.05% caprylic acid + 0.03% SDS (pH 3.4)	−[d]	−[d]	−[d]	−[d]	−[d]	−[d]
0.05% caprylic acid + 0.05% SDS (pH 3.2)	−[d]	−[d]	−[d]	−[d]	−[d]	−[d]
0.5% acetic acid + 0.05% SDS (pH 3.0)	−[d]	−[d]	−[d]	−[d]	−[d]	−[d]
0.5% lactic acid + 0.05% SDS (pH 2.5)	−[d]	−[d]	−[d]	−[d]	−[d]	−[d]

[a] +, positive by enrichment culture but not by direct plating (minimum detection level set at 0.7 log CFU/ml); −, negative by direct plating and enrichment culture.
[b] The actual time 0 was delayed by 5 to 10 s because of time needed for sample processing.
[c] Not significantly different from the control ($P > 0.05$).
[d] Significantly different from the control ($P < 0.05$).

chemical solution for 0, 1, 2, and 5 min. The bags were agitated in a vertical shaker (Stovall Life Science Inc., Greensboro, NC) at 150 rpm with intermittent hand massage (every 30 s). After chemical treatment, each chicken wing was placed in a Whirl-Pak bag containing 50 ml of 0.1 M PBS. The bag was agitated in the vertical shaker for 2 min at 150 rpm with intermittent hand massage. A 1-ml sample of the cell suspension was serially diluted (1:10) in 9 ml of 0.1% peptone to 10^{-6} CFU/ml, and 0.1-ml portions of each dilution were surface plated in duplicate on XLD and TSA plates. The plates were incubated at 37°C for 24 and 48 h for enumeration of *Salmonella* and aerobic bacteria, respectively. Samples treated with PBS only were used as the negative control.

Determination of *Salmonella* inactivation on chicken skin. Chicken skin was separated and one square sample (5 by 5 cm) was removed immediately before the experiment. *Salmonella* Enteritidis at 10^7 to 10^8 CFU in a volume of 0.5 ml in pure culture or in feces was inoculated onto the skin and air dried in a laminar flow hood for 20 min. The inoculated skin was placed into a stomacher bag containing the antimicrobial solution (200 ml solution for each skin sample) at 21°C for a contact time of 0, 1, 2, and 5 min with intermittent hand massage (every 30 s). At each sampling time, the samples were placed in Whirl-Pak bags, each containing 9 ml of PBS, and pummeled for 1 min in a stomacher blender at 150 beats per min. The suspension was serially diluted (1:10) in 0.1% peptone, and 0.1 ml from each dilution was plated on XLD and TSA plates in duplicate. The plates were incubated at 37°C for 48 h. Presumptive *Salmonella* colonies were enumerated according to the procedures described above. Samples treated with PBS only were used as the negative control.

Statistical analysis. Duplicate trials were conducted for each treatment. The general linear models procedure of the Statistical Analysis System (SAS Institute, Cary, NC) was used to analyze data. The least significant difference test was used to determine significant differences ($P \leq 0.05$) between mean populations of *Salmonella* Typhimurium, *Salmonella* Enteritidis, and *E. coli* O157:H7 (log CFU per milliliter, gram, or square centimeter) in samples of PBS-treated and chemical-treated solutions, lettuce, and chicken wings, feces, or feathers. Values of 0.7 log CFU/ml for pure culture assay and 1.7 log CFU/ml, log CFU/g, or log CFU/cm^2 for lettuce and chicken feces, wings, and skin were used for statistical analysis when pathogens were undetectable by the direct plating method.

RESULTS

Salmonella Enteritidis was inactivated by ca. ≤1 log CFU/ml in suspensions of 0.1 to 2% levulinic acid for 30 min at 21°C (Table 1). Inactivation was greater in 3% levulinic acid, with a 3.4-log CFU/ml reduction at 30 min (Table 1). Treatments with 0.5% acetic acid and 0.5% lactic acid for 30 min reduced *Salmonella* Enteritidis populations by 0.7 and 2.0 log CFU/ml, respectively. *Salmonella* Enteritidis and *E. coli* O157:H7 populations were decreased

by 1.0 and 0.7 log CFU/ml, respectively, by treatment with 0.5% SDS for 30 min (Tables 1 and 2). *Salmonella* Enteritidis cell numbers were reduced by 3.3 log CFU/ml in 1% SDS for 30 min and by >5 log CFU/ml in 1.5 and 2.0% SDS for 30 min. *E. coli* O157:H7 cell numbers were reduced by 2.1, 5.4, and >6 log CFU/ml in 1.0, 1.5, and 2.0% SDS for 30 min, respectively (Table 2).

All organic acids evaluated in combination with 0.03 to 0.05% SDS were effective in reducing *Salmonella* populations to undetectable levels (>7-log reduction) within ca. 10 s (Table 1). The population of *Salmonella* was quickly reduced from 10^7 CFU/ml to undetectable by enrichment culture with a contact time of 5 to 10 s (processing time) (Table 1).

Neither 0.5% levulinic acid nor 0.5% SDS when used independently provided a substantive (<0.5 log CFU/ml) killing effect on *E. coli* O157:H7 or *Salmonella* Enteritidis within 30 min at 21°C. However, a combination of 0.5% levulinic acid and 0.05% SDS provided a ca. 7-log reduction of *E. coli* O157:H7, *Salmonella* Enteritidis, and *Salmonella* Typhimurium DT104 within 1 min (Tables 1 through 3).

Treatment of *Salmonella*-inoculated lettuce with 0.3% levulinic acid plus 0.05% SDS for 1 min reduced *Salmonella* Enteritidis cell numbers by ca. 4.2 log CFU/g, whereas a 0.5% levulinic acid plus 0.05% SDS treatment for 1 min reduced *Salmonella* Typhimurium on lettuce by ca. 4.4 log CFU/g (Table 4). Similarly, *E. coli* O157:H7 on lettuce was reduced by 4.5 log CFU/g when treated with 0.5% levulinic acid plus 0.05% SDS for 1 min (Table 4). Increasing the concentrations of levulinic acid to 3% and SDS to 1.0% substantially increased the antimicrobial activity on lettuce, with no *E. coli* O157:H7 and *Salmonella* Typhimurium detected by enrichment culture (ca. 7-log reduction) after a 1 to 2 min of exposure (Table 4).

Treating *Salmonella*-inoculated chicken skin with 0.5% levulinic acid plus 0.05% SDS for 2 min reduced *Salmonella* Enteritidis populations by 3.7 log CFU/cm^2 (Table 4). Neither *Salmonella* nor *E. coli* O157:H7 were detectable by direct plating or enrichment culture in the chemical solutions after they were used to treat fresh produce or chicken skin (Table 4).

The levulinic acid plus SDS combination treatment was evaluated for killing *Salmonella* Enteritidis in water containing chicken feces or feathers. Results revealed that feather contamination did not substantially affect the antimicrobial activity of 1.0% levulinic acid plus 0.1% SDS, with the *Salmonella* Enteritidis population reduced by >7 log CFU/ml at 2 min (Table 5). However, the presence of chicken feces negatively influenced the antimicrobial activity, with *Salmonella* Enteritidis detectable by enrichment culture but not by direct plating after 5 to 30 min in feces-contaminated water treated with 1.0% levulinic acid plus 0.1% SDS (Table 5). Increasing the concentrations of levulinic acid to 3% and of SDS to 2% reduced *Salmonella* to undetectable levels (>7-log reduction) within 2 min in water heavily contaminated with chicken feces (Table 5).

APCs in water containing chicken feces at a ratio of 1:100 (wt/vol) were reduced by 4.6 log CFU/ml when treat-

TABLE 2. *Counts of* E. coli *O157:H7 in pure culture and after treatment with levulinic acid and SDS individually and in combination at 21°C*

Chemical treatment	Mean ± SD *E. coli* O157:H7 counts (log CFU/ml) at minute[a]:							
	0[b]	1	2	5	10	20	30	60
E. coli O157:H7 only (control)	7.1 ± 0.1	7.2 ± 0.1	7.0 ± 0.2	7.2 ± 0.2	7.1 ± 0.3	7.1 ± 0.0	7.2 ± 0.1	7.2 ± 0.1
0.5% levulinic acid (pH 3.0)	7.0 ± 0.2[c]	6.7 ± 0.3[c]	6.8 ± 0.1[c]	6.7 ± 0.2[c]	6.9 ± 0.1[c]	6.8 ± 0.3[c]	6.8 ± 0.3[c]	6.4 ± 0.6[c]
0.05% SDS (pH 6.1)	7.1 ± 0.0[c]	6.9 ± 0.3[c]	7.1 ± 0.1[c]	7.0 ± 0.1[c]	6.9 ± 0.3[c]	6.9 ± 0.2[c]	7.1 ± 0.0[c]	7.0 ± 0.2[c]
0.5% SDS (pH 6.0)	6.6 ± 0.3[c]	6.6 ± 0.4[c]	6.5 ± 0.1[c]	6.6 ± 0.2[c]	6.5 ± 0.5[c]	6.4 ± 0.5[c]	6.5 ± 0.2[c]	6.4 ± 0.4[c]
1.0% SDS (pH 6.0)	6.5 ± 0.1[c]	6.7 ± 0.3[c]	6.6 ± 0.1[c]	6.5 ± 0.1[c]	6.2 ± 0.2[c]	5.5 ± 0.4[d]	5.1 ± 0.6[d]	4.5 ± 0.5[d]
1.5% SDS (pH 6.0)	4.0 ± 0.4[d]	3.0 ± 0.0[d]	2.7 ± 0.0[d]	2.4 ± 0.8[d]	2.7 ± 0.4[d]	2.0 ± 0.5[d]	1.8 ± 0.1[d]	+[d]
2.0% SDS (pH 6.0)	5.6 ± 0.2[d]	4.8 ± 0.1[d]	3.7 ± 0.2[d]	2.4 ± 0.5[d]	+[d]	+[d]	+[d]	+[d]
0.5% levulinic acid + 0.05% SDS (pH 3.0)	−[d]	−[d]	−[d]	−[d]	−[d]	−[d]	−[d]	−[d]

[a] +, positive by enrichment culture but not by direct plating (minimum detection level set at 0.7 log CFU/ml); −, negative by direct plating and enrichment culture.
[b] The actual time 0 was delayed by 5 to 10 s because of time needed for sample processing.
[c] Not significantly different from the control ($P > 0.05$).
[d] Significantly different from the control ($P < 0.05$).

TABLE 3. *Counts of* Salmonella *Typhimurium DT104 in pure culture and after treatment with levulinic acid and SDS individually and in combination at 21°C*

Chemical treatment	Mean ± SD *Salmonella* Typhimurium DT104 counts (log CFU/ml) at minute[a]:							
	0[b]	1	2	5	10	20	30	60
Salmonella Typhimurium only (control)	6.9 ± 0.3	7.0 ± 0.0	7.0 ± 0.1	7.0 ± 0.1	7.0 ± 0.2	6.9 ± 0.3	7.0 ± 0.1	7.0 ± 0.0
0.5% levulinic acid (pH 3.0)	6.8 ± 0.1[c]	6.7 ± 0.2[c]	6.6 ± 0.3[c]	6.5 ± 0.4[c]	6.7 ± 0.3[c]	6.6 ± 0.6[c]	6.4 ± 0.5[c]	5.9 ± 0.8[c]
0.05% SDS (pH 6.0)	7.0 ± 0.0[c]	7.0 ± 0.0[c]	6.8 ± 0.3[c]	6.9 ± 0.2[c]	6.8 ± 0.1[c]	6.9 ± 0.1[c]	6.9 ± 0.2[c]	6.9 ± 0.0[c]
0.5% levulinic acid + 0.05% SDS (pH 3.0)	+[d]	–[d]	–[d]	–[d]	–[d]	–[d]	–[d]	–[d]

[a] +, positive by enrichment culture but not by direct plating (minimum detection level set at 0.7 log CFU/ml); –, negative by direct plating and enrichment culture.
[b] The actual time 0 was delayed by 5 to 10 s because of time needed for sample processing.
[c] Not significantly different from the control ($P > 0.05$).
[d] Significantly different from the control ($P < 0.05$).

ed with 1% levulinic acid plus 0.1% SDS for 2 min (Table 5). APCs decreased by ca. 5.5 log CFU/ml in water containing chicken feces at a ratio of 1:20 (wt/vol) when treated with 3% levulinic acid plus 2.0% SDS for 2 min.

Three combinations of different concentrations of levulinic acid and SDS were evaluated at 8°C to reduce *Salmonella* Enteritidis populations on chicken wings. Treatments of 2% levulinic acid plus 1% SDS or 3% levulinic acid plus 1% SDS treatment for 1 min reduced *Salmonella* Enteritidis populations on chicken wings by 2.6 and 4.0 log CFU/g, respectively. Increasing the concentrations of levulinic acid and SDS to 3 and 2%, respectively, reduced the *Salmonella* Enteritidis population by ca. 7 log CFU/g and the APC by >7 log CFU/g within 2 min (Table 6).

DISCUSSION

Previous studies have revealed that the killing effects of organic acids and other antimicrobial chemicals on pathogens can be increased substantially in the presence of elevated temperature, high pressure, electrolyzed oxidizing water, ozonated water, or surfactants *(3, 4, 7, 8, 10, 12, 13, 18, 20–23, 27, 29–31).* Levulinic acid was selected as the primary focus of this study because it can be produced at low cost and in high yield from renewable feedstocks *(6, 9).* The antimicrobial properties of levulinic acid have been reported previously *(3).* The safety of this compound for humans has been widely tested, and it has been designated by the U.S. Food and Drug Administration (FDA) *(25)* as generally recognized as safe for direct addition to food as a flavoring substance or adjunct (21 CFR 172.515). Its application in fresh produce may extend shelf life because levulinic acid can arrest light-induced chloroplast development during greening and can be removed by washing the leaves to restore the developmental process without any apparent toxic effect *(11).* We confirmed this property by soaking whole Romaine lettuce in a 3% levulinic acid plus 1% SDS solution for either 30 or 60 s and in a 0.5% levulinic acid plus 0.05% SDS solution for either 15 or 30 min. The lettuce was then rinsed with water three times, and the treated lettuce and lettuce rinsed with water only (control) was stored at 5°C for up to 14 days to observe the color change. There were no visual differences between the lettuce treated with 3% levulinic acid plus 1% SDS for 30 or 60 s or with 0.5% levulinic acid plus 0.05% SDS for 15 or 30 min and the lettuce rinsed with water only (unpublished data). This finding is in contrast with results obtained after treating lettuce with 1.5% lactic acid plus 2.0% hydrogen peroxide at 22°C for 5 min, in which browning occurred within 6 days *(15).*

The bactericidal effect of 1% levulinic acid alone is not sufficient to kill more than 1 log CFU/ml *Salmonella* within 30 min, but its bactericidal activity was increased to 3.4 log CFU/ml when the levulinic acid concentration was increased to 3%. These results indicate that application of 3% levulinic acid alone cannot ensure elimination of high levels of *Salmonella* contamination on produce and poultry after relatively short time exposures.

SDS also is generally recognized as safe by the FDA *(24)* for multipurpose additives (21 CFR 172.822). This

TABLE 4. *Counts of* Salmonella *Enteritidis,* E. coli *O157:H7, and* Salmonella *Typhimurium DT104 inoculated onto fresh produce or chicken skin that was then treated with levulinic acid plus SDS at 21°C*

Treatment	Mean ± SD bacterial counts (log CFU/g or cm^2) at minute[a]:				
	0[b]	1	2	5	5 (in treatment solution)
Pathogen counts on Romaine lettuce					
Inoculated with *Salmonella* Enteritidis					
Pathogen alone (control)	7.7 ± 0.9	7.3 ± 1.1	7.4 ± 0.5	7.3 ± 0.7	7.4 ± 0.2
0.3% levulinic acid + 0.05% SDS (pH 3.1)	3.1 ± 0.2[c]	3.1 ± 0.4[c]	2.7 ± 0.3[c]	2.6 ± 0.6[c]	+[c]
Inoculated with *Salmonella* Typhimurium DT104					
PBS (control)	7.4 ± 1.4	7.3 ± 0.8	7.4 ± 0.2	7.3 ± 0.5	7.4 ± 0.0
0.5% levulinic acid + 0.05% SDS (pH 3.1)	2.8 ± 0.4[c]	2.9 ± 0.9[c]	2.9 ± 0.5[c]	2.7 ± 0.1[c]	+[c]
3% levulinic acid + 1% SDS (pH 2.7)	+[c]	−[c]	−[c]	−[c]	−[c]
Inoculated with *E. coli* O157:H7					
PBS (control)	7.4 ± 0.0	7.5 ± 0.3	7.2 ± 0.2	7.2 ± 0.1	7.4 ± 0.1
0.5% levulinic acid + 0.05% SDS (pH 3.0)	3.1 ± 0.7[c]	3.0 ± 0.4[c]	3.0 ± 0.2[c]	2.9 ± 0.4[c]	−[c]
3% levulinic acid + 1% SDS (pH 2.7)	+[c]	+[c]	−[c]	−[c]	−[c]
Pathogen counts on chicken skin inoculated with *Salmonella* Enteritidis					
Pathogen alone (control)	7.1 ± 0.4	7.3 ± 0.6	7.2 ± 0.2	7.0 ± 0.5	6.8 ± 0.3
0.5% levulinic acid + 0.05% SDS (pH 3.0)	6.7 ± 0.9[d]	4.4 ± 0.2[c]	3.5 ± 0.5[c]	1.7 ± 0.1[c]	−[c]
Aerobic plate counts on Romaine lettuce					
Inoculated with *E. coli* O157:H7					
PBS (control)	8.1 ± 0.3	ND[e]	ND	8.2 ± 0.2	8.0 ± 0.5
3% levulinic acid + 1% SDS	2.8 ± 0.7[c]	1.7 ± 0.1[c]	2.3 ± 0.2[c]	1.7 ± 0.3[c]	−[c]
Inoculated with *Salmonella* Typhimurium DT104					
PBS (control)	7.8 ± 0.4	ND	ND	7.9 ± 0.5	7.8 ± 0.3
3% levulinic acid + 1% SDS	2.8 ± 0.1[c]	2.0 ± 0.2[c]	1.7 ± 0.1[c]	1.7 ± 0.1[c]	−[c]

[a] +, below minimum detection level (<1.7 log CFU/g or cm^2) by direct plating, but positive by selective enrichment culture; −, negative by direct plating and enrichment culture.
[b] The actual time 0 was delayed by 10 to 20 s because of time needed for sample processing.
[c] Significantly different from the control ($P < 0.05$).
[d] Not significantly different from the control ($P > 0.05$).
[e] ND, not determined.

compound has been widely studied as a surfactant and is used in household products such as toothpastes, shampoos, shaving foams, and bubble baths. SDS is approved for use in a variety of foods, including egg whites, fruit juices, vegetable oils, and gelatin as a whipping or wetting agent *(24).* The SDS molecule has a tail of 12 carbon atoms attached to a sulfate group, giving the molecule the amphiphilic properties required of a detergent. SDS can denature protein surfaces and damage cell membranes, and its bactericidal effect can be increased when pH is reduced to between 1.5 and 3.0 *(1, 7, 23, 28).* Our results revealed that 0.05 to 0.5% SDS by itself at a pH 6.0 has very limited antimicrobial activity, which confirms previous findings that a common feature for the *Enterobacteriaceae* is their tolerance to SDS; many bacteria in this family can grow in the presence of 5% SDS *(14, 17, 28).* However, the weak antimicrobial properties of SDS can be greatly enhanced when SDS is mixed with levulinic acid or other organic acids.

From the time of harvest until consumption, produce products are in a race against time with ongoing ripening and spoilage processes. Spray washers can remove most but not all soil and attached debris that interfere with many commonly used biocides such as chlorine. The organic load on produce can neutralize much of the antimicrobial activity of chlorine. Chlorine is pH sensitive, so the pH must be controlled to optimize the pathogen killing effect. In the meat and poultry industry, many pathogen reduction interventions involve the use of acids or antimicrobial chemical treatments, but no individual intervention application has been effective for eliminating *E. coli* O157:H7 from beef or *Salmonella* from poultry *(8, 13, 21).* In 2007 there were 22 recalls of ground beef contaminated with *E. coli* O157:H7 *(19),* and the contamination of *Salmonella* on poultry in 2006 had not decreased substantially since 1998, indicating that more effective antimicrobial interventions are needed for the meat and poultry industries *(2).*

Combining levulinic acid with SDS dramatically increased the bactericidal activity of these two chemicals. The enhanced antimicrobial activity of a combination of levulinic acid and SDS on *E. coli* O157:H7 and *Salmonella* was validated on fresh produce, chicken wings and skin, and water contaminated with chicken feces or feathers. The combined bactericidal activity remained effective in an organic-rich environment containing fecal matter or feathers. Although the combination of levulinic acid and SDS had

TABLE 5. *Counts of* Salmonella *Enteritidis and aerobic bacteria in water containing chicken feces or feathers that was then treated with levulinic acid plus SDS at 21°C*

Treatment	Mean ± SD bacterial counts (log CFU/ml) at minute[a]:					
	0[b]	2	5	10	20	30
Salmonella Enteritidis counts						
Water plus chicken feces (1:100, wt/vol)						
Pathogen only (pH 6.8) (control)	7.6 ± 0.1	7.5 ± 0.5	7.5 ± 0.5	7.6 ± 0.1	7.5 ± 0.5	7.6 ± 0.3
1.0% levulinic acid + 0.1% SDS (pH 4.0)	4.9 ± 0.6[c]	1.8 ± 0.4[c]	+[c]	+[c]	+[c]	+[c]
Water plus chicken feces (1:20, wt/vol)						
Pathogen only (pH 6.7) (control)	7.7 ± 0.1	7.8 ± 0.3	7.7 ± 0.1	7.7 ± 0.1	7.7 ± 0.1	7.6 ± 0.1
3.0% levulinic acid + 2.0% SDS (pH 4.0)	+[c]	−[c]	−[c]	−[c]	−[c]	−[c]
Water plus chicken feathers (1:100, wt/vol)						
Pathogen only (pH 6.7) (control)	7.5 ± 0.1	7.7 ± 0.4	7.4 ± 0.0	7.5 ± 0.2	7.6 ± 0.3	7.6 ± 0.2
1.0% levulinic acid + 0.1% SDS (pH 3.2)	+[c]	−[c]	−[c]	−[c]	−[c]	−[c]
Aerobic plate counts						
Water plus chicken feces (1:100, wt/vol)						
Aerobic bacteria only (control)	7.4 ± 0.0	7.6 ± 0.3	7.5 ± 0.2	7.4 ± 0.1	7.4 ± 0.2	7.4 ± 0.3
1.0% levulinic acid + 0.1% SDS (pH 4.0)	5.0 ± 0.4[c]	3.0 ± 0.5[c]	2.9 ± 0.5[c]	2.9 ± 0.2[c]	2.0 ± 0.5[c]	2.0 ± 0.2[c]
Water plus chicken feces (1:20, wt/vol)						
Aerobic bacteria only (control)	10.4 ± 0.5	10.4 ± 0.2	10.3 ± 0.2	10.4 ± 0.5	10.4 ± 0.1	10.4 ± 0.2
3.0% levulinic acid +2.0% SDS (pH 4.0)	4.5 ± 0.2[c]	4.9 ± 0.2[c]	5.1 ± 0.7[c]	4.9 ± 0.4[c]	5.1 ± 1.2[c]	5.1 ± 0.5[c]

[a] +, below minimum detection level (<1.7 log CFU/ml) by direct plating, but positive by selective enrichment culture; −, negative by direct plating and enrichment culture.
[b] The actual time 0 was delayed by 10 to 20 s because of time needed for sample processing.
[c] Significantly different from the control ($P < 0.05$).

TABLE 6. *Counts of* Salmonella *Enteritidis and aerobic bacteria on chicken wings treated with levulinic acid plus SDS at 8°C*

Treatment	Mean ± SD bacterial counts (log CFU/g) at minute[a]: 0[b]	1	2	5	5 (in treatment solution)
Salmonella Enteritidis counts					
PBS (7.2) (control)	7.8 ± 0.0	7.0 ± 0.2	6.5 ± 0.1	6.8 ± 0.1	7.3 ± 0.1
2% levulinic acid + 1% SDS (pH 3.0)	7.3 ± 0.2[c]	4.4 ± 0.1[d]	4.9 ± 0.1[d]	3.2 ± 0.2[d]	+[d]
PBS (7.2) (control)	7.4 ± 0.1	6.7 ± 0.4	6.6 ± 0.0	7.0 ± 0.2	6.9 ± 0.1
3% levulinic acid + 1% SDS (pH 2.7)	7.4 ± 0.2[c]	2.7 ± 0.1[d]	2.0 ± 0.5[d]	2.2 ± 0.2[d]	−[d]
PBS (7.2) (control)	6.5 ± 0.5	6.7 ± 0.4	6.9 ± 0.2	6.5 ± 0.3	7.6 ± 0.0
3% levulinic acid + 2% SDS (pH 2.7)	6.1 ± 0.2[c]	+[d]	−[d]	−[d]	−[d]
Aerobic plate counts					
PBS (pH 7.2) (control)	7.9 ± 0.3	7.1 ± 0.2	6.8 ± 0.1	6.7 ± 0.1	6.8 ± 0.2
2% levulinic acid + 1% SDS (pH 3.0)	7.8 ± 0.1[c]	4.9 ± 0.1[d]	5.3 ± 0.1[d]	3.4 ± 0.2[d]	+[d]
PBS (pH 7.2) (control)	7.7 ± 0.5	6.8 ± 0.9	6.8 ± 0.7	7.0 ± 0.3	7.2 ± 0.1
3% levulinic acid + 1% SDS (pH 2.7)	7.6 ± 0.1[c]	3.0 ± 0.6[d]	2.7 ± 0.2[d]	2.7 ± 0.5[d]	+[d]
PBS (pH 7.2) (control)	7.9 ± 0.6	8.2 ± 0.9	8.9 ± 0.4	8.5 ± 0.6	9.1 ± 0.3
3% levulinic acid + 2% SDS (pH 2.7)	7.8 ± 0.8[c]	+[d]	+[d]	+[d]	−[d]

[a] Below minimum detection level (<1.7 log CFU/g) by direct plating, but positive by selective enrichment culture; −, negative by direct plating and enrichment culture.
[b] The actual time 0 was delayed by 10 to 20 s because of time needed for sample processing.
[c] Not significantly different from the control ($P > 0.05$).
[d] Significantly different from the control ($P < 0.05$).

substantial antimicrobial activity in this study, currently levulinic acid and SDS do not have regulatory approval for application to the matrices that were tested. Studies are needed to validate the efficacy of levulinic acid and SDS on produce and poultry in actual production operations. The application of these two compounds may also extend the shelf life of produce and poultry because of their ability to reduce overall bacterial contamination.

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