Journal of Food Protection, Vol. 72, No. 6, 2009, Pages 1201–1208
Copyright ©, International Association for Food Protection

Inactivation of *Listeria monocytogenes, Escherichia coli* O157:H7, and *Salmonella* Typhimurium with Compounds Available in Households

HUA YANG,[1] PATRICIA A. KENDALL,[2] LYDIA MEDEIROS,[3] AND JOHN N. SOFOS[1]*

[1]*Center for Meat Safety & Quality and Food Safety Cluster, Department of Animal Sciences, and* [2]*Department of Food Science and Human Nutrition, Colorado State University, Fort Collins, Colorado 80523; and* [3]*Department of Human Nutrition, Ohio State University, Columbus, Ohio 43210, USA*

MS 08-493: Received 30 September 2008/Accepted 30 January 2009

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/72/6/1201/1680761/0362-028x-72_6_1201.pdf by guest on 08 August 2022

ABSTRACT

Solutions of selected household products were tested for their effectiveness against *Listeria monocytogenes, Escherichia coli* O157:H7, and *Salmonella* Typhimurium. Hydrogen peroxide (1.5 and 3%), vinegar (2.5 and 5% acetic acid), baking soda (11, 33, and 50% sodium bicarbonate), household bleach (0.0314, 0.0933, and 0.670% sodium hypochlorite), 5% acetic acid (prepared from glacial acetic acid), and 5% citric acid solutions were tested against the three pathogens individually (five-strain composites of each, 10^8 CFU/ml) by using a modified AOAC International suspension test at initial temperatures of 25 and 55°C for 1 and 10 min. All bleach solutions (pH 8.36 to 10.14) produced a >5-log reduction of all pathogens tested after 1 min at 25°C, whereas all baking soda solutions (pH 7.32 to 7.55) were ineffective (<1-log reduction) even after 10 min at an initial temperature of 55°C. After 1 min at 25°C, 3% hydrogen peroxide (pH 2.75) achieved a >5-log reduction of both *Salmonella* Typhimurium and *E. coli* O157:H7, whereas undiluted vinegar (pH 2.58) had a similar effect only against *Salmonella* Typhimurium. Compared with 1 min at 25°C, greater reductions of *L. monocytogenes* ($P < 0.05$) were obtained with all organic acid and hydrogen peroxide treatments after 10 min at an initial temperature of 55°C. The efficacies of household compounds against all tested pathogens decreased in the following order: 0.0314% sodium hypochlorite > 3% hydrogen peroxide > undiluted vinegar and 5% acetic acid > 5% citric acid > baking soda (50% sodium bicarbonate). The sensitivity of the tested pathogens to all tested household compounds followed the sequence of *Salmonella* Typhimurium > *E. coli* O157:H7 > *L. monocytogenes.*

Escherichia coli O157:H7, *Salmonella,* and *Listeria monocytogenes* are three major pathogens frequently involved in outbreaks of foodborne disease. Foods such as milk, cheeses, ice cream, raw meat, various ready-to-eat foods, and vegetables that are brought into homes could be contaminated with these pathogenic bacteria *(6).* Areas in the kitchen, particularly food contact surfaces, may acquire pathogens from contaminated foods during food preparation *(9).* The incidence and persistence of *Salmonella, Campylobacter, E. coli,* and *Staphylococcus aureus* in domestic kitchen environments has been established *(9, 10).* In a study of interior surfaces of 342 domestic refrigerators, *S. aureus* was recovered from 6.4% of the examined refrigerators, *L. monocytogenes* and *E. coli* were recovered from 1.2%, and *Yersinia enterocolitica* was recovered from 0.6% *(11).* Gorman et al. *(9)* found that when a lunch was prepared with a total of 25 chickens (2 positive for *Salmonella,* 11 positive for *Campylobacter* and *S. aureus,* and 7 positive for *E. coli*), the frequency of cross-contamination in the kitchen was 18% for *Campylobacter,* 16.6% for *Salmonella,* 9.5% for *E. coli,* and 19.7% for *S. aureus.*

Bacteria may survive on food contact surfaces for hours or days after contamination, and the contaminated areas may serve as niches or harborage sites and sources of cross-contamination of foods by pathogenic microorganisms. At 10^3 and 10 CFU/cm^2, *S. aureus* survived for 4 and 2 days, respectively, on stainless steel, and this microorganism was readily transferred to cucumber and chicken fillet slices at rates of more than 62% *(14).* The home environment may contribute to 90% of *Salmonella* infections *(5).* Thus, cross-contamination with foodborne pathogens via hands, cloths, utensils, handles, and food contact surfaces is a major concern in the home *(4, 9).* Failure to effectively reduce contamination in domestic kitchens can have serious implications for the transmission of foodborne illness. Householders need various methods of inactivating pathogens in their kitchens and of avoiding creation of contamination niches.

Some products commonly available in the household contain chemicals, such as the acetic acid in vinegar and the hypochlorite in bleach, that are capable of reducing microbial numbers *(7, 23).* These common household products are convenient and inexpensive and may be useful for sanitizing surfaces in the home *(23).* Although recipes to formulate cleaning solutions with household products may be available from official or unofficial sources, limited published scientific information exists regarding the effectiveness of these solutions against foodborne pathogens. The purpose of this study was to evaluate selected household products for their efficacy against *L. monocytogenes, E. coli*

* Author for correspondence. Tel: 970-491-7703; Fax: 970-491-0278; E-mail: john.sofos@colostate.edu.

TABLE 1. *Household compounds and their dilutions used in the suspension test*

Compound	Active ingredient	Concn (%)	pH	Dilution method
Clorox bleach	Sodium hypochlorite	0.0314	8.36	1 teaspoon of bleach (5 ml) + 1 qt of water (950 ml)
		0.0933	9.00	1 tablespoon of bleach (15 ml) + 1 qt of water (950 ml)
		0.670	10.14	0.5 cup of bleach (120 ml) + 1 qt of water (950 ml)
Baking soda	Sodium bicarbonate	11	7.32	2 tablespoons of baking soda (30 g) + 1 cup of water (240 ml)
		33	7.50	0.5 cup of baking soda (120 g) + 1 cup of water (240 ml)
		50	7.55	1 cup of baking soda (240 g) + 1 cup of water (240 ml)
White vinegar	Acetic acid	2.5	2.78	1 cup of vinegar (240 ml) + 1 cup of water (240 ml)
		5	2.58	Undiluted
Hydrogen peroxide	Hydrogen peroxide	1.5	2.82	1 cup of hydrogen peroxide (240 ml) + 1 cup of water (240 ml)
		3	2.75	Undiluted

O157:H7, and *Salmonella* Typhimurium. Based on the results, scientific and practical instructions for use of household compounds to decontaminate food contact surfaces could be developed.

MATERIALS AND METHODS

Household compounds. Vinegar, baking soda, household bleach, and hydrogen peroxide were purchased from a local supermarket. Their active ingredients and in-use dilutions are listed in Table 1. These dilution methods were chosen because of the easy availability of the measuring tools (e.g., cup, teaspoon, and tablespoon) in the home. All household compounds at their use dilutions were prepared with sterile distilled water on the day of the evaluation. Solutions of acetic acid (5%, pH 2.70) and citric acid (5%, pH 2.31) were made by adding 2.5 ml of glacial acetic acid (Mallinckrodt Baker Inc., Phillipsburg, NJ) and 2.5 g of citric acid (Fisher Scientific, Fair Lawn, NJ) to 47.5 ml of distilled water, respectively.

Bacterial strains and growth conditions. The five strains each of *L. monocytogenes, E. coli* O157:H7, and *Salmonella* Typhimurium used in the experiment are listed in Table 2 *(2, 8, 21).* All strains were kept on slants at 4°C. *E. coli* O157:H7 and *Salmonella* Typhimurium strains were transferred twice in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) at 35°C for 24 h. *L. monocytogenes* strains were activated by two successive transfers in TSB containing 0.6% yeast extract (Acumedia, Lansing, MI) at 30°C for 24 h. Each strain was washed with phosphate-buffered saline (PBS, pH 7.0) by centrifuging (Eppendorf model 5810 R, Brinkmann Instruments, Inc., Westbury, NY) at 4,629 $\times$ *g* for 15 min at 4°C and resuspended in PBS. Equal volumes (10 ml) of cell suspensions of each strain (5×10^9 CFU/ml) were combined to form composites for the suspension test.

Suspension test. The AOAC suspension test *(1)* was used with some modifications to evaluate the effectiveness of household compounds against *L. monocytogenes, E. coli* O157:H7, and *Salmonella* Typhimurium. The household compounds and distilled water were preequilibrated to an initial temperature of 25 or 55°C in a water bath before testing. Bacterial suspension (0.1 ml of the 5×10^9 CFU/ml culture) was added to 9.9 ml of distilled water and each household compound at the concentrations to be tested, and test tubes were held at room temperature (25°C) for 1 or 10 min. Exposed cultures (1.0 ml) were transferred to 9.0 ml of D/E neutralizing broth (Difco, Becton Dickinson). According to the manufacturer's information (http://www.bd.com/ds/technicalCenter/inserts/D_E_Neutralizing_Agar.pdf), the D/E neutralizing broth used in our study contains five neutralizers: lecithin, polysorbate 80, sodium bisulfite, sodium thioglycollate, and sodium thiosulfate. These neutralizers inactivate a variety of disinfectant and antiseptic chemicals; sodium bisulfite neutralizes aldehydes, sodium thioglycollate neutralizes mercurials, sodium thiosulfate neutralizes iodine and chlorine, lecithin neutralizes quaternary ammonium compounds, and polysorbate 80 (a nonionic surface active agent) neutralizes substituted phenolics.

Initial (pretreatment) inoculum levels were determined by adding 0.1 ml of bacterial suspension to 9.9 ml of distilled water (25°C). After 10 min, 1.0 ml of this solution was transferred to 9.0 ml of D/E neutralizing broth. Neutralized samples were serially diluted 10-fold in 0.1% buffered peptone water (Difco, Becton Dickinson) and surface plated onto both nonselective media, i.e., tryptic soy agar (TSA; Difco) containing 0.6% yeast extract (Acumedia) (TSAYE) for *L. monocytogenes* and TSA for *E. coli* O157:H7 and *Salmonella* Typhimurium, and selective media, i.e., PALCAM agar (Difco, Becton Dickinson) for *L. monocytogenes,* sorbitol-MacConkey agar (Difco, Becton Dickinson) with cefixime-tellurite supplement (Invitrogen Dynal AS, Oslo, Norway) (SMAC-CT) for *E. coli* O157:H7, and xylose lysine deoxycholate agar (XLD; Acumedia) for *Salmonella* Typhimurium. Colonies on SMAC-CT and XLD agar plates were counted after incubation at 35°C for 48 h, and colonies on PALCAM agar plates were counted after incubation at 30°C for 48 h.

Statistical analysis. All tests were performed in two independent replication trials with three samples for each. The reduction of cells due to the action of household compounds at 25°C was calculated as the difference in cell numbers before treatment and after treatment. The reduction of cells due to the action of household compounds at the initial temperature of 55°C for 1 min was calculated as the difference in cell numbers after treatment with water (initial temperature of 55°C, 1 min) and after treatment with each household compound (initial temperature of 55°C, 1 min). The reduction of cells due to the action of household compounds at the initial temperature of 55°C for 10 min was calculated as the difference in cell numbers after treatment with water (initial temperature of 55°C, 10 min) and after treatment with each household compound (initial temperature of 55°C, 10 min). Inoculation levels of the five-strain cocktails of *L. monocytogenes, E. coli* O157:H7, and *Salmonella* Typhimurium and their survival after exposure to the water treatment at an initial temperature of 55°C for 1 and 10 min are summarized in Table 3. Data were analyzed using the general linear mixed model procedure, Proc

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/72/6/1201/1680761/0362-028x-72_6_1201.pdf by guest on 08 August 2022

TABLE 2. L. monocytogenes, E. coli *O157:H7, and* Salmonella *strains used in the suspension test (2, 8, 21)*

Strain	Lineage	Serotype	Source	Antimicrobial resistance pattern[a]
L. monocytogenes				
J1-177	I	1/2b	Human, sporadic	
C1-056	II	1/2a	Human, sporadic	
N3-013	I	4b	Food, epidemic	
R2-499	II	1/2a	Human, epidemic, sliced turkey	
N1-227	I	4b	Food, epidemic	
E. coli O157:H7				
ATCC 43889			Feces of patient with hemolytic uremic syndrome, North Carolina	
ATCC 43888			Human feces	
ATCC 43895			Raw hamburger meat implicated in outbreak of hemorrhagic colitis	
ATCC 43894			Human feces from outbreak of hemorrhagic colitis, Michigan	
EO139			Venison jerky isolate (Dr. M. P. Doyle, University of Georgia, Griffin)	
Salmonella				
Typhimurium ATCC 14028			Tissue, animal	
Typhimurium DT104 ATCC 700408				ACSSuT, trimethoprim-sulfamethoxazole
Typhimurium DT104 UK 1			Equine outbreak isolate (Dr. J. W. Foster, University of South Alabama, Mobile)	
Typhimurium DT104 (var. Copenhagen)			Fed plant	ACSSuT, amoxicillin–clavulanic acid, ampicillin
Typhimurium DT104 (var. Copenhagen)			Fed plant	ACSSuT, amoxicillin–clavulanic acid, trimethoprim-sulfamethoxazole

[a] ACSSuT, ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline.

Mixed, in SAS release 9.1.3 (SAS Institute, Cary, NC). To determine the significance of the effects ($P < 0.05$), least-square means were compared using *t* tests.

RESULTS

Baking soda solutions (11, 33, and 50% sodium bicarbonate) caused <1-log reductions based on counts from nonselective media for all the tested foodborne pathogens under all evaluated conditions (data not shown). Because baking soda did not have a sufficient bactericidal effect against the tested foodborne pathogens, the data for this compound were not included in the statistical analysis. All tested concentrations of household bleach solutions (0.0314, 0.0933, and 0.670% sodium hypochlorite) reduced all foodborne pathogens tested from the inoculum level of approximately 8 log CFU/ml to below the detection level of 2 log CFU/ml after 1 min of exposure at 25°C (Table 4).

After 1 min at 25°C, treatment with undiluted vinegar and 5% acetic acid resulted in >5-log reductions of *Salmonella* Typhimurium (Tables 5 and 6). The other organic acid treatments (Tables 5 through 7) achieved <5-log reductions after 1 min of exposure at 25°C. Therefore, extending the exposure time to 10 min and/or increasing the

TABLE 3. *Initial inoculum levels of five-strain cocktails of* L. monocytogenes, E. coli *O157:H7, and* Salmonella *Typhimurium and their surviving populations after exposure to water treatment*

Exptl time	Initial temp (°C)	Exposure time (min)	Medium[a]	Mean ± SD population (log CFU/ml)		
				L. monocytogenes	*E. coli* O157:H7	*Salmonella* Typhimurium
At inoculation	25	10	Nonselective	7.79 ± 0.13	8.05 ± 0.15	8.37 ± 0.05
			Selective	7.77 ± 0.11	7.83 ± 0.34	8.18 ± 0.12
After water treatment	55	1	Nonselective	7.04 ± 0.20	7.32 ± 0.10	7.36 ± 0.06
			Selective	6.85 ± 0.17	7.16 ± 0.08	7.10 ± 0.05
	55	10	Nonselective	6.62 ± 0.29	7.25 ± 0.13	7.18 ± 0.05
			Selective	6.55 ± 0.26	5.80 ± 0.75	6.91 ± 0.07

[a] Nonselective: TSAYE for *L. monocytogenes* and TSA for *E. coli* O157:H7 and *Salmonella* Typhimurium. Selective: PALCAM agar for *L. monocytogenes*, SMAC-CT for *E. coli* O157:H7, and XLD agar for *Salmonella* Typhimurium.

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/72/6/1201/1680761/0362-028x-72_6_1201.pdf by guest on 08 August 2022

TABLE 4. *Surviving populations of five-strain cocktails of* L. monocytogenes, E. coli *O157:H7, and* Salmonella *Typhimurium on nonselective and selective media after treatments with bleach*

% sodium hypochlorite	Initial temp (°C)	Exposure time (min)	Medium[a]	Mean population (log CFU/ml)[b]		
				L. monocytogenes	*E. coli* O157:H7	*Salmonella* Typhimurium
0.0314	25	1	Nonselective	<2.00	<2.00	<2.00
			Selective	<2.00	<2.00	<2.00
0.0933	25	1	Nonselective	<2.00	<2.00	<2.00
			Selective	<2.00	<2.00	<2.00
0.670	25	1	Nonselective	<2.00	<2.00	<2.00
			Selective	<2.00	<2.00	<2.00

[a] Nonselective: TSAYE for *L. monocytogenes* and TSA for *E. coli* O157:H7 and *Salmonella* Typhimurium. Selective: PALCAM agar for *L. monocytogenes*, SMAC-CT for *E. coli* O157:H7, and XLD agar for *Salmonella* Typhimurium.

[b] The initial cell density of the suspensions is given in Table 3. Survival data are the mean of six samples. All values were below the detection limit (2.00 log CFU/ml). None of the results were significantly different from each other ($P < 0.05$).

initial temperature to 55°C was evaluated for improving effectiveness. Significantly greater reductions ($P < 0.05$) in *L. monocytogenes* and *E. coli* O157:H7 with undiluted vinegar and 5% acetic acid and in *Salmonella* Typhimurium with 5% citric acid were achieved by either extending the exposure time to 10 min or increasing the initial temperature to 55°C (Tables 5 through 7). Increasing the initial temperature to 55°C was more effective than extending the exposure time to 10 min for reducing *L. monocytogenes* and *E. coli* O157:H7 with 1:1 diluted vinegar (Table 5). Combined effects of the initial temperature of 55°C and 10 min always caused significantly greater reductions ($P < 0.05$) in all three foodborne pathogens tested (Tables 6 and 7).

Undiluted hydrogen peroxide reduced *Salmonella* Typhimurium (8.37 log CFU/ml) and *E. coli* O157:H7 (8.05 log CFU/ml) to below the detection limit of 2 log CFU/ml after 1 min at 25°C (Table 8). The 1:1 diluted hydrogen peroxide reduced pathogenic bacteria to below the detection limit after 1 min at 25°C for *Salmonella* Typhimurium, 10 min at 25°C or 1 min at the initial temperature of 55°C for *E. coli* O157:H7, and 10 min at the initial temperature of 55°C for *L. monocytogenes* (Table 8).

The tested pathogens were not significantly different ($P \geq 0.05$) in their sensitivity to the bleach treatments. The sensitivity of the tested pathogens to the organic acid and hydrogen peroxide treatments was dependent on initial temperature and exposure time. The sensitivity of *Salmonella*

TABLE 5. *Surviving populations of five-strain cocktails of* L. monocytogenes, E. coli *O157:H7, and* Salmonella *Typhimurium on nonselective and selective media after treatments with white vinegar*

White vinegar treatment	Initial temp (°C)	Exposure time (min)	Medium[a]	Mean ± SD population (log CFU/ml)[b]		
				L. monocytogenes	*E. coli* O157:H7	*Salmonella* Typhimurium
Undiluted (5% acetic acid)	25	1	Nonselective	5.99 ± 0.29 EF	6.01 ± 0.46 E	<2.00 A
			Selective	4.26 ± 1.44 ab	2.63 ± 0.35 a	<2.00 a
	25	10	Nonselective	2.22 ± 0.32 A	2.72 ± 0.73 A	<2.00 A
			Selective	2.22 ± 0.34 a	<2.00 a	<2.00 a
	55	1	Nonselective	<2.00 A	<2.00 A	<2.00 A
			Selective	<2.00 a	<2.00 a	<2.00 a
	55	10	Nonselective	<2.00 A	<2.00 A	<2.00 A
			Selective	<2.00 a	<2.00 a	<2.00 a
1:1 diluted (2.5% acetic acid)	25	1	Nonselective	7.04 ± 0.10 F	7.36 ± 0.27 E	5.10 ± 0.02 CD
			Selective	6.89 ± 0.15 c	5.52 ± 0.43 b	3.29 ± 0.11 a
	25	10	Nonselective	7.03 ± 0.10 F	6.09 ± 0.04 E	<2.00 A
			Selective	6.90 ± 0.10 c	<2.00 a	<2.00 a
	55	1	Nonselective	4.82 ± 1.29 DE	2.82 ± 0.42 AB	<2.00 A
			Selective	4.30 ± 1.52 b	<2.00 a	<2.00 a
	55	10	Nonselective	2.73 ± 0.45 AB	<2.00 A	<2.00 A
			Selective	<2.00 a	<2.00 a	<2.00 a

[a] Nonselective: TSAYE for *L. monocytogenes* and TSA for *E. coli* O157:H7 and *Salmonella* Typhimurium. Selective: PALCAM agar for *L. monocytogenes*, SMAC-CT for *E. coli* O157:H7, and XLD agar for *Salmonella* Typhimurium.

[b] The initial cell density of the suspensions is given in Table 3. Survival data are the mean of six samples. Some values were below the detection limit (2.00 log CFU/ml). Within both rows and columns, means not followed by the same uppercase letters are significantly different ($P < 0.05$). Within both rows and columns, means not followed by the same lowercase letters are significantly different ($P < 0.05$).

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/72/6/1201/1680761/0362-028x-72_6_1201.pdf by guest on 08 August 2022

TABLE 6. *Surviving populations of five-strain cocktails of* L. monocytogenes, E. coli *O157:H7, and* Salmonella *Typhimurium on nonselective and selective media after treatments with 5% acetic acid (prepared from glacial acetic acid)*

Initial temp (°C)	Exposure time (min)	Medium[a]	Mean ± SD population (log CFU/ml)[b]		
			L. monocytogenes	*E. coli* O157:H7	*Salmonella* Typhimurium
25	1	Nonselective	6.02 ± 0.42 B	5.49 ± 0.78 B	<2.00 A
		Selective	5.24 ± 0.48 b	<2.00 a	<2.00 a
25	10	Nonselective	<2.00 A	<2.00 A	<2.00 A
		Selective	<2.00 a	<2.00 a	<2.00 a
55	1	Nonselective	<2.00 A	<2.00 A	<2.00 A
		Selective	<2.00 a	<2.00 a	<2.00 a
55	10	Nonselective	<2.00 A	<2.00 A	<2.00 A
		Selective	<2.00 a	<2.00 a	<2.00 a

[a] Nonselective: TSAYE for *L. monocytogenes* and TSA for *E. coli* O157:H7 and *Salmonella* Typhimurium. Selective: PALCAM agar for *L. monocytogenes*, SMAC-CT for *E. coli* O157:H7, and XLD agar for *Salmonella* Typhimurium.

[b] The initial cell density of the suspensions is given in Table 3. Survival data are the mean of six samples. Some values were below the detection limit (2.00 log CFU/ml). Within both rows and columns, means not followed by the same uppercase letters are significantly different ($P < 0.05$). Within both rows and columns, means not followed by the same lowercase letters are significantly different ($P < 0.05$).

Typhimurium to the organic acid treatments for 1 min at 25°C was higher ($P < 0.05$) than the sensitivity of *E. coli* O157:H7 and *L. monocytogenes* except for treatment with 5% citric acid, whereas *Salmonella* Typhimurium and *E. coli* O157:H7 had significantly higher sensitivity than *L. monocytogenes* to hydrogen peroxide treatments. The sensitivity of *Salmonella* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* was not different ($P \geq 0.05$) when the exposure time was extended to 10 min and the initial temperature was increased to 55°C.

The minimum required conditions to reduce the tested foodborne pathogens from approximately 8 log CFU/ml (Table 3) to below the detection limit of 2 log CFU/ml is summarized in Table 9. In most cases, after 1 min at 25°C, *Salmonella* Typhimurium was reduced to below the detection limit by all the tested household compounds except the 1:1 diluted vinegar and 5% citric acid. The higher initial temperature of 55°C was necessary for reducing *L. monocytogenes* to below the detection limit except for treatment with household bleach (25°C for 1 min). When 1:1 diluted hydrogen peroxide and 1:1 diluted vinegar were used, increasing exposure time to 10 min resulted in a reduction of *E. coli* O157:H7 similar to that achieved with undiluted hydrogen peroxide and vinegar.

DISCUSSION

Five products commonly available in households were tested for their effectiveness against common foodborne pathogens. Baking soda (sodium bicarbonate) is a naturally occurring mild alkali. White vinegar is a weak organic acid containing 5% acetic acid. Citric acid (5%), also a weak organic acid, was used to simulate lemon and lime juice.

TABLE 7. *Surviving populations of five-strain cocktails of* L. monocytogenes, E. coli *O157:H7, and* Salmonella *Typhimurium on nonselective and selective media after treatments with 5% citric acid*

Initial temp (°C)	Exposure time (min)	Medium[a]	Mean ± SD population (log CFU/ml)[b]		
			L. monocytogenes	*E. coli* O157:H7	*Salmonella* Typhimurium
25	1	Nonselective	7.13 ± 0.06 F	6.84 ± 0.30 EF	7.13 ± 0.16 EF
		Selective	7.12 ± 0.06 c	6.15 ± 0.36 b	5.72 ± 0.14 b
25	10	Nonselective	5.96 ± 0.17 DE	6.59 ± 0.10 DE	4.01 ± 0.01 AB
		Selective	5.75 ± 0.12 b	5.38 ± 0.08 b	<2.00 a
55	1	Nonselective	4.73 ± 0.77 CD	4.45 ± 0.56 C	<2.00 A
		Selective	4.53 ± 0.94 b	3.32 ± 0.75 a	<2.00 a
55	10	Nonselective	<2.00 A	<2.00 A	<2.00 A
		Selective	<2.00 a	<2.00 a	<2.00 a

[a] Nonselective: TSAYE for *L. monocytogenes* and TSA for *E. coli* O157:H7 and *Salmonella* Typhimurium. Selective: PALCAM agar for *L. monocytogenes*, SMAC-CT for *E. coli* O157:H7, and XLD agar for *Salmonella* Typhimurium.

[b] The initial cell density of the suspensions is given in Table 3. Survival data are the mean of six samples. Some values were below the detection limit (2.00 log CFU/ml). Within both rows and columns, means not followed by the same uppercase letters are significantly different ($P < 0.05$). Within both rows and columns, means not followed by the same lowercase letters are significantly different ($P < 0.05$).

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/72/6/1201/1680761/0362-028x-72_6_1201.pdf by guest on 08 August 2022

TABLE 8. *Surviving populations of five-strain cocktails of* L. monocytogenes, E. coli *O157:H7, and* Salmonella *Typhimurium on nonselective and selective media after treatments with hydrogen peroxide*

Hydrogen peroxide	Initial temp (°C)	Exposure time (min)	Medium[a]	Mean ± SD population (log CFU/ml)[b]		
				L. monocytogenes	*E. coli* O157:H7	*Salmonella* Typhimurium
Undiluted (3%)	25	1	Nonselective	4.02 ± 0.55 B	<2.00 A	<2.00 A
			Selective	2.59 ± 0.69 a	<2.00 a	<2.00 a
	25	10	Nonselective	2.37 ± 0.37 A	<2.00 A	<2.00 A
			Selective	<2.00 a	<2.00 a	<2.00 a
	55	1	Nonselective	<2.00 A	<2.00 A	<2.00 A
			Selective	<2.00 a	<2.00 a	<2.00 a
	55	10	Nonselective	<2.00 A	<2.00 A	<2.00 A
			Selective	<2.00 a	<2.00 a	<2.00 a
1:1 diluted (1.5%)	25	1	Nonselective	6.04 ± 0.23 C	4.04 ± 0.90 B	<2.00 A
			Selective	5.44 ± 0.16 b	<2.00 a	<2.00 a
	25	10	Nonselective	4.21 ± 0.11 B	<2.00 A	<2.00 A
			Selective	2.14 ± 0.36 a	<2.00 a	<2.00 a
	55	1	Nonselective	5.48 ± 0.91 C	<2.00 A	<2.00 A
			Selective	4.45 ± 1.30 b	<2.00 a	<2.00 a
	55	10	Nonselective	<2.00 A	<2.00 A	<2.00 A
			Selective	<2.00 a	<2.00 a	<2.00 a

[a] Nonselective: TSAYE for *L. monocytogenes* and TSA for *E. coli* O157:H7 and *Salmonella* Typhimurium. Selective: PALCAM agar for *L. monocytogenes*, SMAC-CT for *E. coli* O157:H7, and XLD agar for *Salmonella* Typhimurium.

[b] The initial cell density of the suspensions is given in Table 3. Survival data are the mean of six samples. Some values were below the detection limit (2.00 log CFU/ml). Within both rows and columns, means not followed by the same uppercase letters are significantly different ($P < 0.05$). Within both rows and columns, means not followed by the same lowercase letters are significantly different ($P < 0.05$).

Household bleach (sodium hypochlorite as the active ingredient) and hydrogen peroxide kill microorganisms by oxidation. Most of these materials are not harsh chemicals and are economical, safe, and environmentally friendly. To our knowledge, this is the first report of the effectiveness of a variety of household compounds against the foodborne pathogens *L. monocytogenes, E. coli* O157:H7, and *Salmonella* Typhimurium.

Our results suggested that the antimicrobial activities of these household compounds (at 25°C for 1 min) from the highest to the lowest were 0.0314% sodium hypochlorite > 3% hydrogen peroxide > undiluted vinegar and 5%

TABLE 9. *Summary of reaction time and initial solution temperature needed to reduce tested foodborne pathogens to below the detection limit of 2 log CFU/ml on nonselective media*[a]

Household compound	Ingredient and concn	pH	Reaction temp and time		
			L. monocytogenes	*E. coli* O157:H7	*Salmonella* Typhimurium
Clorox bleach	Sodium hypochlorite (0.0314%)	8.36	25°C, 1 min	25°C, 1 min	25°C, 1 min
	Sodium hypochlorite (0.0933%)	9.00	25°C, 1 min	25°C, 1 min	25°C, 1 min
	Sodium hypochlorite (0.670%)	10.14	25°C, 1 min	25°C, 1 min	25°C, 1 min
Hydrogen peroxide	3%	2.75	55°C, 1 min	25°C, 1 min	25°C, 1 min
	1.5%	2.82	55°C, 10 min	25°C, 10 min or 55°C, 1 min	25°C, 1 min
White vinegar	Acetic acid (5%)	2.58	55°C, 1 min	55°C, 1 min	25°C, 1 min
	Acetic acid (2.5%)	2.78	NA[b]	55°C, 10 min	25°C, 10 min or 55°C, 1 min
Acetic acid (prepared from glacial acetic acid)	5%	2.70	55°C, 1 min	25°C, 10 min or 55°C, 1 min	25°C, 1 min
Citric acid	5%	2.31	55°C, 10 min	55°C, 10 min	55°C, 1 min

[a] Initial concentrations of the pathogens are indicated in Table 3.

[b] NA, not available; none of the tested conditions reduced the tested pathogen to below the detection limit.

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/72/6/1201/1680761/0362-028x-72_6_1201.pdf by guest on 08 August 2022

acetic acid > 5% citric acid > baking soda (50% sodium bicarbonate, which exhibited no antibacterial activity under the conditions tested). The effectiveness of household bleach and the lack of detectable effectiveness of baking soda are in agreement with previously published findings *(15, 16, 23).* Vijayakumar and Wolf-Hall *(23)* reported that the efficacy against *E. coli* on iceberg lettuce was hydrogen peroxide > white vinegar > lime and lemon juice.

Bleach was the only household compound that was effective against all the tested foodborne pathogens after 1 min of exposure at room temperature (25°C). In spite of bleach's effectiveness, concerns about its unpleasant odor, corrosiveness on metal surfaces, and safe preparation of use solutions from concentrated stocks may limit its application in the home environment. Hydrogen peroxide was the next most effective household compound tested after household bleach. Compared with bleach, hydrogen peroxide is easier to handle and is considered more ''natural'' because it always decomposes into oxygen and water. Hydrogen peroxide at 3% concentration is widely available in supermarkets. Because it degrades quickly when exposed to light, freshly poured hydrogen peroxide should be used within a short period of time. Our results for the other household products used for pathogen inactivation, especially against *L. monocytogenes,* indicate that these products should be applied as warm solutions (55°C initial temperature) for maximum efficacy.

The effectiveness of organic acids is dependent on variables including type of acidulant, concentration, pH, temperature, and contact time *(12).* When compared on an equal pH basis, the order of antimicrobial activity against *L. monocytogenes* was acetic acid > citric acid, whereas based on equal molar concentration, the order was citric acid > acetic acid *(20).* In some studies, acetic acid was more effective than citric acid for inhibiting *E. coli* O157:H7 *(12, 17).* In this study, the antimicrobial activities of acetic acid and citric acid were compared at 5% concentration because this is the concentration available in homes. At 5%, the calculated molarities for acetic acid and citric acid solutions should be 0.83 and 0.26 M, respectively, with corresponding pH values of 2.41 and 1.86. However, the actual pH values of 5% acetic acid and citric acid solutions made in the laboratory were 2.70 and 2.31, respectively. This deviation between calculated and actual pH values may be primarily due to the ionic strength of the distilled water used. The results of our study suggested that at 5% concentration, vinegar was more effective than citric acid against all foodborne pathogens tested.

In this study, pathogenic bacteria surviving exposure to the household compounds were enumerated on both selective and nonselective media. The difference between the bacterial counts on these two media types indicates the extent of cell injury. Organic acids and hydrogen peroxide injured cells when applied at 25°C for 1 min. The exposure of foodborne pathogens to a sublethal environment may result in survivors with increased tolerance to acidic conditions *(13, 18).* Increasing temperature and/or increasing contact time have been reported as efficient methods to improve sanitizer efficacy *(19, 20, 22).* In the present study, increasing the initial temperature of 1:1 diluted vinegar to 55°C enhanced inactivation of *L. monocytogenes* and *E. coli* O157:H7 more than did extending the exposure time to 10 min. Significantly higher efficacy ($P < 0.05$) was always achieved at the initial temperature of 55°C for 10 min than at 25°C for 1 min, indicating that preparation of household compounds with warm water or prewarming household compounds before use plus a longer contact time would be helpful for limiting the survival of acid-adapted bacterial cells.

The differences in the sensitivities of foodborne pathogens to household compounds may be explained by differences in membrane structures between gram-positive and gram-negative bacteria. It is generally believed that the undissociated molecules of organic acids diffuse into the cell, release protons in the cytoplasm, and cause cell death *(3).* Gram-positive bacteria have a much thicker peptidoglycan layer than do gram-negative bacteria, which may cause some difficulties in the diffusion of organic acids. *L. monocytogenes* was the pathogen least sensitive to the household compounds tested. *Salmonella* Typhimurium was the most sensitive to organic acid and hydrogen peroxide treatments, although the strains used in the present study were multidrug resistant *(2).*

In conclusion, the efficacies of household compounds against all tested pathogens from the highest to the lowest (at 25°C for 1 min) were 0.0314% sodium hypochlorite > 3% hydrogen peroxide > undiluted vinegar and 5% acetic acid > 5% citric acid > baking soda (50% sodium bicarbonate). The sensitivity of tested foodborne pathogens to organic acid and hydrogen peroxide treatments at 25°C for 1 min was *Salmonella* Typhimurium > *E. coli* O157:H7 > *L. monocytogenes*. The household bleach solution (0.0314% sodium hypochlorite) prepared by adding 1 teaspoon (5 ml) of bleach into 1 quart (950 ml) of water was the most effective against all tested foodborne pathogens when applied at 25°C for 1 min. When the other household products were used against *L. monocytogenes,* an initial temperature of 55°C was required for maximum effectiveness. It is important to note that our data were obtained with a suspension test in which microorganisms are highly susceptible to inactivation. Therefore, the results presented may not fully demonstrate the exact efficacy of these household compounds against microorganisms on surfaces, especially in the presence of food debris. Hard surface sanitation studies should be undertaken to further determine the effectiveness of these household compounds and to compare their effectiveness with that of commercial sanitizers.

ACKNOWLEDGMENTS

This work was supported by the National Integrated Food Safety Initiative of the U.S. Department of Agriculture Cooperative State Research, Education, and Extension Service (agreements 2004-51110-02160 and 2005-51110-03278) and by the Colorado State University Agricultural Experiment Station.

REFERENCES

1. AOAC International. 2000. Germicidal and detergent sanitizing action and disinfectants. Official method 960 09. *In* Official methods

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/72/6/1201/1680761/0362-028x-72_6_1201.pdf by guest on 08 August 2022

of analysis of AOAC International, 17th ed. AOAC International, Gaithersburg, MD.
2. Bacon, R. T., J. R. Ransom, J. N. Sofos, P. A. Kendall, K. E. Belk, and G. C. Smith. 2003. Thermal inactivation of susceptible and multiantimicrobial-resistant *Salmonella* strains grown in the absence or presence of glucose. *Appl. Environ. Microbiol.* 69:4123–4128.
3. Bjornsdottir, K., F. Breidit, Jr., and R. F. McFeeters. 2006. Protective effect of organic acids on survival of *Escherichia coli* O157:H7 in acidic environments. *Appl. Environ. Microbiol.* 72:660–664.
4. Bloomfield, S. F., and E. Scott. 1997. Cross-contamination and infection in the domestic environment and the role of chemical disinfectants. *J. Appl. Microbiol.* 83:1–9.
5. Collins, J. E. 1997. Impact of changing consumer lifestyles on the emergence/reemergence of foodborne pathogens. *Emerg. Infect. Dis.* 3:471–479.
6. Doyle, M. P., L. R. Beuchat, and T. J. Montville (ed.). 2001. Food microbiology: fundamentals and frontiers. ASM Press, Washington, DC.
7. Entani, E., M. Asai, S. Tsujihata, Y. Tsukamoto, and M Ohta. 1998. Antibacterial action of vinegar against food-borne pathogenic bacteria including *Escherichia coli* O157:H7. *J. Food Prot.* 61:953–959.
8. Fugett, E., E. Fortes, C. Nnoka, and M. Wiedmann. 2006. International Life Sciences Institute North America *Listeria monocytogenes* strain collection: development of standard *Listeria monocytogenes* strain sets for research and validation studies. *J. Food Prot.* 69: 2929–2938.
9. Gorman, R., S. Bloomfield, and C. C. Adley. 2002. A study of cross-contamination of food-borne pathogens in the domestic kitchen in the Republic of Ireland. *Int. J. Food Microbiol.* 76:143–150.
10. Humphrey, T. 2001. The spread and persistence of *Campylobacter* and *Salmonella* in the domestic kitchen. *J. Infect.* 43:50–53.
11. Jackson, V., I. S. Blair, D. A. McDowell, J. Kennedy, and D. J. Bolton. 2005. The incidence of significant foodborne pathogens in domestic refrigerators. *Food Control* 18:346–351.
12. Khurana, A., G. B. Awuah, B. Taylor, and E. Enache. 2006. Efficacy of selected acidulants in pureed green beans inoculated with pathogens (*Escherichia coli* O157:H7 and *Listeria monocytogenes*). *J. Food Prot.* 69:1865–1869.
13. Koutsoumanis, K. P., P. A. Kendall, and J. N. Sofos. 2003. Effect of food processing-related stresses on acid tolerance of *Listeria monocytogenes. Appl. Environ. Microbiol.* 69:7514–7516.
14. Kusumaningrum, H. D., G. Riboldi, W. C. Hazeleger, and R. R. Beumer. 2003. Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. *Int. J. Food Microbiol.* 85:227–236.
15. Parnes, C. A. 1997. Efficacy of sodium hypochlorite bleach and ''alternative'' products in preventing transfer of bacteria to and from inanimate surfaces. *Environ. Health* 59:14–20.
16. Rutala, W. A., S. L. Barbee, N. C. Aguiar, M. D. Sobsey, and D. J. Weber. 2000. Antimicrobial activity of home disinfectants and natural products against potential human pathogens. *Infect. Control Hosp. Epidemiol.* 21:33–38.
17. Ryu, J. H., Y. Deng, and L. R. Beuchat. 1999. Behavior of acid-adapted and unadapted *Escherichia coli* O157:H7 when exposed to reduced pH achieved with various organic acids. *J. Food Prot.* 62: 451–455.
18. Samelis, J., J. N. Sofos, P. A. Kendall, and G. C. Smith. 2002. Effect of acid adaptation on survival of *Escherichia coli* O157:H7 in meat decontamination washing fluids and potential effects of organic acid interventions on the microbial ecology of the meat plant environment. *J. Food Prot.* 65:33–40.
19. Shin, J. H., S. Y. Lee, R. H. Dougherty, B. Rasco, and D. H. Kang. 2006. Combined effect of mild heat and acetic acid treatment for inactivating *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* Typhimurium in an asparagus puree. *J. Appl. Microbiol.* 101:1140–1151.
20. Sorrells, K. M., D. C. Enigl, and J. R. Hatfield. 1989. Effect of pH, acidulant, time, and temperature on the growth and survival of *Listeria monocytogenes. J. Food Prot.* 52:571–573.
21. Stopforth, J. D., P. N. Skandamis, I. Geornaras, and J. N. Sofos. 2007. Acid tolerance of acid-adapted and nonacid-adapted *Escherichia coli* O157:H7 strains in beef decontamination runoff fluids or on beef tissue. *Food Microbiol.* 24:530–538.
22. Tsujihata, S., E. Entani, M. Asai, Y. Tsukamoto, and M. Ohta. 1998. Mathematical modeling to predict the bactericidal effect of processed vinegar on *Escherichia coli* O157:H7. *Int. J. Food Microbiol.* 43: 135–138.
23. Vijayakumar, C., and C. E. Wolf-Hall. 2002. Evaluation of household sanitizers for reducing levels of *Escherichia coli* on iceberg lettuce. *J. Food Prot.* 65:1646–1650.

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/72/6/1201/1680761/0362-028x-72_6_1201.pdf by guest on 08 August 2022