Journal of Food Technology 3 (3): 464-468, 2005

Inactivation of *Escherichia coli* O157:H7 on Apples by Caprylic Acid

Patrick Marek, Thirunavukkarasu Annamalai and Kumar Venkitanarayanan
Department of Animal Science, Unit-40, University of Connecticut, 3636 Horsebarn Hill Road Extension, Storrs, Connecticut 06269

Abstract: The efficacy of caprylic acid as a wash treatment for reducing *E. coli* O157:H7 on apples was investigated. Apples inoculated with a five-strain mixture of *E. coli* O157:H7 (10^9 CFU) were subjected to washing in sterile deionized water (control) or deionized water containing 50 mM, 75 mM, or 100 mM caprylic acid for 5 or 10 min at 24°C (treatment) with gentle shaking. Immediately after washing in caprylic acid solution or water, apples were transferred to separate Whirl-Pak bags containing 50 ml of sterile Tryptic Soy Broth (TSB) and agitated at 200 rpm for 2 min. One ml of wash suspension from each bag was serially diluted (1:10) with 9 ml of sterile PBS and 0.1-ml portions from appropriate dilutions were spread plated on duplicate Tryptic soy agar pates. The plates were incubated at 37°C for 24 h before counting the colonies. Immersion of apples in caprylic acid solutions (50, 75, or 100 mM) for 5 or 10 min significantly reduced ($P < 0.05$) *E. coli* O157:H7 population, in comparison to that on apples soaked in sterile water (control). Moreover, no *E. coli* O157:H7 was detected in caprylic acid wash solution, whereas a substantial population of the pathogen survived in the control wash water. Results indicate that caprylic acid treatment could effectively be used to kill *E. coli* O157:H7 on apples, but sensory and quality characteristics of apples treated with caprylic acid need to be determined.

Key words: Antimicrobial, Apple, caprylic acid, *escherichia coli* o157:h7, medium-chain fatty acid

INTRODUCTION

Enterohemorrhagic *Escherichia coli* O157:H7 is a major foodborne pathogen in the United States. The Centers for Disease Control and Prevention, Atlanta, Georgia estimated that *E. coli* O157:H7 accounts for more than 73,000 cases of foodborne illness each year in the United States[1]. *E. coli* O157:H7 primarily colonizes the rumen and colon of cattle[2,3]. Cattle have been implicated as the principal reservoir of *E. coli* O157:H7[4-7], with fecal contamination of food products being an important source of human infection. Although most of the *E. coli* O157:H7 foodborne outbreaks are associated with the consumption of undercooked ground beef[2,9,8], many *E. coli* O157:H7 outbreaks involving non-bovine foods, such as fruits and vegetables are linked to cross contamination of the implicated food with contaminated bovine manure [10]. Moreover, fresh apple cider has been implicated in many disease outbreaks involving *E. coli* O157:H7 in the United States[11,12]. Fresh apple cider (turbid non-fermented apple juice)[13] is a ready-to-eat product, which often receives no microbial inactivation steps in its manufacturing. Apple cider potentially gets contaminated with *E. coli* O157:H7 by the usage of ground apples contaminated with the pathogen present in animal fecal material in soil[10]. Therefore, effective methods for reducing or eliminating *E. coli* O157:H7 on apples are critical. Treatment of apples with water or water containing chlorine or commercial sanitizing agents approved for fruits and vegetables cannot bring about significant reductions in bacterial load[14,15]. Effective methods that reduce pathogenic microorganisms on fruits and vegetables will help in the successful implementation of Hazard Analysis Critical Control Points (HACCP) programs by the fresh produce industry.

Caprylic acid (octanoic acid,) is a natural, eight-carbon fatty acid present in breast milk, bovine milk [16] and coconut oil[17,18]. Caprylic acid is a food-grade chemical approved by the FDA as GRAS (CFR 184.1025). Previous studies conducted in our laboratory revealed that caprylic was very effective in killing *E. coli* O157:H7 [19] and *Salmonella* Enteritidis[20] in bovine rumen fluid and chicken cecal contents, respectively. The objective of this study was to determine the efficacy of caprylic acid as a wash treatment for reducing *E. coli* O157:H7 on apples.

MATERIALS AND METHODS

Bacterial culture and media: Five different isolates of *E. coli* O157:H7 were used for the study. The cultures were obtained from Dr. Michael P. Doyle at the Center for Food Safety, University of Georgia, Griffin, Georgia. The five strains of *E. coli* O157:H7 were E06 (milk isolate), E08 (meat isolate), E10 (meat isolate), E16 (meat isolate) and

Corresponding Author: Kumar Venkitanarayanan, Department of Animal Science, Unit-40, University of Connecticut, 3636 Horsebarn Hill Road Extension, Storrs, Connecticut 06269
Tele: (860)-486-0947 Fax: (860)-486-4375

E22 (calf feces isolate). The five strains of *E. coli* O157:H7 were individually cultured in 10 ml of Tryptic Soy Broth (TSB, Difco, Sparks, MD) at 37 °C for 24 h with agitation (150 rpm). Following incubation, the cultures were sedimented by centrifugation (3600 X *g* for 15 min), washed twice and resuspended in 1 ml of sterile 10 mM Phosphate Buffered Saline (PBS, pH 7.4). The bacterial population in each culture was verified by plating 0.1-ml portions of the appropriately diluted culture on Tryptic Soy Agar plates (TSA, Difco), with incubation at 37 °C for 24 h. Equal portions from each of the five strains were combined and 100 µl (approximately 10^9 CFU) of the suspension was used as the inoculum. The bacterial count of the five-strain mixture of *E. coli* O157:H7 was also confirmed by plating 0.1-ml portions of appropriate dilutions on TSA plates and by incubating the plates at 37 °C for 24 h.

Samples: The samples for the study included Red Delicious apples purchased from a local grocery store. The wax coating on apples was removed as per the method of Venkitanarayanan *et al.*[21]. Briefly, apples were dipped in food-grade ethyl alcohol (Florida Distillers Co., Lake Alfred, FL) for 20 sec and wiped with a paper towel. The fruits were then rinsed with plenty of cool tap water towel and stored in sterile plastic trays at 4°C for at least 24 hours prior to inoculation.

Sample inoculation: Apples (27 for each replicate) were inoculated with the five-strain mixture of *E. coli* O157:H7 as per the method of Venkitanarayanan and others[21]. The samples were placed on a sterile plastic tray and 100 µl of inoculum (10^9 CFU) was applied in drops around the stem end of each fruit. Following inoculation, the samples were transferred to a laminar flow hood and dried at room temperature (24°C) for 2 h. After drying, the population of *E. coli* O157:H7 was determined on three apples (baseline). The remaining apples were immersed in separate, 100 ml sterile Whirl-Pak bags (Nasco, Fort Atkinson, WI) containing 50 ml of sterile deionized water containing 0 mM (control), 50 mM, 75 mM, or 100 mM caprylic acid (Sigma Chemical Co, St. Louis, MO). The Whirl-Pak bags were placed on a bench-top orbitary shaker (New Brunswick Scientific, NJ) at 24°C and agitated gently at 80 rpm for 5 or 10 min, making sure that the inoculated stem end was completely immersed in the treatment solution. At the end of treatment, each apple was transferred to a separate sterile, Whirl-Pak bag containing 50 ml of sterile TSB, taking care that the inoculated stem end of each fruit was completely submerged in the medium.

Bacteriological analysis: The Whirl-Pak bag containing 50 ml of TSB and apple was sealed and placed on a bench-top orbitary shaker (New Brunswick Scientific, NJ) and agitated at 200 rpm for 2 min. One ml of wash suspension from each bag was serially diluted (1:10) with 9 ml of sterile PBS and 0.1-ml portions from appropriate dilutions were spread plated on duplicate TSA plates. A volume of 0.1-ml portions of wash suspension from each Whirl-Pak bag was also directly plated on duplicate TSA plates without dilutions. The plates were incubated at 37°C for 24 h before counting the colonies. Each Whirl-Pak bag containing TSB and apple was incubated at 37°C for 24 h. Following enrichment in TSB, the culture was streaked on Sorbitol MacConkey Agar (SMA, Oxoid Division, Unipath Co., NY) containing 0.1% 4-Methylumbelliferyl-β-D-Glucuronide (MUG, Oxoid) and incubated at 37°C for 24 h. Representative colonies of bacteria from SMA and TSA were confirmed for *E. coli* O157:H7 by API-20E bacterial identification kit (Biomerieux, Hazelwood, MO) and *E. coli* O157 latex agglutination test (Oxoid Division, Ogdensburg, NY).

Triplicate samples of apples were analyzed for baseline, each treatment and control. The entire study was replicated three times. A total of 81 apples were used for the entire study.

Statistical analysis: The data from independent replicate trials were pooled and were analyzed using the general linear model of Statistical Analysis software (SAS institute, Inc., Cary, NC). The significant differences ($P < 0.05$) between the antimicrobial effect of different treatments and controls were determined by Least Significant Difference (LSD) test.

RESULTS

Although a treatment temperature ranging from 4°C to 50°C for soaking apples has been reported in the literature, a temperature of 24°C was used in this study because it is the most practical treatment temperature that can be easily applied and maintained in a factory. Further, the Food and Drug Administration has indicated that the temperature of wash water used for treating fruits and vegetables be at least 10°F warmer than that of produce to prevent penetration of water and contamination of the inside flesh of the produce with microorganisms[14]. The mean pH of wash water (control) and water containing 50, 75, or 100 mM caprylic acid were 6.20, 3.59, 3.57, 3.56, respectively. The results of inactivation of *E. coli* O157:H7 on apples by different concentrations of caprylic acid are provided in Table 1. The mean

Table 1: Inactivation of *E. coli* O157:H7 on apples by caprylic acid

Treatment	Surviving bacterial population (mean log CFU/apple)* after exposure for: 5 min		10 min	
	Apple	Wash	Apple	Wash
0 mM (control)	5.80 ± 0.20^a	7.90 ± 0.13^a	6.00 ± 0.28^a	7.94 ± 0.13^a
50 mM	2.52 ± 0.72^b	0.00 ± 0.00^b	1.62 ± 0.66^b	0.00 ± 0.00^b
75 mM	1.73 ± 0.85^{bc}	0.00 ± 0.00^b	0.90 ± 0.57^{bc}	0.00 ± 0.00^b
100 mM	1.14 ± 0.74^c	0.00 ± 0.00^b	0.67 ± 0.67^c	0.00 ± 0.00^b

± Standard Error of the Mean

* Means in the same column with different letters are significantly different. (P < 0.05)

population of *E. coli* O157:H7 recovered from apples after inoculation (baseline) was approximately 8.0 log CFU/apple. Immersion of apples in caprylic acid solutions (50, 75, or 100 mM) for 5 or 10 min significantly reduced (P < 0.05) *E. coli* O157:H7 population, in comparison to that on apples soaked in sterile water (control). Washing of apples in 50 mM, 75 mM, or 100 mM of caprylic acid solution for 5 min reduced the population of *E. coli* O157:H7 by approximately 5.5 log CFU, 6.3 log CFU and 7.0 log CFU/apple, respectively, with greater reductions in the pathogen counts on apples subjected to 10 min immersion (Table 1). Although the magnitude of reduction in *E. coli* O157 counts on treated apples increased with increase in caprylic acid concentration, only the difference in counts between apples immersed in 50 and 100 mM caprylic acid were significant statistically (P < 0.05). Moreover, the populations of *E. coli* O157:H7 recovered from apples subjected to 5 and 10 min of immersion were not significantly different (P > 0.05) at any tested concentration of caprylic acid. Immersion of apples in water (control) for 5 or 10 min resulted in a reduction of approximately 2.0 log CFU of *E. coli* O157:H7/apple. It was also found that no *E. coli* O157:H7 was detected in the treatment (caprylic acid) wash solution, whereas greater than 7.0 log CFU/ml of the pathogen was recovered from the control wash solution (Table 1), thus representing a potential source of cross-contamination or recontamination in case the same water is used for washing apples.

DISCUSSION

The U.S. Food and Drug Administration proposed that antimicrobial treatments for fruits and vegetables should be capable of reducing bacterial load by a minimum of 5.0 log CFU[22]. Several GRAS (Generally Regarded As Safe) chemicals and sanitizers have been evaluated for killing *E. coli* or *E. coli* O157:H7 on apples. Wright *et al.*[23] investigated the efficacy of hypochlorite (200 ppm), acetic acid (5%), acetic acid (5%) plus hydrogen peroxide (3%), phosphoric acid (0.3%), or peroxyacetic acid (80 ppm) at 25°C for 2 min for killing *E. coli* O157:H7 on apples and reported that a treatment with 5% acetic acid or peroxyacetic acid was most effective, reducing *E. coli* O157:H7 populations on apples by 3.1 log CFU/cm^2 and 2.6 log CFU/cm^2, respectively. Winsniewsky *et al.*[24] evaluated the efficacy of a number of commercially available sanitizers for inactivating *E. coli* O157:H7 on apples and found that none of chemicals resulted in a 5-log CFU reduction on apples. Sapers *et al.* [25] reported that dipping of apples in a solution containing 5% hydrogen peroxide with or without an acid surfactant at 50°C for 1 min reduced *E. coli* on apples by less than 3.0 log CFU/g of *E. coli* on apples. It is evident from the results of the aforementioned studies that none of the treatments resulted in a 5-log CFU reduction on apples. However, we found that treatment of apples with caprylic acid at room temperature decreased *E. coli* O157:H7 populations on apples by greater than 5.0 log CFU.

Numerous hypotheses have been suggested to explain the general mode of antimicrobial activity of fatty acids. Freese *et al.*[26] reported that cell membranes are the primary target for the antimicrobial effect of fatty acids. Short- and medium-chain fatty acids diffuse into bacterial cells in their undissociated form and dissociate within the protoplasm, thereby leading to intracellular acidification [27]. A lower intracellular pH can lead to inactivation of intracellular enzymes[28] and inhibition of amino acid transport[26]. Another explanation on the antimicrobial effect of fatty acids proposes interference with bacterial signal transduction and inhibition of expression of virulence factors and antibiotic resistance genes [29,30].

This study revealed that immersion of apples in caprylic acid solution at room temperature for as low as 5 min can reduce substantial populations of *E. coli* O157:H7 on the fruit. Although washing apples in deionized water reduced *E. coli* O157:H7 counts by approximately 2.0 log CFU/fruit, a large population of the pathogen survived in the wash water. Our future studies will determine the sensory and quality characteristics of apples treated with caprylic acid wash solution.

REFERENCES

1. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin and R. V. Tauxe, 1999. Food-related illness and death in the United States. Emerg. Infect. Dis., 5: 607-625.

2. Rasmussen, M. A., W. C. Cray, T. A. Casey and S. C. Whipp, 1993. Rumen contents as a reservoir of enterohemorragic *Escherichia coli*. FEMS Microbiol. Lett., 114: 79-84.
3. Brashears, M. M., D. Jaroni and J. Trimble, 2003. Isolation, selection and characterization of lactic acid bacteria for a competitive exclusion product to reduce shedding of *Escherichia coli* O157:H7 in cattle. J. Food. Prot., 66: 355-363.
4. Chapman, P. A., D. J. Wright, P. Norman, J. Fox and E. Crick, 1993. Cattle as a possible source of verocytotoxin-producing *Escherichia coli* O157:H7 infections in man. Epidemiol. Infect., 111: 439-447.
5. Zhao, T., M. P. Doyle, J. Shere and L. Garber, 1995. Prevalence of enterohemorrhagic *Escherichia coli* O157:H7 in a survey of dairy herds. Appl. Environ. Microbiol., 61: 1290-1293.
6. Shere, J. A., K. J. Bartlett and C. W. Kaspar, 1998. Longitudinal study of *Escherichia coli* O157:H7 dissemination on four dairy farms in Wisconsin. Appl. Environ. Microbiol., 64: 1390-1399.
7. Laegreid, W. W., R. O. Elder and J. E. Keen, 1999. Prevalence of *Escherichia coli* O157:H7 in range beef calves at weaning. Epidemiol. Infect., 123: 291-298.
8. Padhye, N. V. and M. P. Doyle, 1992. *Escherichia coli* O157:H7 Epidemiology, pathogenesis and methods for detection in food. J. Food. Prot., 55: 555-565.
9. Hancock, D. D., T. E. Besser, M. L. Kinsel, P. I. Tarr, D. H. Rice and M. G. Paros, 1994. The prevalence of *Escherichia coli* O157:H7 in dairy and beef cattle in Washington state. Epidemiol. Infect., 113: 199-207.
10. McLellan, M. R. and D. F. Splittstoesser, 1994. Reducing risk of *E. coli* in apple cider. Food Technol., 50:174.
11. Besser, R. E., S. M. Lett, J. T. Weber, M. P. Doyle, T. J. Barret, J. G. Wells and P. M. Griffin, 1993. An outbreak of diarrhea and hemolytic syndrome from *Escherichia coli* O157:H7 in fresh pressed apple cider. J. American Med. Assoc., 269: 2217-2220.
12. Centers for Disease Control and prevention (CDC), 1997. Outbreaks of *Escherichia coli* O157:H7 infection and Cryptsporidiosis associated with drinking unpasteurized apple cider- Connecticut and New York, October 1996. Morbid Mortal Weekly Rep., 46: 4-8.
13. Uljas, H. E. and S. C. Ingham, 1999. Combination of intervention treatments resulting in 5-$\log_{10}$ unit reductions in numbers of *E. coli* O157:H7 and Salmonella Typhimurium DT104 organisms in apple cider. Appl. Environ. Microbiology., 65: 1924-1929.
14. Food and Drug Administration (FDA), 1997. Guide to minimize microbial food safety hazards for fresh fruits and vegetables. U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Washington, D.C.
15. Sapers, G. M., R. L. Miller and A. M. Mattrazzo, 1999. Effectiveness of sanitizing agents in inactivating *Escherichia coli* on Golden Delicious apples. J. Food. Sci., 64: 734-737.
16. Jensen, R. G., 2002. The composition of bovine milk lipids: January 1995 to December 2000. J. Dairy. Sci., 85: 295-350.
17. Jensen, R. G. , A. M. Ferris, C. J. Lammi-Keefe and R. A. Henderson, 1990. Lipids of bovine and human milks: A comparison. J. Dairy. Sci., 73: 223-240.
18. Sprong, R. C., M. F. Hulstein and M. F. Van der Meer, 2001. Bactericidal activities of milk lipids. Antimicrob. Agents. Chemother., 45: 1298-1301.
19. Annamalai, T., M. K. M. Nair, P. Marek, P. Vasudevan, D. Schreiber, R. Knight, T. Hoagland and K. Venkitanarayanan, 2004. *In vitro* inactivation of enterohemorrhagic Escherichia coli O157:H7 in bovine rumen fluid by caprylic acid. J. Food. Prot., 67: 884-888.
20. Vasudevan, P., P. Marek, M. K. M. Nair, T. Annamalai, M. Darre, M. Khan and K. Venkitanarayanan, 2005. *In vitro* inactivation of Salmonella Enteritidis in chicken cecal contents by caprylic acid. (Accepted in Journal of Applied Poultry Research).
21. Venkitanarayanan, K. S., C. M. Lin, H. Bailey and M. P. Doyle, 2002. Inactivation of *Escherichia coli* O157:H7, *Salmonella* Enteritidis and *Listeria monocytogenes* on apples, oranges and tomatoes by lactic acid with hydrogen peroxide. J. Food. Protect., 65: 100-105.
22. Food and Drug Administration. 1998. Hazard Analysis and Critical Control point (HACCP), procedures for the safe and sanitary processing and importing of juice; food labeling: Warning and notice statements; labeling of juice products; proposed rules. Federtal Register, 63: 20450-20493.
23. Wright, J. R., S. S. Sumner, C. R. Hackney, M. D. Pierson and B. W. Zoecklein, 2000. Reduction of *Escherichia coli* O157:H7 on apples using wash and chemical sanitizer treatments. Dairy Food Environ. Sanit., 20: 120-126.
24. Wisniewsky, M. A., B. A. Glatz, M. L. Gleason and C. A. Reitmeier,2000. Reduction of *Escherichia coli* O157:H7 counts on whole fresh apples by treatment with sanitizers. J. Food. Prot., 63: 703-708.

25. Sapers, G. M., R. L. Miller, M. Jantschke and A. M. Mattrazzo, 2000. Factors limiting the efficacy of hydrogen peroxide washes for decontamination of apples containing *Escherichia coli* . J. Food. Sci., 65: 529-532.
26. Freese, E., C. W. Sheu and E. Galliers, 1973. Function of lipophilic acids as antimicrobial food additives. Nature, 241: 321-325.
27. Sun, C. Q., C. J. O'Connor, S. J. Turner, G. D. Lewis, R. A. Stanley and A. M. Roberton, 1998. The effect of pH on the inhibition of bacterial growth by physiological concentrations of butyric acid: implications for neonates fed on suckled milk. Chem. Biol. Interact., 113: 117-131.
28. Viegas, C. A. and I. Sa-Correia, 1991. Activation of plasma membrane ATPase of *Saccharomyces cerevisiae* by octanoic acid. J. Gen. Microbiol., 137: 645-651.
29. Petschow, B. W., R. P. Batema and L. L. Ford, 1996. Susceptibility of *Helicobacter pylori* to bactericidal properties of medium-chain monoglycerides and free fatty acids. Antimicrob. Agents. Chemother., 40: 302-306.
30. Ruzin, A. and R. P. Novick, 1998. Glycerol monolaurate inhibits induction of vancomycin resistance in *Enterococcus faecalis*. J Bacteriol., 180: 182-185.