Determination of effect of sodium ion pretreatment on *Escherichia coli* O157:H7 after selected organic acid treatments

by

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DEDICATION

This work is dedicated to my parents
and to my husband:

William and Susan Ahern

and

Wayne K. Wixom

TABLE OF CONTENTS

INTRODUCTION

In 1980, Escherichia coli was considered to be a disappearing causative agent of diarrhea in developed countries like the United States or Canada. That opinion changed with the discovery of E. coli O157:H7. E. coli O157:H7 belongs to the enterohemorrhagic group of pathogenic E. coli. Like other E. coli, it is a gram-negative bacillus. However, unlike most E. coli, it does not ferment sorbitol within twenty-four hours and it also does not possess b-glucuronidase activity. Most E. coli have a typical growth temperature range from 21°C to 45°C. E. coli O157:H7 has a different temperature range from most E. coli, with limited growth above 42°C. Due to this temperature difference, it has not been detected by normal methods for identifying fecal contaminants in food (Mermelstein, 1993; Padye and Doyle, 1992; Doyle, 1991).

Since 1982, E. coli O157:H7 has been recognized as a food associated pathogen. This recognition was due to two major outbreaks occurring that year in Michigan and Oregon. Outbreaks are usually recognized by large numbers of

hospitalizations of people with hemorrhaghic colitis, or as clustering occurrences of hemolytic uremic syndrome (HUS) or thrombotic thrombocytopenic purpura (TTP). HUS is severe renal failure and TTP involves impairment of the Central Nervous System. E. coli O157:H7 was isolated from both patients and implicated ground beef in these initial outbreaks. The same strain was later isolated from humans and beef in another outbreak. Since then, the majority of outbreaks with E. coli O157:H7 have been caused by foodborne transmission - principally ground beef.

After the first few outbreaks of E. coli O157:H7 were associated with ground beef, cattle were suspected as the reservoir for the microbial pathogen. Since most ground beef comes from dairy cattle, many surveys have been conducted on dairy cows. Generally, the fecal isolation rate of E. coli O157:H7 in most surveys has ranged from 1%, and lower, to as high as 5.3% (Griffin and Tauxe, 1991). Fecal isolation of E. coli O157:H7 is rare in most healthy cattle.

Bacterial contamination of beef carcasses during the slaughtering process is inevitable and unwelcomed. Basically, the internal muscle tissue of the carcass starts out sterile

and becomes contaminated with microorganisms during the hide removal process. This contamination is usually from exposure of the carcass to soil or fecal matter present in the abattoir or hide. Although contamination of carcasses with bacteria is unwanted, most organisms are not pathogenic. However, the pathogen E. coli O157:H7 has been detected on beef carcasses due to fecal contamination. Although there was a small number of positive carcasses, the bacterial number of E. coli O157:H7 measured from 0.3 to 3.0 cells per 900 square centimeters on the beef carcass surface area.

Since E. coli O157:H7 has been indicated on beef carcasses, control methods have been sought. One of the traditional methods of controlling bacterial contamination is the application of organic acid sprays. However, E. coli O157:H7 appears to be more resistant to organic acids than most pathogens (Cutter and Sirgusa, 1994). In 1972, Sato reported that sodium ions induced a sub-lethal injury of E. coli cells due to the loss of intracellular magnesium (Sato et al., 1972). With this knowledge, this study sought to evaluated the antimicrobial effectiveness of selected organic acids in combination with various sodium ion compounds. From

preliminary results, one combination of sodium ion and organic acid was selected for its antimicrobial effectiveness. Various conditions of this combination were further analyzed and established in this study.

Thesis Organization

This thesis consists of an introduction, a literature review, one manuscript that will be submitted for publication, a summary and discussion, an appendix and acknowledgments. The literature cited for both the literature review and manuscript are listed in a single "literature cited" section. The master's degree candidate, Peggy J. Wixom, is the senior author and principal investigator for the manuscript.

LITERATURE REVIEW

Food Association of E. coli O157:H7

During November 15, 1992 to February 28, 1993, one of the largest occurrences of hemorrhagic colitis and hemolytic uremic syndrome occurred in the states of Washington, Idaho, California and Nevada. In this outbreak, more than 475 people were identified as being infected. In most of these cases, E. coli O157:H7 was attributed as the main causative agent of the infection. One example of the severity of the infection is shown in the number of cases associated with the E. coli O157:H7 outbreak in the state of Washington. During this outbreak, E. coli O157:H7 was isolated from 447 cases -- with three children being fatally infected (Mermelstein, 1993).

Investigation into this outbreak showed that undercooked hamburger contaminated with E. coli O157:H7 was the vehicle of transmission. Although the ground beef could be traced to one Southern California meat supplier, the Centers for Disease Control could only implicate five United States slaughterhouses and one Canadian slaughterhouse as the

possible sources of contaminated beef carcasses (Mermelstein, 1993; Dorn, 1993).

The majority of outbreaks attributed to E. coli O157:H7 infection has been linked to the consumption of undercooked ground beef. Dairy cattle are often implicated in the human outbreaks of E. coli O157:H7 since hamburger primarily contains more meat from dairy cattle then beef cattle (Padhye and Doyle, 1992). E. coli O157:H7 has been found in healthy cattle in many countries, such as -- Canada, United Kingdom, Spain, Germany, Argentina, Egypt and the United States of America. Only two countries, Spain and Argentina, have reported finding it in cattle with diarrhea (Dorn, 1993; Griffin and Tauxe, 1991).

In 1985 and 1986, surveys of retail fresh meats in stores in Alberta, Canada and Wisconsin showed a wide range of E. coli O157:H7 contamination. In the meat samples, 3.7% of the 164 beef samples, 2.0% from 205 lamb samples, 1.5% from chicken and turkey samples and 1.5% of pork sample contained E. coli O157:H7 (Griffin and Tauxe, 1991; Doyle, 1991). In 1987 and 1989 , a United States national retail survey showed

a lower frequency of isolation of E. coli O157:H7 contamination. In this survey, E. coli O157:H7 was found in only 0.12% of 1668 beef samples and not in any of the 3,997 chicken samples (Griffin and Tauxe, 1991).

Lactic Acid

Lactic acid (pK_a 3.83) is listed as a "generally recognized as safe" compound (GRAS) in the United States (21 CFR 184.1061). Levels up to 1500 mg per kg or more can be tolerated by most mammals (Smulders et al., 1986). It is widely known that the absence of acute or chronic toxicity has made lactic acid an acceptable choice for a decontaminating agent. The inhibitory capacity of this acid lies in its ability to lower pH levels. This reduction may allow the undissociated molecules to act as a toxic factor and interfere with cellular metabolism or biological activity (M. R. Adams and C. J. Hall, 1988; C. A. Cherrington et al., 1991) or by decreasing the internal pH level of the cells (Gill and Newton, 1982).

The use of lactic acid as a sanitizer has been evaluated of several different animal carcasses. On pig carcasses, concentrations of 2.4% showed bacterial reduction but also irreversible carcass discoloration (Smulders et al., 1986). These authors also stated that, on lamb carcasses, a 1974 study found higher concentrations of 12% lactic to have no adverse effect on the carcass. Lactic acid has been used in the poultry industry to reduce bacterial populations. One percent lactic acid treatments reduced general bacterial numbers on broiler chickens by 1 $\log_{10}$ cycle per skin gram (van der Marcel et al., 1988). Pork livers have also been shown to be decontaminated when immersed in lactic acid (Woolthius et al., 1984). These authors reported immersion of pork liver into a 0.2% lactic acid solution for five minutes showed a total bacterial reduction of 2 to 3 $\log_{10}$ cycles.

The most common way of applying lactic acid in the commercial beef slaughterhouse is by spraying the carcasses. Lactic acid concentrations have been evaluated on beef carcasses for bacterial reduction and color analysis. Although bacterial reductions are greatest at the higher lactic acid concentrations, the discoloration of the carcass

is also at its highest. In one study, concentrations were evaluated from 0.75 to 2.5%. At concentrations up to 2.0%, discoloration appeared to be limited to the subcutaneous fat but at 2.5% concentration lean and cut up surfaces started to show denaturation (Woolthius and Smulders, 1985). Dickson and Anderson (Dickson and Anderson, 1992) showed that carcass washing with 1.25% lactic acid reduced the total aerobic bacteria by 1 $\log_{10}$ cycle.

General reduction in bacterial population with lactic acid have been shown to vary from 1 to 3 $\log_{10}$ cycles. Although lactic acid has shown its usefulness against total bacterial reductions, its has not been shown to be effective against E. coli O157:H7. Strains of E. coli O157:H7 have been shown to be more resistant to lactic acid than other pathogens (Cutter and Siragusa, 1994).

Consideration should be given to the temperature at which the lactic acid is sprayed. Commonly on beef tissues the higher the concentration of lactic acid and temperature, the greater the bacteria reduction. With 2 and 3% lactic acid at 55°C, significant bacterial reduction has been noticed. At temperature of 70°C and a concentration of 3% lactic acid, the

largest reduction of total aerobic bacteria was about 1.80 $\log_{10}$ (Anderson and Marshall, 1990b). However, it should be noted at that temperature discoloration of the meat is possible and that at 3% lactic acid the surface pH was slow to return (5.2 in 24 hours). In trying to determine the effectiveness of increasing temperature of lactic acid on E. coli O157:H7, temperatures from 20°C to 55°C have been tested (Brackett et al., 1994). With lactic acid concentrations up to 1.5%, no effect on the E. coli 0157:H7 population on beef tissue was reported at these temperatures.

Acetic Acid

Acetic acid (pK_a 4.75) is generally regarded as safe (GRAS) for "miscellaneous and general usage" (21 CFR 184.1005). The acceptable daily intake is unlimited for humans. The antimicrobial effect of acetic acid has been attributed to undissociated acid molecules that interfere with cellular metabolism or decrease the biological activity due to the pH change of the bacterial cell's environment (Doores, 1993). When compared to other organic acids, such as lactic

and citric at equal pH values, acetic acid was far more effective antimicrobial on Listeria monocytogenes than the other two organic acids (Young and Foegeding, 1993).

Many studies have been conducted using acetic acid at various combinations on pork carcasses. In 1973, acetic acid at pH of 1.5, 2.0, and 3.0 were sprayed on pork carcasses inoculated with Salmonella enteritidis. Biemuller et al. (1973) reported a 4 log reduction of total plate count with pH 1.5 acetic acid, a 2 log reduction with pH of 2.0 and no observable reduction with the pH 3.0 spray. Mixtures of acetic acid and propionic acid (60:40) have also been evaluated on pork carcasses. From a plant study of these mixtures, reduction of microbial population was approximately 2 logs with little discoloration at both 1.36M and 1.67M concentrations (Reynolds and Carpenter, 1974). When pork chops were sprayed with 1.36M acetic and propionic acid in a 60:40 mixture at pH 2.6, reduction in microbial populations of 2.4 $\log_{10}$ was noted after twenty-four hours storage (Carpenter et al., 1986). With broiler carcasses, 0.5% acetic acid in the scald water has been shown to effectively control

Salmonella cross-contamination in water due to the bactericidal effect of the acid (Lillard et al., 1986).

Acetic acid has been evaluated on lamb carcasses at concentrations of up to 24%. Although reductions in bacterial populations were reported and increased as the concentration increased, bleaching of the lamb carcasses occurred at the 12% concentration (Ockerman et al., 1974).

In examining the effect of acetic acid on beef, dipping in a 1.2% of acetic acid solution reduced bacterial counts from 45.8% for E. coli to 77.2% for Pseudomonas aeruginosa with only slight discoloration of the meat (Bell et al., 1986). Bacterial populations of S. typhimurium were reduced by 0.5 to 0.8 $\log_{10}$ cycles when beef samples were treated with 2% acetic acid solution (Dickson, 1992). Acetic acid has been tested on beef and other meat in various combinations with other acids (Adams and Hall, 1988; Bell et al., 1986; Dickson and Anderson, 1992; Anderson et al., 1992; Dixon et al., 1987; Anderson and Marshall, 1990). Combinations of acetic, lactic, citric, and ascorbic acids were studied with no effective difference in bacterial reduction when compared to the acetic and lactic by themselves (Anderson and Marshall, 1992).

Growth studies have suggested a synergistic effect existing with lactic and acetic acid mixtures (Adams and Hall,1988; Dickson and Anderson, 1992). This effect caused a bacterial reduction for the acid mixture to occur between the two acids.

E. coli O157:H7 was found to be more resistant to acetic acid, regardless of the acid is being delivered in calcium alginate or singly as a dip (Sirgusa and Dickson, 1993). When a log reduction factor for E. coli O157:H7 was calculated for a beef carcass model, treatment with acetic acid was found to be not significantly different than lactic or citric acid treatments. However with acetic acid, E. coli O157:H7 was almost two logs more resistant than P. fluorescens using the same experimental design (Cutter and Siragusa, 1994). In general, as the temperature of acetic acid and the acid concentration increases - the bacterial reduction increases (Anderson and Marshall, 1989). However, with E. coli O157:H7, acetic acid concentrations up to 1.5% at the different temperatures had little disinfecting value regardless of temperature (Brackett et al., 1994).

Citric Acid

Citric acid (pK1 3.13) is a tricarboxycylic acid that is approved as a generally safe (GRAS) substance for "miscellaneous and general purpose usage" (21 CFR 182.1033). It is commonly used to adjust the pH of foods and as a synergistic antioxidant to prevent rancidity of food products during storage (Doores, 1993).

Although the inhibitory effect of citric acid on bacteria increases as pH decreases, it appears not to be totally dependent on pH. The undissociated form of citric acid has been shown to have a more inhibitory effect on L. monocytogenes Scott A than either acetic or lactic acid (Ita and Hutkins, 1991; Young and Foegeding, 1993). These results suggest that the inhibition effect of citric acid on L. monocytogenes is caused not by a decrease in the pH but more likely due to the undissociated acid disrupting the biological activity within the bacterial cell.

Citric acid has been shown to be less effective against reported acid-tolerant food pathogens, such as Yersinia enterocolitica, when compared with acetic acid (Karapinar and

Gonul, 1992). Citric acid has been suggested as a possible agent to increase shelf life of canned liver paste against thermophilic bacteria such as Bacillus and Clostridium (Houben and Krol, 1991). However, the inhibitory effect that citric acid has on Clostridium species may be due to its chelation of divalent metal ions. Therefore, that effect may vary considerably with the amount of divalent metal ions, like calcium, available in food. The lower the amount of divalent metal ions available, the greater the inhibitory effect of citric acid (Graham and Lund, 1986; Doores, 1993).

Citric acid has been tested on beef tissue samples in mixtures with lactic, acetic and ascorbic acid for its antimicrobial activity. The results of these experiments suggest the mixtures were not as effective in inhibiting bacteria as lactic and acetic acid solutions by themselves (Dixon et al., 1987; Anderson and Marshall, 1990; Dickson and Anderson, 1993). When E. coli was grown in Tryptic soy broth and then adjusted to a pH of 3.7 with citric acid, 90% of the bacteria were injured in 21 minutes (Anderson and Marshall, 1990). However, E. coli O157:H7 appears to be more resistant to citric acid than to other organic acids. A concentration

of 5% citric acid, applied as a spray, resulted in the greatest bacterial reduction (log_{10}) but this log reduction was still not as high as that obtained with lactic acid (Cutter and Siragusa, 1994). Although the bacterial reduction generally increases as temperature increases, concentrations of citric acid up to 1.5% at 20°C or 55°C showed little inhibitory effect on beef samples inoculated with E. coli O157:H7 (Brackett et al., 1994).

Sodium Ion Compounds

Sodium lactate has been used in the food industry for twenty years as a humectant and is currently being used in the meat industry for flavor enhancement and extension of shelf-life. Sodium lactate concentrations of 2 to 3% inhibit development of off-flavors in aerobically packaged fresh ground pork with only 1-2 log_{10} reduction of bacteria during refrigerated storage (Brewer et al., 1992). Cooked, refrigerated roast beef with 3-4% sodium lactate injected into it had an increased microbial shelf-life of up to 84 days compared to uninjected control (Papadopoulos et al., 1991a).

Redness of cooked roast beef was increased with a maximum addition of 2% sodium lactate (Papadopoulos et al., 1991b).

The antimicrobial effect of sodium lactate can not be completely explained by its ability to lower water activity. The inhibitory action of sodium lactate appears to depend upon how the various microorganisms can effectively cope with this compound and the food product itself (de Wit and Rombouts, 1990). In pork liver sausage, 2 to 3% concentration of sodium lactate suppressed L. monocytogenes cell growth (Weaver and Shelef, 1993). In cooked ground beef, the number of surviving of L. monocytogenes decreased during a two day storage with sodium lactate. However, sodium lactate appeared to have no significant effect in decreasing L. monocytogenes in raw ground beef (Harmayani et al., 1993).

At any particular water activity, sodium lactate has been demonstrated to be a more effective inhibitor of food pathogens than sodium chloride (Houtsma, 1993). Five percent sodium lactate in culture medium inhibited the growth of Staphylococcus aureus and S. typhimurium. However, the growth of E. coli was hardly effected at this concentration (de Wit and Rombouts, 1990). A combination of sodium lactate and

lactic acid in chill - water has been shown to reduce Salmonella on broiler carcasses (Waldroup, 1993).

Phosphates are currently being used in food products based on the amount needed to achieve the functional objectives rather than antimicrobial activity. In meat products, functions of phosphates include the following: water binding, retardation of oxidation, emulsification, color development and color stabilization.

Little data has been gathered on the antimicrobial effect of trisodium phosphate. Phosphates have been suggested as a possible substitute for nitrites in cured meat because toxin production of C. botulinum may be delayed (Wagner, 1986). Sodium tripolyphosphate was shown to inhibit colony formation of Moraxella - Acinetobacter (Firstenberg-Eden et al., 1981). Trisodium phosphate has been demonstrated to be a sanitizer on poultry to reduce Salmonella. Poultry dipped into 8 to 12% trisodium phosphate solutions showed a 35% bacterial reduction (Gises, 1993). In lean beef tissue, S. typhimurium, L. monocytogenes and E.coli O157:H7 were reduced by 1 to 1.5 $\log_{10}$ cycles in 8 to 12% trisodium phosphate concentrations. The

same bacterial species were reduced by 2 to 2.5 $\log_{10}$ cycles on adipose beef tissues. The bacterial populations were further reduced as the temperature of the trisodium phosphate solutions increased (Dickson et al., 1994).

Sodium glutamate monohydrate is generally recognized a safe substance (GRAS) to be used as a food component or additive (21 CFR 182.1). Although it has been used in the form of "kombu" seaweed in ancient Chinese cooking, it was not until 1908 that the Japanese isolated monosodium glutamate. Recently research in Europe, the United States and Japan has suggested that glutamates may provide a fifth basic taste - "Umami" (Anonymous, 1987). "Umami" is an independent taste that falls outside the other four basic taste of sweet, sour, bitter and salty. In the past couple of decades, some consumers and medical doctors have complained of a sensitivity to monosodium glutamate. The symptoms of this sensitivity vary from slight headaches to severe asthma and brain damage, and organizations like NOMSG (no monosodium glutamate) would like it taken off the GRAS list. However, other reports are in direct conflict with this organization's data. To date, the Food and Drug Administration has not change the GRAS

status of monosodium glutamate monohydrate (Leibman, 1991). There is no reported antimicrobial activity for sodium glutamate.

SURVIVAL OF Escherichia coli O157:H7 AFTER TREATMENT WITH SODIUM COMPOUNDS IN COMBINATION WITH SELECTED ORGANIC ACIDS UNDER DIFFERENT CONDITIONS

A paper to be submitted for publication in the Journal of Food Protection

Peggy Wixom and J. S. Dickson

Abstract

In an attempt to reduce bacterial contamination during the slaughtering process, organic acid sprays have been implemented. In this study the survival of Escherichia coli O157:H7 on beef tissue slices following sodium ion pretreatments and organic acid treatment was examined. At room temperature, sodium lactate, trisodium phosphate and sodium glutamate, at concentrations of 1%, 2%, 3% and 5%, were evaluated in combination with lactic, acetic, citric and their acid mixtures (50:50) at a final concentration of 2%. Selected pretreatment - treatment combinations of 1% sodium

lactate and 1% trisodium phosphate in combination with 2% acetic, 2% lactic, and the acid mixture were further evaluated at 52°C and 5°C. Colony counts were determined using both tryptic soy agar and MacConkey's agar with sorbitol under aerobic conditions. Although there was no significant bacterial reduction with all pretreatment - treatment combinations, the greatest reduction of approximately of two log was obtained using sodium lactate and 2% acetic at 52°C.

Introduction

Escherichia coli O157:H7 is a gram negative bacillus that belongs in the enterohemorrhagic group of pathogenic E. coli due to its Shiga like toxin production (Griffin and Tauxe, 1991). It was originally isolated in 1975 at the Centers for Disease Control from a California woman. In 1982, two outbreaks of hemorrhagic colitis, occurring in Oregon and in Michigan, brought about the recognition of E. coli O157:H7 as a food associated pathogen (Padhye and Doyle, 1992).

Clinical manifestations of E. coli O157:H7 bacterial infection include hemorrhagic colitis, which is characterized by severe abdominal cramps, bloody stools and little or no

fever. Illness caused by E. coli O157:H7 usually causes no severe consequences in most patients. However some may develop hemolytic uremic syndrome (HUS), which is severe renal failure, or thrombotic thrombocytopenic purpura (TTP), which causes impairment of the central nervous system. Both syndromes can lead to death in the young, the elderly or the immune deficient (Doyle, 1991).

The vehicle of transmission in most E. coli O157:H7 outbreaks has been identified as ground raw beef (Dorn, 1993). Typically, bacterial contamination of beef carcasses is due to fecal or soil contamination at the slaughtering process. One approach for decontamination of beef carcasses at the commercial slaughter house is the application of organic acid sprays (Dickson and Anderson, 1992). The antimicrobial effect of organic acid has been suggested to be due to the undissociated acid molecule either interfering with cellular growth and metabolism or a decrease in the biological activity of the bacteria due to the pH change in the cell's environment (Doores, 1993). Studies have suggested that E. coli O157:H7 is more resistant to organic acids than most bacteria (Cutter and Siragusa, 1994). Concentrations up to 5% of citric,

acetic and lactic acid did not completely eliminate the bacterium. Recently it has been shown that little bacterial reduction occurs with citric, lactic and acetic acid at concentration up to 1.5% at various temperatures (Brackett et al., 1994).

The antimicrobial effect of sodium chloride on E. coli cells has been documented. In some studies, the concentration of sodium chloride appeared to inhibit the growth of the cell due to sublethal injury. This antimicrobial effect may be due to the cells intracellular loss of magnesium ions (Glass et al., 1992; Sato et al., 1972). It has also been demonstrated that sodium chloride solutions in combination with acid solutions appear to increase the effectiveness of the acid solution by changing the surface moisture of the tissue (Dickson, 1990). Besides sodium chloride, the antimicrobial effect of trisodium phosphate has reportedly reduced bacterial population on beef tissue (Dickson et al., 1994). Also, sodium lactate in combination with lactic acid has decreased the incidence of Salmonella on poultry carcasses (Waldroup, 1993).

The specific objectives of this research was to determine the effectiveness of trisodium phosphate, sodium lactate and sodium glutamate in combination with lactic, acetic and citric acid to eliminate E. coli O157:H7 on contaminated beef tissue at various temperatures. In addition, contamination of beef tissue with E. coli O157:H7 bovine fecal material was tested to evaluate the effect the chosen pretreatment - treatment combination would have in a simulated slaughterhouse contamination.

Materials and Methods

Bacterial cultures

Six strains of E. coli O157:H7, ATCC 43890 (human isolate, American Type Culture Collection, Rockville, MD), ATCC 43894(human isolate American Type Culture Collection, Rockville, MD), N801-3-1 (bovine isolate, National Veterinary Service Laboratories, Ames, IA), N886-2-1 (bovine isolate, National Veterinary Service Laboratories, Ames, IA), WS3062 (human isolate, University of Washington, Seattle, WA) and 397 (human isolate, Oregon Department of Health, Portland, OR) were statically cultured in tryptic soy broth (Accumedia,

Baltimore, MD) at 37°C for 15.5 hours. One milliliter of each culture were combined to make a "cocktail". Five ml of the combined bacterial culture was diluted with 15 ml of 0.1 % buffered peptone water (Accumedia, Baltimore, MD). The inoculum typically contained 10^7 colony forming units (cfu's)/ml with each individual strain containing on the average 1 to 2 X 10^7 cfu's/ml. Strain cultures were maintained on tryptic soy agar slants (Accumedia, Baltimore, MD) at 5°C.

Tissue preparation

Beef muscle tissue was obtained from the Iowa State University Meat Laboratory (Ames, IA). The beef was sliced into 0.5 cm thick slices, vacuum - packed (Multivac, Germany) and frozen at -20°c until use. Tissue prepared in this manner typically contained less than 25 cfu's/cm^2. The tissue slices were cut into 2.0 by 2.0 by 0.5 cm pieces for a total surface area of 12 cm^2, and these pieces were immersed into the diluted culture for five minutes.

In the fecal inoculation method, bovine feces packaged in plastic bags were irradiated to a dose of 3.7 - 4.6 kGy at the

Linear Accelerator Facility (Iowa State University, Ames, IA; dosimeter measured by alanine tablets) and frozen at -20°C until use. This typically caused the feces to contain less than 25 cfu's/cm^2. Five ml of the bacterial "cocktail", prepared as previously described, were combined with 15 grams of feces and allowed to acclimate for five minutes at room temperature. Beef tissue, prepared as previously described, were submerged into the fecal inoculum for five minutes.

Sodium ion compounds and organic acid treatments

The inoculated tissues were immersed into a 20 ml volume of one of the sodium ion pretreatment solutions (Table 1) and vortexed for ten seconds at 75% of maximum setting with a Genie 2 Vortex (Scientific Industries, Bohemia, NY). The pretreatment solution was then decanted and the tissues were then treated with 20 ml of one of the organic acid treatment (Table 2) and vortexed for ten seconds at 75% of maximum setting. This protocol was replicated with each pretreatment and treatment solution applied at 5°C, room temperature (ca 25°C) or 52°C for broth inoculum and at room temperature only

Table 1. Pretreatment compounds and their concentrations used for determining the elimination of *E. coli* O157:H7.

COMPOUNDS	CONCENTRATIONS
Sodium Lactate (60%)	1%, 2%, 3%, 5% (v/v)
Trisodium Phosphate	1%, 2%, 3%, 5% (w/v)
Sodium Glutamate	1%, 2%, 3%, 5% (w/v)
Buffered Peptone Water	0.1%

Table 2. Treatment compounds and their concentrations used for determining the elimination of E. coli O157:H7.

COMPOUNDS	CONCENTRATIONS
Lactic Acid (88%)	2% (v/v, pH 2.42)
Acetic Acid	2% (v/v, pH 2.60)
Citric Acid	2% (v/v, pH 2.51)
Lactic Acid : Acetic Acid	2%:2% (50:50, pH 2.32)
Lactic Acid : Citric Acid	2%:2% (50:50, pH 2.19)
Citric Acid : Acetic Acid	2%:2% (50:50, pH 2.22)
Buffered Peptone Water (Control)	0.1%

for fecal inoculum.

Enumeration of bacteria

After decanting the acid solution, the tissues were homogenized in buffered peptone water with a Stomacher Mark II (Tekmar, Inc., Cincinnati, OH) for two minutes at normal speed. The homogenized samples were then plated using a Spiral plater Model D (Spiral Systems, Inc., Cincinnati, OH) on tryptic soy agar (TSA) and MacConkey's agar with 1% sorbitol (MSA, Accumedia, Baltimore, MD). Plates were incubated to 18 to 24 hours under aerobic conditions and populations were enumerated using methods appropriate for spiral plates (Peeler and Maturin, 1992).

Statistical analysis

Bacterial counts were expressed in $\log_{10}$ cfu/cm^2. The reduction of bacterial populations were calculated using the following formula:

$$\text{log reduction} = (\log_{10} \text{initial population/cm}^2 - \log_{10} \text{final population/cm}^2)$$

Statistical analysis was conducted using the General Linear Model procedure of the Statistical Analysis System (SAS, 1985). Each experiment was independently replicated three times. Initial inoculum values were used as covariates in the analysis to normalize data between replications. Statistical analysis revealed no significant difference between plate counts ($p \geq .05$). As a result, TSA and MSA counts were pooled.

Results

Effects of sodium ion compounds with acids at room temperature

The effects of sodium lactate, trisodium phosphate, and sodium glutamate at four concentration in combination with three acids and acid mixtures were initially evaluated at room temperature. The results of this study were varied, but, there was no significant effect ($p \geq 0.05$) of the sodium ion compounds in reducing bacterial populations on meat tissues. However, the greatest numerical log reductions with sodium lactate in combination with acids was noticed at 1% sodium lactate with 2% acetic (1.71 log reduction) and 3% sodium lactate with 2% lactic acid (1.73 log reduction; see Figure

1). The greatest reduction with sodium lactate in combination with mixed acids, was at 1% and 3% sodium lactate in combination with 2% lactic: 2% acetic - 50:50 (Figure 2).

As previously noted, there was no significant difference between sodium ion compounds ($p \geq 0.05$). In analyzing the trisodium phosphate concentrations with the organic acids, the highest numerical log reduction was with lactic acid, at approximately 1.70 (Figure 3), at all trisodium phosphate concentrations. As with the sodium lactate, the greatest numerical log reduction was with 1% trisodium phosphate in combination with 2% lactic: 2% acetic - 50:50 with a log reduction of 1.84 (Figure 4).

In viewing the results of sodium glutamate in combination with organic acids, the greatest numerical reduction was with 1% sodium glutamate in combination with 2% citric with a log reduction of 1.88 (Figure 5). As with the other sodium ion compounds, sodium glutamate at all concentrations was most effective with 2% lactic: 2% acetic - 50:50 with a log reduction of approximately 1.70 (Figure 6). However as stated previously, there was no significant effect ($p \geq 0.05$)

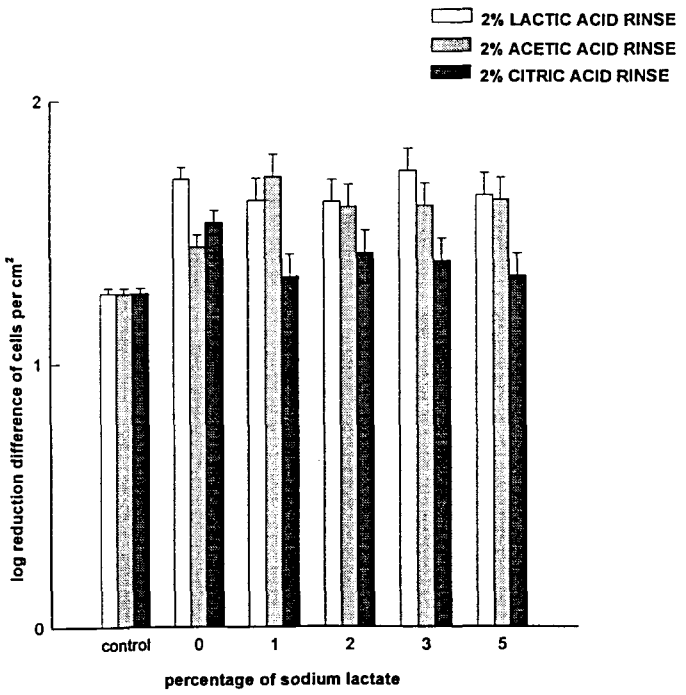

Figure 1. Log reduction difference of E. coli O157:H7 cells per cm^2. Sodium lactate % with organic acids at room temperature. Control is buffered peptone water with no acid treatment and 0% is buffered peptone with acid treatment.

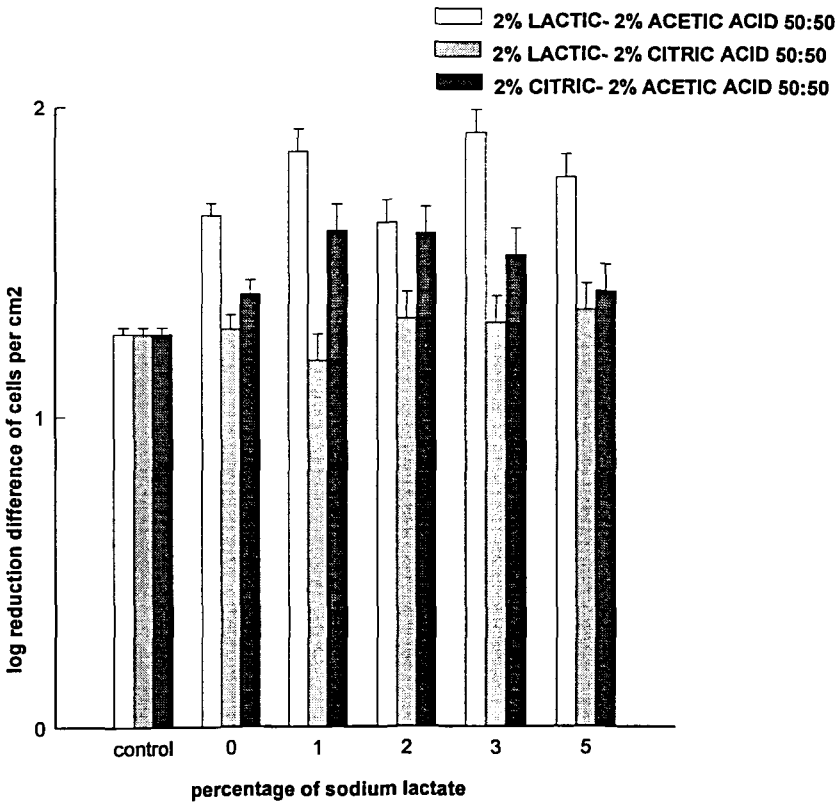

Figure 2. Log reduction difference of E. coli O157:H7 cells per cm^2. Sodium lactate % in combination with mixed acid solutions at room temperature. Control is buffered peptone water with no acid treatment and 0% is buffered peptone water with acid treatment.

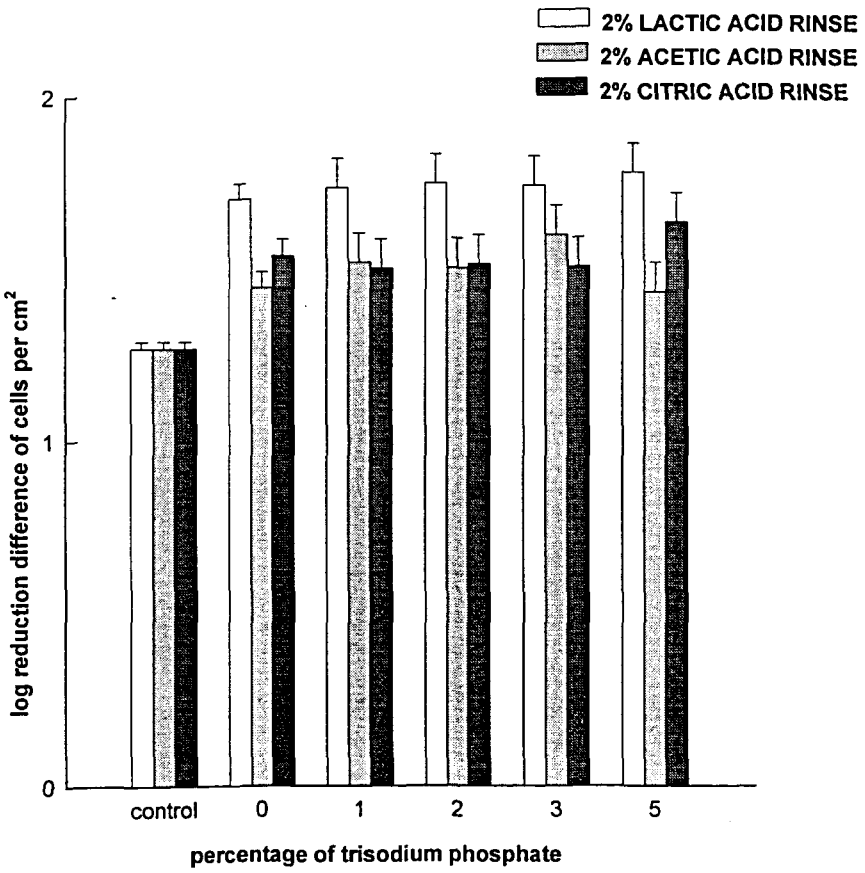

Figure 3. Log reduction difference of E. coli O157:H7 cells per cm^2. Trisodium phosphate % with organic acids at room temperature. Control is buffered peptone water with no acid treatment and 0% is buffered peptone water with acid treatment.

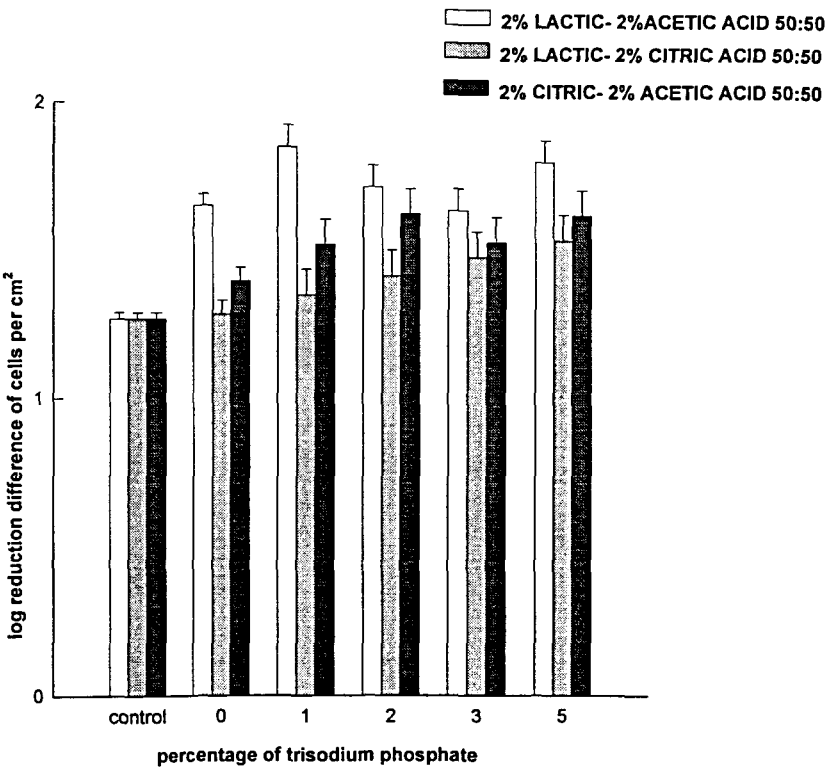

Figure 4. Log reduction difference of E. coli O157:H7 cells per cm^2. Trisodium phosphate % in combination with mixed acid solutions at room temperature. Control is buffered peptone water with no acid treatment and 0% is buffered peptone water with acid treatment.

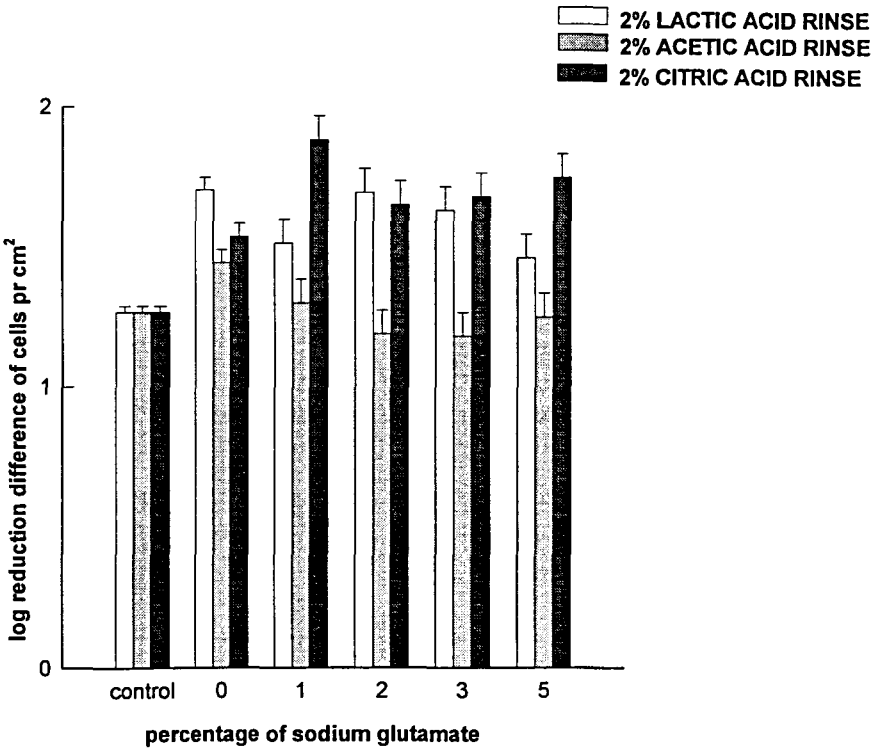

Figure 5. Log reduction difference of E. coli O157:H7 cells per cm^2. Sodium glutamate % with organic acids at room temperature. Control is buffered peptone water with no acid treatment and 0% is buffered peptone water with acid treatment.

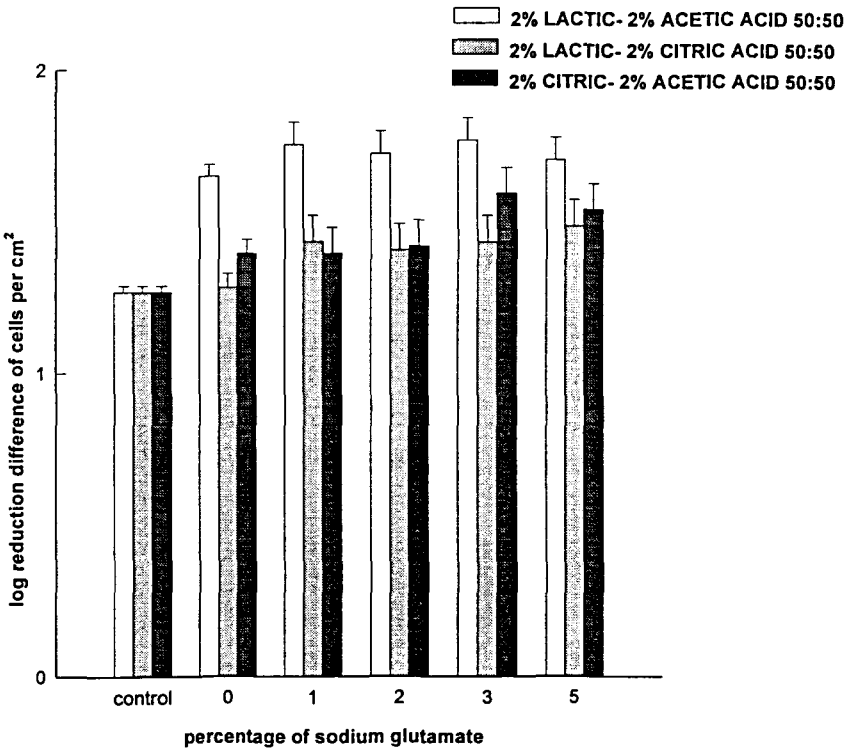

Figure 6. Log reduction difference of E. coli O157:H7 cells per cm^2. Sodium glutamate % in combination with mixed acid solutions at room temperature . Control is buffered peptone water with no acid treatment and 0% is buffered peptone water with acid treatment.

for any of the sodium ion compounds. Because of the chemical instability and susceptibility to contamination, sodium glutamate was not investigated further.

Further investigations were conducted with 1% and 3% concentrations of sodium lactate and trisodium phosphate in combination with 2% lactic, 2% acetic, and 2% lactic: 2% acetic - 50:50 mixed acid.

Effect of sodium compound and acid at 5°C and 52°C

The temperature of solutions appeared to affect the reduction of E. coli O157:H7. Overall, solutions at 52°C had the greater log reduction than at 5°C ($p \geq .05$). However, as previously stated no significant effect was noted between sodium ion compounds ($p \geq 0.05$). At 5°C, the greatest numerical log reduction was with peptone buffered water and 2% lactic acid at 1.88 (Figure 7). Although no sodium compound combination with acid had the greatest log reduction, acetic acid in combination 3% sodium lactate at 5°C had a numerical log reduction of 1.87. At 52°C, the highest numerical log reduction was with 1% sodium lactate combined with 2% acetic

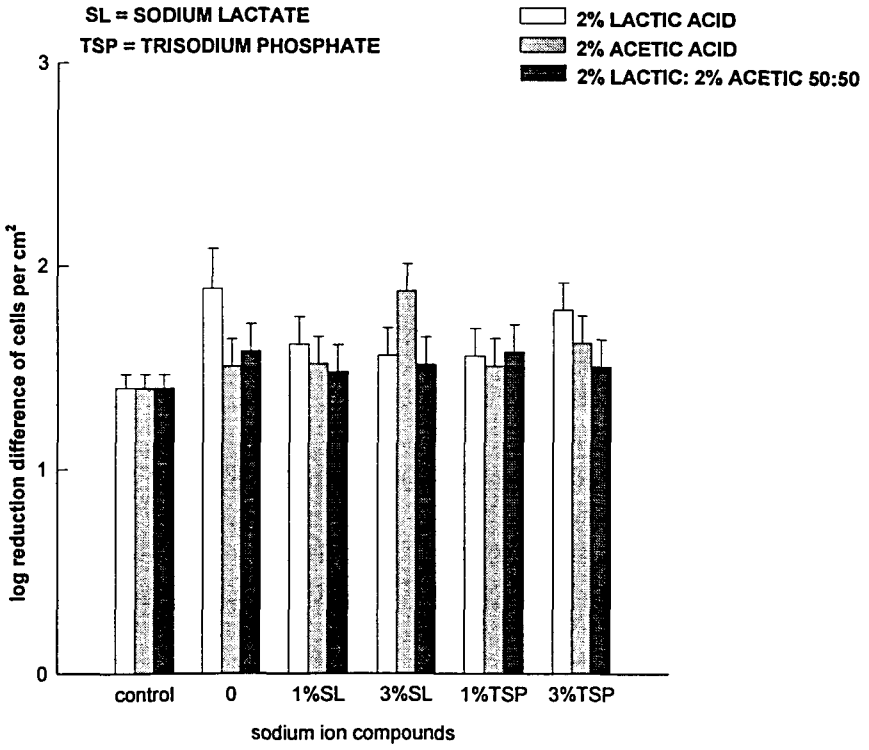

Figure 7. Log reduction difference of E. coli O157:H7 cells per cm^2. 1% and 3% of both sodium lactate (SL) and trisodium phosphate (TSP) in combination with chosen acids at $5^{\circ}C$. Control is buffered peptone water with no acid treatment and 0% is buffered peptone water with acid treatment.

and 2% lactic: 2% acetic -50:50 (Figure 8).

Effect of fecal bovine inoculation

In the final part of this study, a concentration of 1% sodium lactate in combination with 2% lactic, 2% acetic and 2% lactic: 2% acetic - 50:50 at room temperature was evaluated on beef tissue inoculated with contaminated bovine feces. The greatest numerical log reduction was with 1% sodium lactate and 2% lactic acid combination with a log reduction of 1.45. The lowest log reduction was 1% sodium lactate with the 2% lactic: 2% acetic - 50:50 acid mixture. This reduction was even less than the peptone buffer water with the acid mixture (Figure 9).

Discussion

Many reports have evaluated organic acids as an intervention strategy for bacterial decontamination in the slaughtering process. In general, bacteria population reduction has been shown to vary from 1 to 3 logs for organic acids. Overall, the greater bacterial reduction is noticed with higher concentrations and higher temperatures of

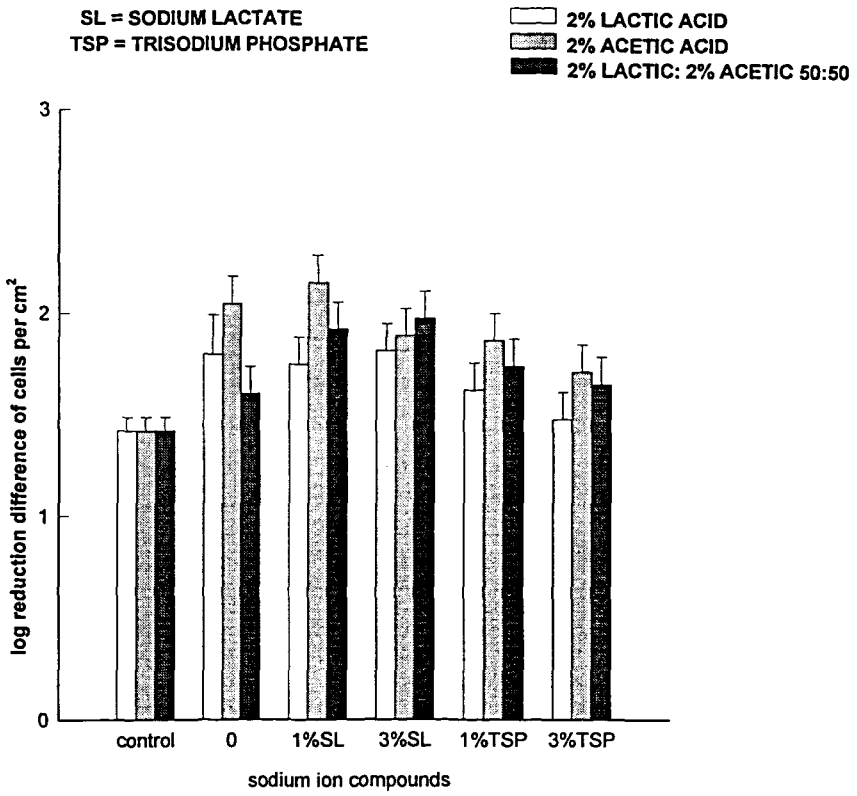

Figure 8. Log reduction difference of E. coli O157:H7 cells per cm2. 1% and 3% of both sodium lactate (SL) and trisodium phosphate (TSP) in combination with chosen organic acids at 52°C. Control is buffered peptone eater with no acid treatment and 0% is buffered peptone water with acid treatment.

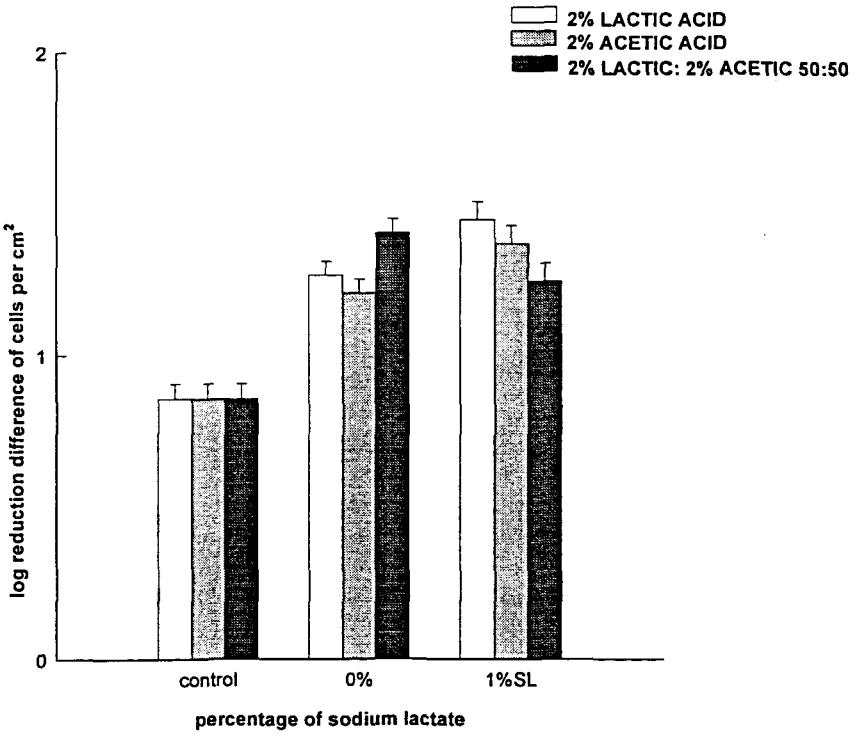

Figure 9. Log reduction difference of E. coli O157:H7 cells per cm^2. 1% sodium lactate (SL) in combination with chosen organic acids on beef tissue inoculated with bovine fecal material at room temperature. Control is buffered peptone water with no acid treatment and 0% is buffered peptone water with acid treatment.

the acids (Dickson and Anderson, 1992). However some strains of E. coli O157:H7 have been shown to be more resistant to organic acids than other bacteria. Cutter and Siragusa (1994) showed that certain strains of E. coli O157:H7, such as ATCC 43895, had lower bacterial reductions than Pseudomonas fluorescens at 1%, 3% and 5% lactic, acetic, and citric acids. The largest log reduction was noticed at 5% lactic acid with a log reduction of 2.60 (Cutter and Siragusa, 1994). Although our initial findings with acids did not result in as large of reduction as other studies, E. coli O157:H7 appeared to be more resistant to lactic acid (data not shown). This is similar to earlier observed results that E. coli was resistant to lactic acid (Anderson and Marshall, 1990a).

In evaluating the effect of the sodium ion pretreatment, the effect against the initial log reduction of acid must be examined. Overall, trends suggested an increase in the effectiveness of the organic acids with a pretreatment (data not shown). In particular, the calculated log reduction of lactic acid against E. coli O157:H7 was increased by sodium lactate and trisodium phosphate. However, this overall increased reduction is diminished when the numerical log

reduction of phosphate buffer in combination with lactic acid is compared to it. Trends also indicated a noticeable increase in the numerical log reduction with citric acid combined with the sodium glutamate pretreatment but not with the other sodium ion compounds. With acetic acid, a large bacterial reduction was notice with 1% sodium lactate. In analyzing the general tendency of acetic acid with sodium ion compounds, a slight increase of the calculated bacterial reduction was noticed, but a larger numerical increase was obtained with 1% sodium lactate. Our initial acid results for acetic acid differed slightly from the reported 3 log reduction due to acetic acid but our study was only after a brief time exposure and different concentrations (Dickson, 1991). Our initial results were similar to other findings for organic acids with E. coli O157:H7 (Brackett et al., 1994; Cutter and Siragusa, 1994).

However, our results were unlike the findings of Brackett et al. (1994), which stated that there seemed to be no significant difference between acids at temperatures of 20°C and 55°C. Our general findings suggest that there was an increase in the numerical log reduction at the temperature of

52°C - especially for acetic acid. This limited increase in calculated log reduction at a higher temperature is supported by the overall findings of Anderson and Marshall with the increased temperature of lactic, acetic and acid mix solution (Anderson and Marshall, 1989; Anderson and Marshall, 1990a; Anderson and Marshall, 1990b). Our findings also seem to agree with the suggested synergistic effect of lactic and acetic acids (Adams and Hall, 1989) - even increasing with combinations of sodium ion compounds regardless of temperature.

However all of these findings were reversed when bovine fecal inoculation method was used. Lactic acid with 1% sodium lactate had the highest calculated log reduction, while 2% lactic: 2% acetic -(50:50) had the lowest numerical log reduction - even lower than the phosphate buffer in combination with the mixed acid. Previous results of 1% sodium lactate in combination with acetic acid showed a higher log reduction than the lactic acid - sodium lactate combination. Results with the fecal inoculation showed the sodium lactate with lactic acid had a greater log reduction than the acetic - sodium lactate combination. These findings

also suggest that the lactic and acetic acid synergistic effect may not occur in the presence of fecal contamination on beef tissue - perhaps due to the added organic material present.

Our study confirms the previous report that trisodium phosphate can decrease bacterial populations (Dickson et al., 1994). However, our reported bacterial reduction with trisodium phosphate in combination with organic acids (1.70 $\log_{10}$) was not as great as the previously mentioned study reported amount for trisodium phosphate alone (2.5 $\log_{10}$). Our study's lower reduction may be due to the lower concentration (3% compared to 12%) of trisodium phosphate used in our study. However, Dickson et al. earlier reported findings did not reported much difference between 8% and 12% trisodium phosphate. Another difference between this study and Dickson et al. study is that our study used a six strain "cocktail" for evaluation so individual strain sensitivity may have been diminished.

It has been suggested that when E. coli is stressed by acids, that populations may be underreported if plated on selective media (Anderson and Marshall, 1990b). It was

further reported that differences of E. coli O157:H7 population recovery could be impaired by the isolation media (Abdul-Raouf et al., 1993), and that MacConkey's agar with sorbitol was not as accurate for enumerating E. coli O157:H7 bacterial counts as tryptic soy agar - if the cells were acid-stressed. Our study did not confirm this result. In our study, there were no significant differences ($p \geq 0.05$) in populations enumerated on MacConkey's agar with sorbitol and tryptic soy agar plates for any of the treatments. These results would suggest that the cells were lethally injured.

The main purpose of this study was to determine the effectiveness of a sodium ion compound in combination with organic acids. Although sodium ion compounds did increase the effectiveness of these acids, the numerical increase was only slight and not significant. This increase trend was further lessened when compared to a phosphate buffer wash in combination with the acids. Perhaps increased contact times for the pretreatments and different application method or amount would change this. These items were not evaluated in this study. The major point is that some increase in calculated bacterial reduction did occur with pretreatments,

regardless of type applied, and this may be the start into new ideas to more intervention strategies.

SUMMARY AND DISCUSSION

In this research study, the effect of sodium ion compounds in combination with organic acids was evaluated in various conditions on E. coli O157:H7 inoculated beef tissue. Originally, acids and acid mixtures were tested to determine the most effective organic acids to use in the following study (Figures A1, A2).

In the first experiment, four levels of sodium lactate, trisodium phosphate and sodium glutamate (1%, 2%, 3% and 5%) were separately combined with the individually organic acids of 2% lactic, 2% acetic, 2% citric and 50:50 mixed acids solutions (2% lactic: 2% acetic, 2% lactic: 2% citric, and 2% citric: 2% acetic). The results suggest that neither sodium ion compound was overall more effective (Figures 1-6). Individually, sodium lactate and trisodium phosphate decreased the E. coli O157:H7 resistance to lactic acid. There was a numerical increase in log reduction with 1% sodium lactate and 2% acetic.

An increased calculated log reduction for 2% citric acid was noticed with sodium glutamate was obtained in this first experiment. This increase in bacterial reduction with sodium

glutamate combined with citric may be an artifact, since it did not carry through to the mixed acid results. It may also be an indication that the chemicals are recombining briefly. New chemicals, like sodium citrate or glutamic acid, perhaps are being produced that may have a greater effect then the original chemicals. However, sodium glutamate was easily susceptible to contamination and chemical unstable when analyzing the pH.

In 1972, Sato et al. (1972) had determined that sodium chloride provided a sub-lethal injury to E. coli cells. Since the numerical log reduction of our initial results were not as great as was expected, it was decided to compare these with sodium chloride percentages. Our results with sodium chloride did not show any dramatic calculated increase in log reduction (Figure A3). The results showed similar reductions to the reported results for the other sodium ion compounds. This information demonstrated that our original chosen sodium ions were applicable to continue for further study.

Further analysis of selected sodium ion compounds with specific acids was conducted at 52°C and 5°C. The bacterial reduction of 1% and 3% sodium lactate and trisodium phosphate

in combination with 2% lactic, 2% acetic and 2% lactic; 2% acetic - 50:50 was determined. These results supports previous findings that increased temperature of solutions increased bacterial reduction (Anderson and Marshall, 1989; Anderson and Marshall, 1990a; Anderson and Marshall, 1990b). The reduction was a significant increase in the effectiveness of 1% sodium lactate with 2% acetic and 2% lactic: 2% acetic - 50:50 between 5°C to 52°C (Figures 7,8).

After the temperature results, one sodium ion compound was chosen for further study. The sodium ion compound was 1% sodium lactate since a trend of increased calculated bacterial reduction was noticed for the above mentioned conditions. Once that item was chosen, it was decided to determine the about of recovery of cells that may occur due to sub-lethal injury. The beef tissue was subjected, as for the previous studies, and stored at 5°C for 24 hr (Figure A4). The main trend to notice is that there was no drastic recovery of cells during that storage period. This would suggest that these cells were lethally injured during the pretreatment - treatment combination.

The last part of this study was to take the results of broth inoculation of beef tissue and determine if the results held true when beef tissue is inoculated by fecal material. This proved not to be the case. The largest reduction was with 1% sodium lactate and 2% lactic acid (Figure 9). Although previously one of the higher reductions, 1% sodium lactate and 2% lactic: 2% acetic - 50:50 reported the lowest reduction effect of the sodium combinations studied with fecal inoculum.

This study suggest a few possible areas that may be addressed that may be addressed further. The retesting and determining the cause for the increase in numerical reduction of citric acid with sodium glutamate would be one area. However, this may be difficult to conduct. Increasing the exposure time and amount of solutions used to treat the beef tissue would be an easy and plausible continuation. Trying to determine the number of reversibly attached cells that are recovered from just the sodium ion and phosphate buffer pretreatment may also be valuable information. This may demonstrate the suggested trend that any pretreatment increases bacterial reduction.

In analyzing this study's data, an increased bacterial reduction was indicated with the usage of sodium ion compounds combined with organic acids. However, the effective increase reduction is not as significant when viewed with the values of the peptone buffer water and organic acids. Regardless of this, the results may still identify a potentially useful intervention strategy in the slaughtering process. The information gathered in this study showed that a pretreatment, regardless of the type, may numerical increase the bacterial reduction. When surveys of positive beef samples showed only .3 to 3.0 cells per 900 cm^2, any increase in the calculated log reduction may demonstrate an effective strategy, such as suggesting an increase in the postevisceration spraying.

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APPENDIX

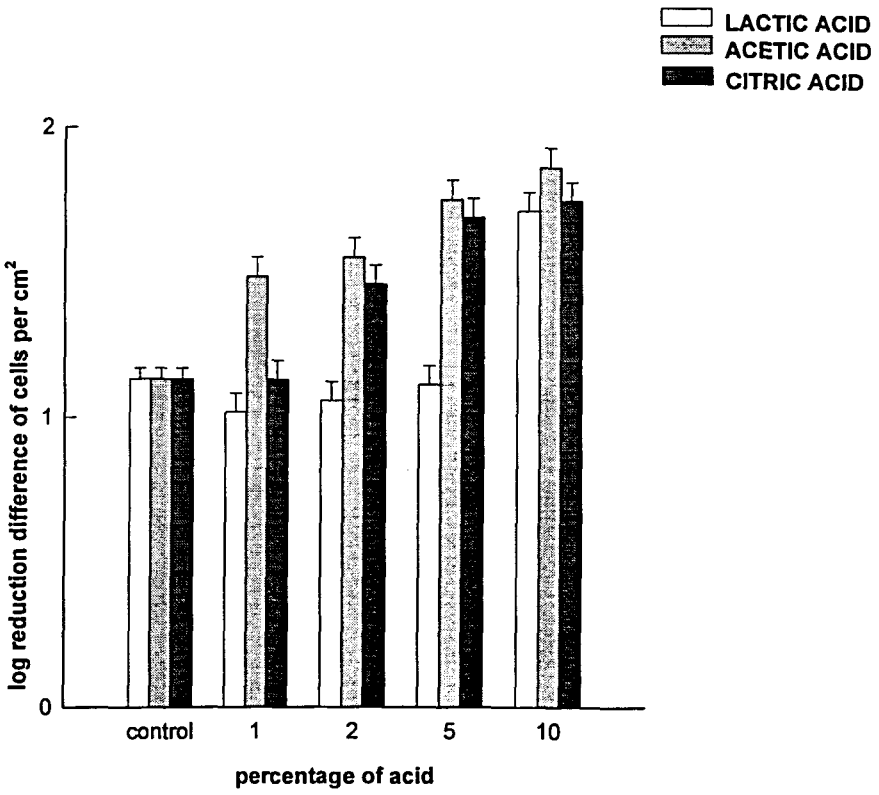

Figure A1. Log reduction difference of E. coli O157:H7 cells per cm^2 with organic acids at room temperature. Control is buffered peptone water.

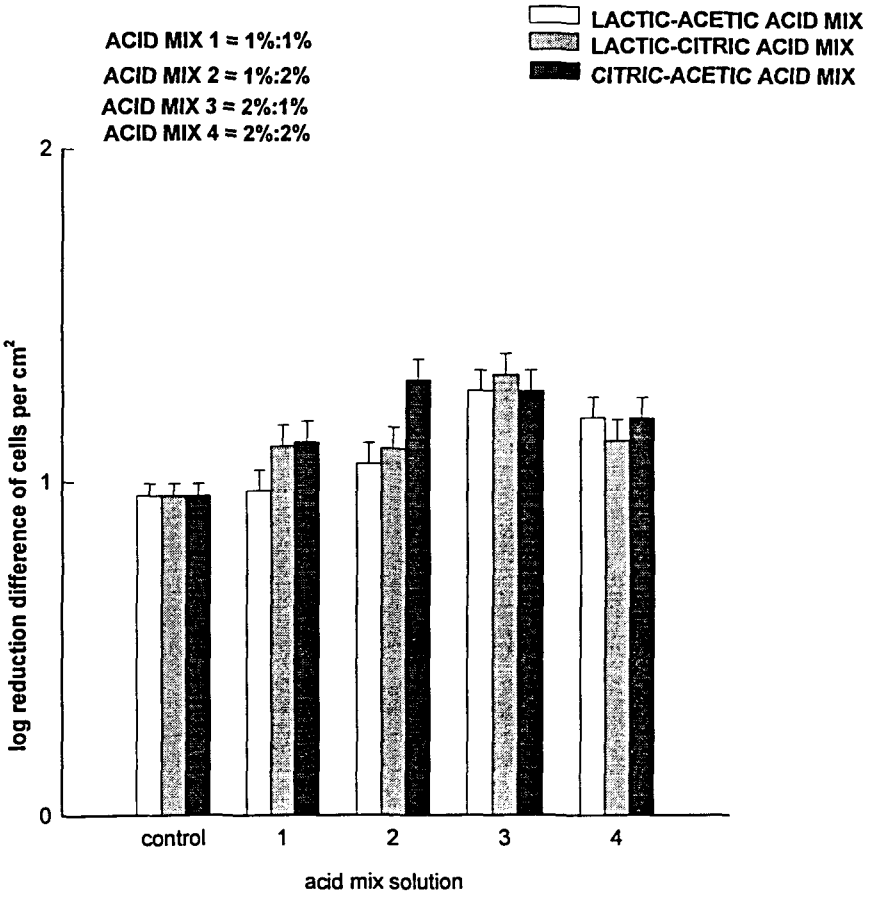

Figure A2. Log reduction difference of E. coli O157:H7 cells per cm^2 with 50:50 mixed acids solutions at room temperature. Control is buffered peptone water.

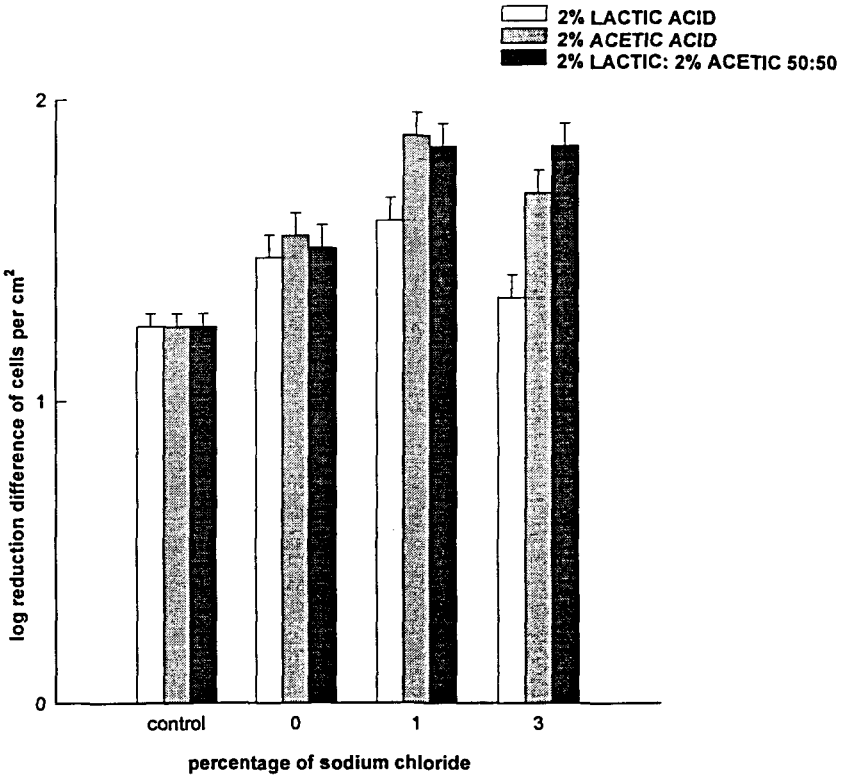

Figure A3. Log reduction difference of E. coli O157:H7 cells per cm^2. Sodium chloride % in combination with chosen organic acids at room temperature. Control is buffered peptone water with no acid treatment and 0% is buffered peptone water with acid treatment.

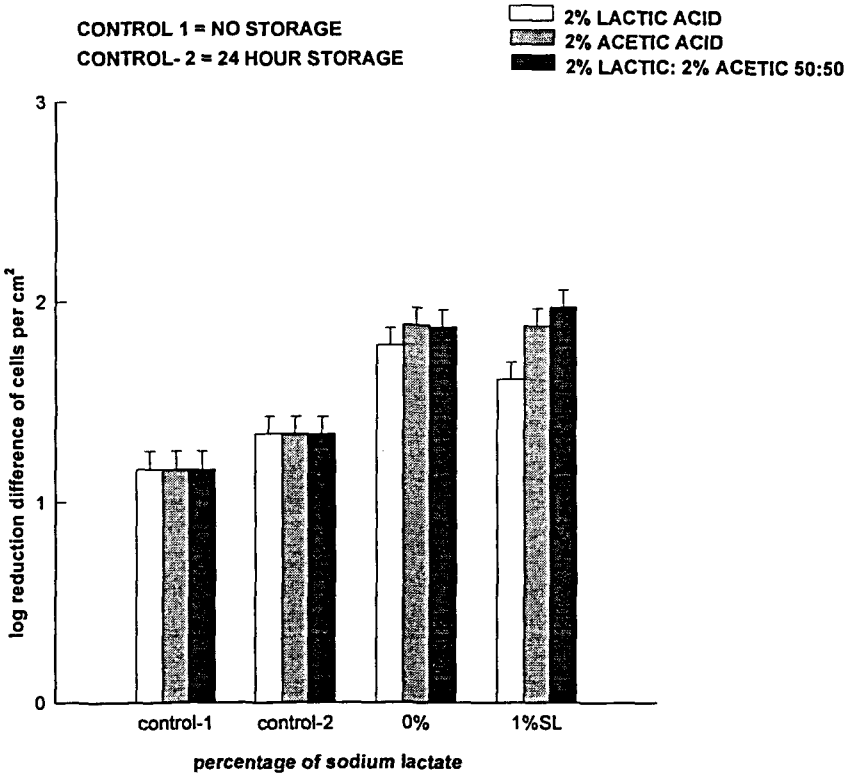

Figure A4. Log reduction difference of E. coli O157:H7 cells per cm^2. 1% sodium lactate (SL) in combination with chosen organic acids at room temperature followed by 24 hr storage at 5^0C. Control 1 is buffered peptone water with no acid treatment or storage. Control 2 is buffered peptone water with no acid treatment and 24 hr storage. 0% is buffered peptone water with acid treatment.