DISSERTATION

RESPONSES OF PATHOGENS ON FRESH BEEF AND READY-TO-EAT MEAT PRODUCTS SUBJECTED TO PROCESSING, PREPARATION, AND CONSUMPTION ASSOCIATED STRESSES

Submitted by

Jarret Dean Stopforth

Department of Animal Sciences

In partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Fall 2004

COLORADO STATE UNIVERSITY

August 3, 2004

WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY JARRET DEAN STOPFORTH ENTITLED "**RESPONSES OF PATHOGENS ON FRESH BEEF AND READY-TO-EAT MEAT PRODUCTS SUBJECTED TO PROCESSING, PREPARATION, AND CONSUMPTION ASSOCIATED STRESSES**" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Robert P. Ellis

Gary C. Smith

Pat Kendall
Co-Advisor

Advisor

William R. Woodward
Department Head

ABSTRACT OF DISSERTATION

RESPONSES OF PATHOGENS ON FRESH BEEF AND READY-TO-EAT MEAT PRODUCTS SUBJECTED TO PROCESSING, PREPARATION, AND CONSUMPTION ASSOCIATED STRESSES

This research was undertaken to elucidate the responses of *Escherichia coli* O157:H7 and *Salmonella* spp. on fresh beef and *Listeria monocytogenes* on ready-to-eat (RTE) meat products exposed to stresses associated with processing, preparation and consumption. Initially, studies evaluated the use of decontamination interventions to reduce *E. coli* O157:H7 populations on beef tissue. In general, it appeared that pathogen reductions achieved with treatments combining two 5% lactic acid applications or at least one hot (82°C) and one 5% lactic acid application were greater than those achieved with treatments including one or more hot (82°C) application or a single 5% lactic acid application. In another study, the effect of chemicals included in a simulated spray-chilling process was evaluated on beef tissue inoculated with acid- or nonacid-habituated *E. coli* O157:H7. Previous acid-habituation of *E. coli* O157:H7 rendered the cells more resistant to the effects of spray-chilling, especially with acid; however, reductions achieved, irrespective of acid-habituation, followed the order spray-chilling with water = nonspray-chilling < spray-chilling with chemical solutions. In the third study, the effect of strain interaction on growth and acid tolerance response of *E. coli* O157:H7 inoculated in meat decontamination runoff fluids (washings) and on meat was evaluated. Results indicated that mixing and subsequent co-habitation of *E. coli* O157:H7 strains prior to

inoculation of meat and washings did not affect their growth or acid tolerance. Acid-adaptation of *E. coli* O157:H7 on meat or washings at refrigeration temperatures may promote its resistance to subsequent interventions, especially acidic. The fourth study involved use of *E. coli* O157:H7 inocula of different histories to evaluate resistance responses during exposure to stresses simulating incomplete cooking and consumption. It was evident that heat and acid tolerance of the pathogen were influenced by inoculum history and more so by temperature of storage and transfer from anaerobic to aerobic conditions during storage. In the final study, survival of *L. monocytogenes* on ready-to-eat (RTE) pork frankfurters with antimicrobials as ingredients and/or dipping treatments was determined after exposure to a simulated gastric fluid. The combination of formulation of frankfurters with formulated with 0.25% sodium diacetate and dipping in 2.5% lactic acid appeared to increase the pathogen's acid tolerance. In general, the greater the growth of *L. monocytogenes* on frankfurters during storage, the higher the chance of survival in simulated gastric fluid.

Jarret Dean Stopforth
Department of Animal Sciences
Colorado State University
Fort Collins, CO 80523
Fall 2004

ACKNOWLEDGEMENTS

I have finally reached the pinnacle of my academic training but with the same token not even near the top of the mountain in my education. With this chapter of my life completed I look forward to the next steps and strive to continue with the same vigor I have up to now. There are so many people to thank for their support and assistance through my period of instruction over the last few years in my academic career. First and foremost, I express my thanks daily to the Lord for the blessing of the opportunities with which I have been presented. With my Savior at the helm I feel that there can be no task to big because whenever my strength starts to wane and doubt sets in I just remember that the single footprints in the sand are not mine. I also extend my absolute praises to my parents, Ian and Elizabeth Stopforth, who have been steadfast for me over the last few years, whose prayers have been the pillars of my efforts. I will never be able to repay you guys for the selfless sacrifices made on my behalf to get me to where I am but I promise to do my best through my achievements and in the way that I present myself to others in life. A special thanks goes out to my advisor, Dr. John Sofos. I could not possibly express my thanks to you for all that you have done for me while I have been under your instruction. I thank Dr. Sofos for caring very dearly about all of us and for his vested interest in all of us as individuals primarily and then as scientists. During my stay here, you have been tough on me but never once did I feel you were tough for the wrong reasons, all has always been in my best interest and for that I thank you. I take away much knowledge and many lessons from our acquaintance; the most important lessons

you have taught me are: the value of being honest and straightforward, the importance of good ethics in science, and most importantly to sometimes just "shut-up". I now have a far greater arsenal of tools in my bag with which to tackle the world. I thank my family, loved-ones and friends near and far for their support in my education, especially my Aunt Shan who remains up to date with all my happenings. I extend a special thanks to my dear friend Yohan Yoon. We have been together here for almost four years and you have always been a true friend and a great colleague. I hope that our collaborations and our professional and social activities are continued in the future.

DEDICATION

This thesis is dedicated to my beautiful wife, Maria. Thank you for being the hand of support in this challenging chapter of my life. A wise man once said "the true test of friendship comes in the times of difficulty" and Maria aced it with flying colors.

TABLE OF CONTENTS

	Page
ABSTRACT OF DISSERTATION	iii
ACKNOWLEDGEMENTS	v
DEDICATION	vii
TABLE OF CONTENTS	viii
LIST OF TABLES	xiii
CHAPTER I	1
INTRODUCTION	1
CHAPTER II	6
LITERATURE REVIEW	6
A. Introduction	6
B. *Escherichia coli* O157:H7	12
B.I. Pathogenic *E. coli* groups	14
B.II. Historical perspective and origins of *E. coli* O157:H7	20
C. Microbial contamination of beef carcasses at slaughter	21
C.I. Microbial contamination of beef carcasses during slaughter	23
D. Decontamination of fresh beef	26
D.I. Current decontamination technologies applied in the meat industry in the U.S	28
D.II. Novel chemicals proposed for decontamination and other approved solutions	36
E. Chilling beef carcasses to reduce microbial contamination	41
E.I. Importance of carcass chilling	41
E.II. Conventional beef carcass chilling	45
E.III. Spray-chilling of beef carcasses	47
E.IV. Use of antimicrobials during spray-chilling of beef carcasses	50
F. Cooking beef to inactivate microorganisms	51
G. *Listeria monocytogenes*	54
G.I. Distribution and disease	56
H. Control of *L. monocytogenes* in RTE meat and poultry products	59
H.I. Strategies to control *L. monocytogenes*	61
I. The digestive tract as a last line of defense against microorganisms	66
I.I. The gastrointestinal tract	67
I.II. The gastrointestinal tract as a defense system against pathogens	69

J. Resistance, adaptation and cross-protection of microorganisms to food processing stresses..... 73
J.I. Resistance and adaptation of microorganisms to food processing stresses..... 73
J.II. Cross-protection of microorganisms to food processing stresses.. 88

CHAPTER III..... 93

EFFECTIVENESS OF EXISTING AND NOVEL CHEMICALS AS SINGLE AND SEQUENTIAL DECONTAMINATION INTERVENTIONS TO REDUCE *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* TYPHIMURIUM ON BEEF TISSUE..... 93

ABSTRACT..... 93

INTRODUCTION..... 94

MATERIALS AND METHODS..... 96
Preparation and inoculation of beef tissue..... 96
Preparation and application of decontamination solutions..... 97
Experimental design..... 98
Microbiological analysis..... 100
Statistical analysis..... 101

RESULTS AND DISCUSSION..... 102
Phase I..... 102
Phase II..... 106

CONCLUSIONS..... 108

CHAPTER IV..... 111

EFFECT OF SIMULATED SPRAY-CHILLING WITH CHEMICAL SOLUTIONS ON ACID-HABITUATED AND NONACID-HABITUATED *ESCHERICHIA COLI* O157:H7 CELLS ATTACHED TO BEEF CARCASS TISSUE..... 111

ABSTRACT..... 111

INTRODUCTION..... 112

MATERIALS AND METHODS..... 115
Preparation of fresh beef decontamination runoff fluids..... 115
Bacterial strains and acid-habituation of *E. coli* O157:H7..... 116
Acid tolerance response (ATR) of inocula..... 117
Preparation of spray-chilling solutions..... 117

Spray-chilling treatments..... 118
Inoculation and spray-chilling of beef carcass tissue..... 119
Microbiological and physical analysis..... 120
Statistical analysis..... 121

RESULTS AND DISCUSSION..... 122
Determination of ATR of acid- and nonacid-habituated *E. coli* O157:H7 inoculum..... 122
Effect of spray-chilling treatments on nonacid-habituated cells..... 122
Effect of chemical spray-chilling treatments on acid- and nonacid-habituated *E. coli* O157:H7 on beef tissue..... 126

CONCLUSIONS..... 132

CHAPTER V..... 134

ACID TOLERANCE RESPONSE OF NONADAPTED AND ACID-ADAPTED *ESCHERICHIA COLI* O157:H7 STRAINS GROWN AS A MIXTURE, OR GRWON INDIVIDUALLY AND MIXED, PRIOR TO INOCULATION IN BEEF DECONTAMINATION RUNOFF FLUIDS OR ON BEEF TISSUE..... 134

ABSTRACT..... 134

INTRODUCTION..... 135

MATERIALS AND METHODS..... 138
Strains and acid-adaptation of *E. coli* O157:H7..... 138
Preparation and inoculation of fresh beef decontamination runoff fluids (washings)..... 139
Preparation and inoculation of fresh beef tissue..... 140
Assessment of acid tolerance and microbial analysis..... 140
Statistical analysis..... 142

RESULTS AND DISCUSSION..... 143
Effect of recovery media..... 143
Changes in pH of meat and washings during storage and acid exposure..... 144
Changes in bacterial populations on meat and in washings inoculated with *E. coli* O157:H7..... 153
ATR of *E. coli* O157:H7 in washings..... 155
ATR of *E. coli* O157:H7 on meat..... 158

CONCLUSIONS..... 160

CHAPTER VI..... 162

REDUCTION OF *ESCHERICHIA COLI* O157:H7 POPULATIONS ON LEAN BEEF TISSUE INOCULATED WITH CULTURES PREPARED UNDER DIFFERENT CONDITIONS AND EXPOSED TO INCOMPLETE COOKING AND SYNTHETIC GASTRIC FLUID..... 162

ABSTRACT..... 162

INTRODUCTION..... 163

MATERIALS AND METHODS..... 166
Bacterial strains..... 166
Inocula preparation..... 167
Inoculation and decontamination of fresh lean beef..... 169
Transfer of vacuum-packaged refrigerated beef to aerobic storage.... 170
Exposure of fresh beef to heat..... 171
Exposure of fresh beef to synthetic gastric fluid..... 171
Microbiological and physical analysis of samples..... 172
Statistical analysis..... 173

RESULTS AND DISCUSSION..... 174

CONCLUSIONS..... 189

CHAPTER VII..... 191

REDUCTION OF *LISTERIA MONOCYTOGENES* POPULATIONS DURING EXPOSURE TO A SIMULATED GASTRIC FLUID FOLLOWING STORAGE OF INOCULATED FRANKFURTERS FORMULATED AND TREATED WITH PRESERVATIVES..... 191

ABSTRACT..... 191

INTRODUCTION..... 192

MATERIALS AND METHODS..... 194
Frankfurter preparation..... 194
Product inoculation..... 195
Frankfurter dipping treatments..... 196
Exposure of *L. monocytogenes* to simulated gastric fluid..... 196
Microbiological and physical analysis of samples..... 197
Statistical analysis..... 198

RESULTS AND DISCUSSION..... 200

CONCLUSIONS..... 213

SUMMARY OF DISSERTATION..... 215

REFERENCES..... 221

LIST OF TABLES

Table number	Title	Page
Table II.1	Impact of foodborne bacterial pathogens annually in the U.S.....	11
Table II.2	USDA-FSIS food product recalls, 2000-2003.....	12
Table III.1	Design of treatment systems.....	100
Table III.2	Least squares means (standard deviations) and pooled (mean values for samples from both pathogens) pH of samples indicating survival and reduction of *Escherichia coli* O157:H7 and *Salmonella* on beef tissue after dipping in decontamination solutions (1.5 L) for 30 s.....	104
Table III.3	Least squares means (standard deviations) indicating survival and reduction of *Escherichia coli* O157:H7 on beef carcass tissue after exposure to sodium metasilicate (SM; 4% [wt/wt]) at different temperatures.....	106
Table III.4	Least squares means (standard deviations) indicating survival and reduction of *Escherichia coli* O157:H7 on beef carcass tissue after exposure to sodium metasilicate (SM) at different concentration-temperature combinations.....	106
Table III.5	Least squares means (standard deviations) and pooled (mean values for samples from both pathogens) pH of samples indicating survival and reduction of *Escherichia coli* O157:H7 and *Salmonella* on beef tissue not dipped (control) or after dipping (for 30 s in 1.5 L) in various treatments.....	110
Table IV.1	Mean (standard deviation) pH values of beef carcass adipose tissue samples inoculated with *Escherichia coli* O157:H7 and exposed to various treatments during the simulated spray-chilling process.....	124
Table IV.2	Mean (standard deviation) surviving bacterial populations on beef carcass adipose tissue samples inoculated with nonacid-habituated *Escherichia coli* O157:H7 and sprayed at 30 min intervals with various chemical solutions during chilling at -3°C for 10 h and then stored at 1°C for 38 h.....	125
Table IV.3	Mean (standard deviation) surviving total bacterial (tryptic soy	

agar; TSA) populations on beef carcass adipose tissue samples inoculated with acid- and nonacid-habituated *Escherichia coli* O157:H7 and sprayed at 30 min intervals with various chemical solutions during chilling at -3°C for 10 h and then stored at 1°C for 38 h..... 128

Table IV.4 Mean (standard deviation) surviving acid- and nonacid-habituated *Escherichia coli* O157:H7 (TSA+Rif) populations inoculated on beef carcass adipose tissue samples and sprayed at 30 min intervals with various chemical solutions during chilling at -3°C for 10 h and then stored at 1°C for 38 h..... 129

Table V.1 Mean (standard deviation) populations (log CFU/ml; tryptic soy agar) of acid-adapted and nonacid-adapted *Escherichia coli* O157:H7 strains grown individually and mixed prior to inoculation into nonacidic (water; pH 7.01) and acidic (water washings adjusted to pH 4.95 with lactic acid [LA] washings) washings and exposed periodically to tryptic soy broth with 0.6% yeast extract (adjusted to pH 3.5 with LA) during storage for 6 d at 4 or 15°C..... 145

Table V.2 Mean (standard deviation) populations (log CFU/ml; tryptic soy agar) of acid-adapted and nonacid-adapted *Escherichia coli* O157:H7 strains grown in a mixture prior to inoculation into nonacidic (water; pH7.01) and acidic (water washings adjusted to pH 4.95 with lactic acid [LA] washings) washings and exposed periodically to tryptic soy broth with 0.6% yeast extract (adjusted to pH 3.5 with LA) during storage for 6 d at 4 or 15°C.. 146

Table V.3 Mean (standard deviation) populations (log CFU/cm^2; tryptic soy agar) of acid-adapted and nonacid-adapted *Escherichia coli* O157:H7 strains grown individually and mixed prior to inoculation on lean beef tissue left untreated or treated (by dipping in hot water [75°] followed by lactic acid [LA; 2%, 55°C]) and exposed periodically to tryptic soy broth with 0.6% yeast extract (adjusted to pH 3.5 with LA) during storage for 6 d at 4 or 15°C..... 147

Table V.4 Mean (standard deviation) populations (log CFU/cm^2; tryptic soy agar) of acid-adapted and nonacid-adapted *Escherichia coli* O157:H7 strains grown in a mixture prior to inoculation on lean beef tissue left untreated or treated (by dipping in hot water [75°] followed by lactic acid [LA; 2%, 55°C]) and exposed periodically to tryptic soy broth with 0.6% yeast extract (adjusted to pH 3.5 with LA) during storage for 6 d at 4 or 15°C.. 148

Table V.5 Mean (standard deviation) populations (log CFU/ml; sorbitol MacConkey Agar with cefixime and tellurite supplement) of acid-adapted and nonacid-adapted *Escherichia coli* O157:H7 strains grown individually and mixed prior to inoculation into nonacidic (water; pH7.01) and acidic (water washings adjusted to pH 4.95 with lactic acid [LA] washings) washings and exposed periodically to tryptic soy broth with 0.6% yeast extract (adjusted to pH 3.5 with LA) during storage for 6 d at 4 or 15°C.. 149

Table V.6 Mean (standard deviation) populations (log CFU/ml; sorbitol MacConkey Agar with cefixime and tellurite supplement) of acid-adapted and nonacid-adapted *Escherichia coli* O157:H7 strains grown in a mixture prior to inoculation into nonacidic (water; pH7.01) and acidic (water washings adjusted to pH 4.95 with lactic acid [LA] washings) washings and exposed periodically to tryptic soy broth with 0.6% yeast extract (adjusted to pH 3.5 with LA) during storage for 6 d at 4 or 15°C..... 150

Table V.7 Mean (standard deviation) populations (log CFU/cm^2; sorbitol MacConkey Agar with cefixime and tellurite supplement) of acid-adapted and nonacid-adapted *Escherichia coli* O157:H7 strains grown individually and mixed prior to inoculation on lean beef tissue left untreated or treated (by dipping in hot water [75°] followed by lactic acid [LA; 2%, 55°C]) and exposed periodically to tryptic soy broth with 0.6% yeast extract (adjusted to pH 3.5 with LA) during storage for 6 d at 4 or 15°C.. 151

Table V.8 Mean (standard deviation) populations (log CFU/cm^2; sorbitol MacConkey Agar with cefixime and tellurite supplement) of acid-adapted and nonacid-adapted *Escherichia coli* O157:H7 strains grown in a mixture prior to inoculation on lean beef tissue left untreated or treated (by dipping in hot water [75°] followed by lactic acid [LA; 2%, 55°C]) and exposed periodically to tryptic soy broth with 0.6% yeast extract (adjusted to pH 3.5 with LA) during storage for 6 d at 4 or 15°C.. 152

Table V.9 Mean (standard deviation) pH values of acid-adapted or nonacid-adapted *Escherichia coli* O157:H7 strains grown individually (IN) and mixed or grown in a mixture (CK) prior to inoculation on lean beef tissue left untreated or treated (by dipping in hot water [75°] followed by lactic acid [LA; 2%, 55°C]) or into nonacidic (water) and acidic (water washings adjusted to pH 4.95 with lactic acid washings) washings..... 154

Table VI.1 Mean (standard deviation) pH values of fresh beef inoculated with *Escherichia coli* O157:H7 of different origins (TSB, WASH, WETB or DRYB), left nondecontaminated or decontaminated with sequential treatments of hot water (75°C) and 2% lactic acid (55°C) for 30 s prior to storage under vacuum at 4 or 12°C for 28 and 16 d, respectively..... 177

Table VI.2 Mean (standard deviation) total bacterial (TSA) populations (log CFU/cm^2) on fresh beef inoculated with *Escherichia coli* O157:H7 of different origins (TSB, WASH, WETB or DRYB), and decontaminated with sequential treatments of hot water (75°C) and 2% lactic acid (55°C) for 30 s prior to storage under vacuum at 4°C for 28 d and periodic exposure to heat (55°C) for 20 min followed by simulated gastric fluid (pH 1.0) for 90 min... 178

Table VI.3 Mean (standard deviation) total bacterial (TSA) populations (log CFU/cm^2) on fresh beef inoculated with *Escherichia coli* O157:H7 of different origins (TSB, WASH, WETB or DRYB), stored under vacuum at 4°C for 28 d and periodically exposed to heat (55°C) for 20 min followed by simulated gastric fluid (pH 1.0) for 90 min..... 179

Table VI.4 Mean (standard deviation) total bacterial (TSA) populations (log CFU/cm^2 ± SD) on fresh beef inoculated with *Escherichia coli* O157:H7 of different origins (TSB, WASH, WETB or DRYB), and decontaminated with sequential treatments of hot water (75°C) and 2% lactic acid (55°C) for 30 s prior to storage under vacuum at 12°C for 16 d and periodic exposure to heat (55°C) for 20 min followed by simulated gastric fluid (pH 1.0) for 90 min..... 180

Table VI.5 Mean (standard deviation) total bacterial (TSA) populations (log CFU/cm^2) on fresh beef inoculated with *Escherichia coli* O157:H7 of different origins (TSB, WASH, WETB or DRYB), stored under vacuum at 12°C for 16 d and periodically exposed to heat (55°C) for 20 min followed by simulated gastric fluid (pH 1.0) for 90 min..... 181

Table VI.6 Mean (standard deviation) *Escherichia coli* O157:H7 (TSA+Rif) populations (log CFU/cm^2) of different origins (TSB, WASH, WETB or DRYB), inoculated onto fresh beef and decontaminated with sequential treatments of hot water (75°C) and 2% lactic acid (55°C) for 30 s prior to storage under vacuum at 4°C for 28 d and periodic exposure to heat (55°C) for 20 min followed by simulated gastric fluid (pH 1.0) for 90 min..... 182

Table VI.7 Mean (standard deviation) *Escherichia coli* O157:H7 (TSA+Rif) populations (log CFU/cm^2) of different origins (TSB, WASH, WETB or DRYB), inoculated onto fresh beef stored under vacuum at 4°C for 28 d and periodically exposed to heat (55°C) for 20 min followed by simulated gastric fluid (pH 1.0) for 90 min..... 183

Table VI.8 Mean (standard deviation) *Escherichia coli* O157:H7 (TSA+Rif) populations (log CFU/cm^2) of different origins (TSB, WASH, WETB or DRYB), inoculated onto fresh beef and decontaminated with sequential treatments of hot water (75°C) and 2% lactic acid (55°C) for 30 s prior to storage under vacuum at 12°C for 16 d and periodic exposure to heat (55°C) for 20 min followed by simulated gastric fluid (pH 1.0) for 90 min..... 184

Table VI.9 Mean (standard deviation) *Escherichia coli* O157:H7 (TSA+Rif) populations (log CFU/cm^2) of different origins (TSB, WASH, WETB or DRYB), inoculated onto fresh beef stored under vacuum at 12°C for 16 d and periodically exposed to heat (55°C) for 20 min followed by simulated gastric fluid (pH 1.0) for 90 min..... 185

Table VII.1 Mean (standard deviation) pH values of nondipped pork frankfurter samples inoculated with *Listeria monocytogenes* and exposed to simulated gastric fluid (pH 1.0; for up to 60 min) periodically during storage under vacuum at 10°C for 40 d..... 201

Table VII.2 Mean (standard deviation) pH values of pork frankfurter samples inoculated with *Listeria monocytogenes* and dipped in 2.5% lactic acid and subsequently exposed to simulated gastric fluid (pH 1.0; for up to 60 min) periodically during storage under vacuum at 10°C for 40 d..... 202

Table VII.3 Mean (standard deviation) pH values of pork frankfurter samples inoculated with *Listeria monocytogenes* and dipped in 2.5% acetic acid and subsequently exposed to simulated gastric fluid (pH 1.0; for up to 60 min) periodically during storage under vacuum at 10°C for 40 d..... 203

Table VII.4 Mean log CFU/cm^2 populations (standard deviations) of *Listeria monocytogenes* on pork frankfurters formulated with antimicrobials and not dipped or dipped in organic acid solutions and later exposed to simulated gastric fluid (pH 1.0) after storage for 0 and 10 days under vacuum at 10°C..... 210

Table VII.5 Mean log CFU/cm^2 populations (standard deviations) of *Listeria*

monocytogenes on pork frankfurters formulated with antimicrobials and not dipped or dipped in organic acid solutions and later exposed to simulated gastric fluid (pH 1.0) after storage for 20 days under vacuum at 10°C..... 211

Table VII.6 Mean log CFU/cm^2 populations (standard deviations) of *Listeria monocytogenes* on pork frankfurters formulated with antimicrobials and not dipped or dipped in organic acid solutions and later exposed to simulated gastric fluid (pH 1.0) after storage for 30 days under vacuum at 10°C..... 212

Table VII.7 Mean log CFU/cm^2 populations (standard deviations) of *Listeria monocytogenes* on pork frankfurters formulated with antimicrobials and not dipped or dipped in organic acid solutions and later exposed to simulated gastric fluid (pH 1.0) after storage for 40 days under vacuum at 10°C..... 213

CHAPTER I

INTRODUCTION

Escherichia coli O157:H7 was first identified as a human pathogen following two outbreaks in the U.S. in 1982 involving undercooked hamburger patties (Riley et al., 1983). Subsequent to these outbreaks and some other less publicized ones in the 1980s, a highly publicized outbreak, involving undercooking of hamburger patties contaminated with *E. coli* O157:H7, occurred with much more extensive consequences, resulting in hundreds of illnesses and the death of four children (Bell et al., 1994). An estimated 62,000 cases of symptomatic *E. coli* O157:H7 infections occur annually in the U.S. due to foodborne exposure, resulting in approximately 1,800 hospitalizations and 52 deaths (Mead et al., 1999). According to the Economic Research Service, the estimated cost of *E. coli* O157 stemming from medical costs, productivity losses and premature deaths annually in the U.S. totals $0.7 billion, while that incurred by *E. coli*, non-O157 STEC is $0.3 billion (available at http://www.ers.usda.gov/briefing/foodbornedisease/). Furthermore, the cost of *E. coli* O157:H7 from an industrial standpoint has accounted for approximately $2.7 billion during the past 10 years (Kay, 2003). Surveillance data indicate that the highest incidence of illness from *E. coli* O157:H7 occurs in children under 5 years of age (Mead et al., 1999). The pathogen may cause severe sequelae in susceptible humans after becoming ingested, including hemorrhagic diarrhea, hemolytic uremic syndrome (HUS) and disseminated intravascular coagulopathy (DIC) (Doyle et al., 1997).

Considering the severity of contamination by this pathogen on fresh beef, the United States Department of of the Agriculture Food Safety and Inspection Service (USDA-FSIS) issued a policy, known as "zero tolerance," requiring the absolute removal of feces, ingesta, and udder contents from beef carcasses, by trimming, before carcass washing, as a means of improving the microbiological quality of carcasses (USDA-FSIS, 1993). This in turn was followed in July 1996, with new regulations for meat and poultry inspection employing the Hazard Analysis Critical Control Point (HACCP) system, which included microbiological performance criteria for *E. coli* biotype 1 and *Salmonella* (USDA-FSIS, 1996). Furthertmore, recent directives, notices, and guidances issued by the USDA-FSIS informed the meat industry to consider this pathogen as a food safety hazard likely to occur in fresh beef. All involved with food, including regulators, educators, consumers, health authorities, research scientists and the industry agree that efforts should be made to reduce incidence and eliminate or control pathogenic bacteria at all stages of the food chain (Sofos et al., 2003). With the regulations and the implications of foodborne illness associated with this pathogen, the meat industry has contributed to the overall effort of improving food safety by supporting development and applying quality assurance programs developed through their associations, and by financially supporting, through their associations, research and development studies on microbial reduction interventions applied during animal slaughter. The principal objective of the meat processing industry has been to improve operations through implementation of HACCP programs, and employment of various carcass decontamination interventions.

On the other hand, *L. monocytogenes* infection in humans results in an estimated 2,493 hospitalizations and 499 deaths annually, most of them affecting elderly, pregnant women and their fetuses, and immunocompromised individuals (Mead et al., 1999). Of estimated case fatality rate (20%) and the highest estimated hospitalization rate (92%) (Mead et al., 1999; Table II.1). The economic burden in the USA due to foodborne listeriosis increased approximately 10-fold over a 7-year period in the 1990s, from $0.2-0.3 billion in 1993 (Buzby et al., 1996), to $2.3 billion in 2000 (http://www.ers.usda.gov/briefing/foodbornedisease/). Currently, there are approximately 6.5 million pregnant women, 4.4 million neonates and 35 million persons ≥65 years of age living in the USA, accounting for approximately 16% of the population. Pregnant women are 17 times more likely than the healthy general population to contract listeriosis, and account for 27% of all cases (Lorber, 1997). Those over the age of 65 have a 7.5 times higher risk of infection with *L. monocytogenes* than healthy adults <65 years of age (Goulet and Marchetti, 1996). Among the elderly, those in nursing homes are at highest risk of listeriosis (Smith, 1998).

The severity of listeriosis has led regulatory agencies since the late 1980s to recommend or require industry to establish control strategies to minimize the presence, survival, and growth of *L. monocytogenes* in foods. In 1991, the National Advisory Committee for Microbiological Criteria in Foods (NACMCF) recommended control strategies such as development of an effective surveillance system for listeriosis, and use of HACCP–based programs to ensure the safety of foods (NACMCF, 1991). Additionally, the USDA-FSIS established a 'zero tolerance' (negative 25 g samples) in RTE meat and poultry products and initiated microbiological testing for *L.*

monocytogenes (USDA-FSIS, 1990). Between 2000 and 2003, there were at least 98 reported recalls of RTE beef, pork and poultry products suspected for *L. monocytogenes* contamination (http://www.fsis.usda.gov/OA/recalls/rec_pr2.htm). More specifically, in 2000, *L. monocytogenes* was considered the primary cause of recalls not only of cooked meats (35 of 39) but also of all recalls due to any bacterial adulteration (35 of 60) (Wallace et al., 2003). These fatal outbreaks and the frequent and highly publicized recalls of meat products potentially contaminated with the pathogen have prompted the industry, public health authorities and researchers to develop and establish effective measures and procedures to maintain product safety and increase consumer confidence in RTE meat products (Bernard and Scott, 1999; Tompkin, 2002; Tompkin et al., 1999). The efforts to control pathogens in meat and meat products rely heavily on the incorporation of antimicrobials in or on the surface of such products during processing and the potential for generating environments with sublethal concentrations of such antimicrobials exists. Generally, exposure of microorganisms to sublethal stresses during food processing may produce stress-hardened pathogens surviving, more readily, antimicrobial treatments aimed at improving the microbiological quality of food, potentially resulting in persistent microbiological populations with higher virulence (Samelis and Sofos, 2003).

The overall goal of the studies included in this dissertation was to elucidate the response of *E. coli* O157:H7 and *L. monocytogenes* on fresh beef and RTE meat, respectively, subjected to adverse conditions during processing and/or preparation of such products for consumption. The first four studies were conducted with *E. coli* O157:H7 on fresh beef tissue while the last study was conducted with *L. monocytogenes*

on RTE pork frankfurters. The objective of the first study was to evaluate the use of chemicals as single and sequential interventions to reduce *Escherichia coli* O157:H7 and *Salmonella* Typhimurium populations on beef tissue. The objective of the second study was to investigate the effectiveness of simulated chilling without spraying vs. spray-chilling using water and chemical solutions in reducing *E. coli* O157:H7 contamination on beef carcass tissue. In addition, this study evaluated the effect of previous acid-habituation (in sublethal acidic meat decontamination runoff fluids) or nonacid-habituation (in water meat decontamination runoff fluids) on subsequent responses of *E. coli* O157:H7 cells on beef carcass tissue exposed to spray-chilling treatments.

The objective of the third study was to assess the acid tolerance response of stationary phase, acid-adapted or nonadapted *E. coli* O157:H7 strains grown individually or in a mixed culture during acid-adaptation and inoculated on beef or into meat decontamination runoff (washings) fluids.

The objective of the fourth study was to evaluate stress responses of different *E. coli* O157:H7 inocula on beef left untreated or decontaminated (sequential hot water and warm acid dipping) and stored under vacuum at different temperatures and periodically transferred to aerobic storage conditions prior to exposure to consumer-induced stresses simulating incomplete cooking and consumption.

The objective of the final study was to evaluate the effect of a simulated gastric fluid on *Listeria monocytogenes*, inoculated postprocessing on pork frankfurters formulated with sodium diacetate and lacate and left untreated or treated by dipping in lactic and acetic acid during storage at 10°C for 40 d.

CHAPTER II

LITERATURE REVIEW

A. Introduction

Food safety has become one of the most important issues in recent times. The aftermath of foodborne illness outbreaks has resulted in many losing their lives, companies going bankrupt and employees losing their jobs as well as public sensitization and skepticism toward the associated food product. Consumers in the United States enjoy the safest food in the history of the world. The International Beef Quality Audit found that U.S. beef had the highest microbiological quality and the lowest incidence of violative levels of chemical residues when compared to 19 other countries from North America, Europe, Asia, Middle East, and the Association of Southeast Asian Nations (Morgan et al., 1995). Even so, there are still millions of Americans stricken by illness every year caused by the food they consume.

For the purpose of discussion in this dissertation, foodborne illnesses are defined as diseases contracted by ingesting microbiologically contaminated food. Contaminated food refers to that food which is rendered unsafe for consumption by the microorganisms themselves or toxins that they produce. There are two main categories of foodborne illness (Prescott et al., 1999), namely infections and intoxications. Infections result from eating food containing pathogenic microbes, which multiply in the body and attack the intestines or other organs directly, causing symptoms such as nausea, vomiting, diarrhea

and fever. Intoxications result from eating food which contains toxins produced by microbes which do not need to grow in the human body to cause illness. Furthermore, there are toxicoinfections characterized by the onset of symptoms due to toxins formed in the body after ingesting food contaminated with the causative agent. The organisms causing these foodborne illnesses are found throughout nature: in plants, in animals, people and soil. Agreement is widespread that the most serious food safety problem is foodborne illness of microbial origin. With the increase in recognition of microorganisms associated with foodborne illnesses there has also been an increase in the number of foods involved in foodborne illnesses (Smith, 2000). Mishandling and poor refrigeration are responsible for most food contamination, with improper cooking, cross-contamination, contaminated equipment, hygiene of the food handlers, and time/temperature abuse being the major causes of mishandling (Smith, 2000). Contamination due to mishandling can occur at any point in the food chain – in processing, at supermarkets, restaurants, or in the home.

One of the possible explanations for the observed increase in foodborne illness in today's society is the advance being made in monitoring and testing methods which facilitates association of pathogens with food contamination (NMA, 1999). Another reason would be more aggressive surveillance due to the activities of regulatory and public health agencies emphasizing the importance of good management of food safety, and the monitoring and regulation of the industry by the government in order to enhance the control of foodborne pathogens.

A major contributor to the current food safety concerns may indeed be our own changes as consumers. Society today has undergone substantial changes in demographics,

population size, lifestyles, food preferences and expectations, life expectancy and education. The number of people actively involved in agriculture and food production has decreased while our population has increased and become more urbanized. The change in lifestyles has been a development under societal and economic pressures. Lifestyle changes over the past two decades have contributed to more opportunities for mishandling of food and greater cross-contamination (Knabel, 1995; NMA, 1999). More and more meals are eaten outside of the home, creating greater demand for mass feeding from foodservice units such as commissary kitchens, restaurants, catering services, and delicatessens where a single outbreak of foodborne illness can involve hundreds of cases. The demand for convenience has resulted in increased reliance on food processing techniques that require careful handling and storage. An American Meat Institute (1996) study details lifestyle changes affecting food behavior, including an increasing number of women in the workforce, limited commitment to food preparation, and a greater number of single head households. Consumers appear to be more interested in convenience and saving time than in proper food handling and preparation (Collins, 1997).

A greater percentage of the population are employed and working longer hours, forcing consumers to eat more meals away from home or causing an increase in the number of "take home" meals and the increase in consumption of RTE meals and other food items that require minimal preparation and offer convenience. Many consumers follow special diets; prefer minimally processed foods and food with an extended shelf-life. In summary, demographics, consumer lifestyles and environmental changes have affected the way consumers in the U.S. purchase, prepare and store food. Some of those transformations include: (a) emerging foodborne pathogens which require new control

techniques; (b) bacterial pathogens have found new modes of transmission; (c) the food supply has become global and many different countries export food to the U.S.; and, (d) a trend of smaller-sized families, a larger share of single-headed households and an increase in two-working-parent families has resulted in: (i) families having less time for shopping and food preparation; (ii) more children preparing food for themselves at home with no adult present; (iii) more youth growing up with little or no instruction on food preparation; and, (iv) more food prepared away from home. These changes, in addition to the fact that declining numbers of Americans believe food safety is their responsibility (FMI, 2000), creates a need for food safety to become a national priority. Many of these factors, individually or combined, may result in food safety risks and pose a challenge to be addressed by appropriate handlers and processors in our food supply. The more processing and handling required to produce a food, the more likely the chance of contamination and abuse and the higher the risk of a foodborne infection.

Even though many activities or everyday events have a higher likelihood of occurring and are more life-threatening than eating a meat product, modern consumers demand zero-risk or expect zero-possibility of becoming ill from food which they consume. A survey in the U.S. found that: 80% of consumers are as concerned about food safety as they are about safe drinking water, crime and health; 75% would alter their food consumption based on negative media stories; and 88% were "very concerned" about bacteria like *Salmonella* (Drovers Journal, 2000; Smith, 2000). Possible food safety concerns about beef (Smith, 2000) include: (a) foodborne pathogens present in meat (of most importance are *Salmonella* spp., *L. monocytogenes*, *E. coli* O157:H7 and *Campylobacter jejuni*); (b) pesticide residues in meat (either one or both hydrocarbons

and organophosphates); (c) antibiotics (fear of the continual use of feed-grade antibiotics resulting in residues in or on meat causing development of antibiotic-resistant strains of human pathogens); (d) residues of livestock growth-promoting compounds in meat (concern for residues of naturally occurring and synthesized growth promotants in or on meat); (e) possibility of beef contaminated with prions responsible for Bovine Spongiform Encephalopathy (BSE); and, (f) risks associated with the potential use of ionizing radiation to destroy foodborne pathogens in/on beef.

It is difficult to determine exactly how many illnesses occur in the U.S. each year. The federal government is actively trying to develop more accurate estimates through the Foodborne Diseases Active Surveillance Network (FoodNet). FoodNet is a joint project by the Department of Health and Human Services (DHHS), state health departments and the USDA to collect more accurate and complete data of occurrences of foodborne illness in the U.S. The magnitude of economic loss, human suffering, and public health impact of foodborne illnesses has been underestimated by the general public and health-care professionals until very recently. The article "Food Related Illness and Death in the United States" released in September 1999 by Mead et al. is regarded as the most up-to-date. The study estimates that about 76 million illnesses per year are caused by foodborne pathogens, with approximately 325,000 hospitalizations per year nationwide. According to Mead et al. (1999), the most likely estimate of deaths annually in the U.S. is close to 5,200. Of the bacterial, viral or parasitic microbial foodborne hazards, it is estimated that bacterial agents are responsible for only 30% of the total foodborne illnesses; however, 72% of total deaths are due to consumption of foods contaminated with bacteria (Mead et al., 1999). According to the latest surveillance data (CDC, 2003),

Salmonella spp. are responsible for causing the highest total number of cases of gastrointestinal illness among bacteria; however, despite the high incidence of illness, the case-fatality rate is <0.05%. *Campylobacter* spp. is responsible for the second highest total number of gastrointestinal illnesses (CDC, 2003) and, like *Salmonella* spp., has a case-fatality rate of <0.05%. Although *E. coli* O157:H7 has a much lower rate of incidence compared to *Campylobacter* and *Salmonella* (CDC, 2003), this organism has a higher case-fatality rate (0.1%). Compared to the above-mentioned pathogens, *L. monocytogenes* has the lowest rate of incidence (CDC, 2003) but a significantly higher (approximately 20%) fatality rate.

Table II.1: Impact of foodborne bacterial pathogens annually in the U.S.

	Total illnesses	% Illnesses	Total deaths	% Deaths	Case fatality rate (% deaths/illness)
All sources*	76,000,000	100	5,200	100	-
Campylobacter	**2,453,926**	3.2	124	**2.4**	<0.05
Salmonella	**1,413,322**	1.9	585	**11.3**	<0.05
E. coli O157:H7	**73,480**	0.1	61	**1.1**	**0.1**
Listeria monocytogenes	2,518	<0.05	504	**9.7**	**20**
Yersinia enterocolitica	96,368	0.1	3	0.1	<0.05
Clostridium botulinum	58	<0.05	4	0.1	6.9
C. perfringens	248,520	0.3	7	0.1	<0.05
Bacillus cereus	27,360	<0.05	0	0	0
Staphylococcus aureus	185,060	0.2	2	<0.05	<0.05

Total of all known and unknown sources
Table adapted from Mead et al. (1999).

Table II.2. USDA-FSIS food product recalls, 2000-2003

Product	*Listeria*	*Salmonella*	*Escherichia coli* O157:H7	Total	**% Recalls/ Product**
Beef	1	-	84	85	**25.3**
Pork	2	-	2	4	**1.1**
Chicken	2	1	0	3	**0.8**
Turkey	5	-	-	5	**1.4**
Other RTE Products	100	9	-	109	**32.4**
Processed Products	16	1	-	17	**5.1**
Other Recalls	-	-	-	113	
Total	126	11	84	336	
% Recalls/Pathogen	**(37.5%)**	**(3.2%)**	**(0.25%)**		

Adapted from USDA-FSIS Recall Information Center database:
http://www.fsis.usda.gov/OA/recalls/rec_pr2.htm

The economic burden of these bacterial agents is evident in the meat product recalls in the period of 2000-2003 (Table II.2). Recognition of the significant impact of microbial foodborne diseases in terms of human suffering and economic costs to society and industry, combined with an increasing global food trade, has underlined the need to change our approaches to management of the safety of our food supply.

B. *Escherichia coli* O157:H7

Escherichia coli, classed in the family *Enterobacteriaceae*, is a normal member of the natural flora inhabiting the digestive tract of humans and many animals (Tauxe, 1997). Although most *E. coli* are not responsible for gastrointestinal illnesses, certain groups of *E. coli* can cause life-threatening diarrhea and severe sequelae, even death (Doyle and Padhye, 1989). All *E. coli* are classed into the family *Enterobacteriaceae* based on their

taxonomic features as gram-negative, asporogenic, straight rods that may be peritrichously flagellated or nonmotile (Ørskov and Ørskov, 1984). The organism is a facultative anaerobe capable of using simple sugars and minimal base media for its growth. Most *E. coli* are able to grow at incubation temperatures of 44-46°C except for *E. coli* O157:H7 which grows more favorably at 30-42°C (Doyle and Padhye, 1992). The organism is able to grow in the presence of 6.5% sodium chloride or at an a_w of 0.95 and grows in the pH range of 4.4-9.0, with the optimum at a pH of 6.0-7.0 (Bacon and Sofos, 2003; Glass et al., 1992).

Escherichia coli are classified on the basis of antigenic differences (serotyping) and virulence factors (virotyping) (Salyers and Whitt, 1994). Two surface components of the *E. coli* bacterium are the primary basis for the serological classification system: (a) the O antigen of the lipopolysaccharide (LPS, O); and, (b) the flagella (H). The serogroup of the strain is identified by the O antigen while the H antigen identifies its serotype (Salyers and Whitt, 1994). The basis for the virotyping system is structured on the characteristics of the bacterium including patterns of attachment to host cells, effects of attachment on host cells, production of toxins and invasiveness (Nataro and Kaper, 1998). It is very useful to use both serotyping and virotyping to classify *E. coli* strains, however, absolute categorization cannot always be made on the basis of these two systems (Nataro and Kaper, 1998). A further difficulty with classification is that the genetic codes for many of the virulence traits reside on plasmids, phages or pathogenicity islands and, thus, the capabilities of a pathogen can be passed from one *E. coli* to another as has been documented by Hedberg et al. (1997). Only a few strains of *E. coli* are pathogenic, and these strains are classified into seven categories (Tauxe, 1997) namely:

enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EaggEC), uropathogenic *E. coli* (UPEC) and neonatal meningitis *E. coli* (NMEC).

B.I. Pathogenic *E. coli* groups

B.I.a. Enteropathogenic *E. coli* (EPEC)

Since it was established that certain serologically distinguishable types of *E. coli* could cause infantile gastroenteritis and serotyping enabled the association of certain serotypes more frequently with this illness, these serotypes became known as EPEC (China et al., 1996; Giammanco et al., 1996). Enteropathogenic *E. coli* are known to adhere to the intestinal mucosa and produce the characteristic "attaching and effacing" lesion in the brush border microvillus membrane causing extensive rearrangement of host cell actin (Beaudry et al., 1996; Knutton, 1995). Much like other *E. coli*, transmission of EPEC occurs via fecal-oral route with contaminated hands or foods (Giammanco et al., 1996). These organisms are associated with adult and infantile diarrhea but are no longer as important a cause of diarrhea in developed countries as they were in the past (Doyle and Padhye, 1989).

B.I.b. Enteroinvasive *E. coli* (EIEC)

Enteroinvasive *E. coli* strains are biochemically, genetically and pathogenically closely related to *Shigella* spp., however, they do not produce Shiga toxin (Acheson and Keusch, 1995). Enteroinvasive *E. coli* are characterized by their positive reaction in Sereny test (a characteristic they share with *Shigella* spp.), in which strains are tested for their ability to cause keratoconjunctivitis in guinea pig eyes (Ephros et al., 1996). Many of the EIEC

strains are non-motile and anaerogenic (Acheson and Keusch, 1995). Recently, it was demonstrated by DNA probes for the invasiveness plasmid that both EIEC and *Shigella* strains produced identical plasmids (Nataro et al., 1995). These 120-140 mDa plasmids, the invasiveness plasmids, encode all the genes necessary for the virulence of EIEC (Nataro et al., 1995). These strains comprise only a small proportion of the diarrheagenic organisms found in non-tropical countries; however, they are still an important cause of morbidity and mortality, particularly in the warmer times of the year. They are an important cause of dysentery-like diarrhea in tropical countries (Doyle and Padhye, 1989). Enteroinvasive *E. coli* infections usually result in watery diarrhea, which is sometimes indistinguishable from the secretory diarrhea associated with ETEC infection (Acheson and Keusch, 1995).

B.I.c. Enterotoxigenic *E. coli* (ETEC)

Enterotoxigenic *E. coli* (ETEC) strains have a strong resemblance to *Vibrio cholerae* in that they adhere to mucosa of the small intestine via surface fimbriae (type 1 pili and colonization factor antigens) and do not produce symptoms by invading the mucosa but by producing one or two enterotoxins (Catani et al., 1996). It was discovered that these organisms produce an enterotoxin similar to cholera toxin and have been associated with conditions known as "Non-Vibrio cholerae cholera-like diarrhea" (Willshaw et al., 1995). These organisms are able to produce one or both of two enterotoxins: a heat stable enterotoxin (ST) and a heat labile enterotoxin (LT) (Wai et al., 1995; Willshaw et al., 1995). The ST is able to survive boiling for 30 min, while the LT is destroyed at this temperature-time combination. In addition, the LT response is more sensitive to low or acid pH while the ST is not (Wai et al., 1995; Willshaw et al., 1995). Most ETEC isolated

from humans produce colonization factor antigens which are human-specific fimbrial antigens (Vasquez et al., 1996). The enterotoxins from ETEC act on intestinal mucosal cells to cause diarrhea and are responsible for traveler's diarrhea and severe, even fatal, diarrhea in infants in developing countries (Roels et al., 1998).

B.I.d. Enterohemorrhagic *E. coli* (EHEC)

Enterohemorrhagic *E. coli* are responsible for causing illness, including hemolytic uremic syndrome (HUS), which is similar to that caused by *Shigella dysenteriae* in the young and elderly (Doyle et al., 1997). The EHEC are also similar to EPEC except EHEC produces one or two Shiga toxins (Griffin, 1995). Humans and most warm-blooded animals carry harmless *E. coli* in their gastrointestinal tract and they are even present on most uncooked foods and on fomites in the environment (Blanco et al., 2001; Bolton et al., 1996). However, many of these *E. coli* harbor genes for one or more of the virulence factors known to be associated with EHEC (Ebel et al., 1996). The factors include the ability to produce Shiga toxin(s), adherence factor(s), enterohemolysin or the somatic antigen which characterizes many EHEC serogroups like O111 or O157 (Ebel et al., 1996; McKee and O'Brien, 1996). However, it appears that unless an *E. coli* cell possesses sufficient quantities of genes associated with the virulence factors, it will not result in disease (Ebel et al., 1996). Another factor of great significance is the size of the infectious dose, which is incredibly small in comparison with that of most other foodborne pathogens (as low as two bacteria per 25 g of food) (Doyle et al., 1997). Although many serotypes of *E. coli* belong to the EHEC group, *E. coli* O157:H7 predominates as a foodborne pathogen (Doyle et al., 1997). Cattle are considered to be the major reservoirs of *E. coli* O157:H7 followed by other ruminants and, although not

common, it has been isolated from dogs, horses and birds (Hancock et al., 1998). *Escherichia coli* O157:H7 (classed by its somatic O and flagellar H antigens) was first identified as a human pathogen following two hemorrhagic colitis outbreaks in the U.S. in 1982 involving undercooked hamburger patties (Riley et al., 1983). Subsequent to these outbreaks and some other less publicized ones in the 1980s, a highly publicized outbreak, involving undercooking of hamburger patties contaminated with *E. coli* O157:H7, occurred with much more extensive consequences, resulting in hundreds of illnesses and the death of four children (Bell et al., 1994). An estimated 62,000 cases of symptomatic *E. coli* O157:H7 infections occur annually in the U.S. due to foodborne exposure, resulting in approximately 1,800 hospitalizations and 52 deaths (Mead et al., 1999). According to the Economic Research Service, the estimated cost of *E. coli* O157 stemming from medical costs, productivity losses and premature deaths annually in the U.S. totals $0.7 billion, while that incurred by *E. coli*, non-O157 STEC is $0.3 billion (available at http://www.ers.usda.gov/briefing/foodbornedisease/). Furthermore, the cost of *E. coli* O157:H7 from an industrial standpoint has accounted for approximately $2.7 billion during the past 10 years (Kay, 2003). Surveillance data indicate that the highest incidence of illness from *E. coli* O157:H7 occurs in children under 5 years of age (Mead et al., 1999). *Escherichia coli* O157:H7 can grow at temperatures ranging from 7 to 46°C, but have an optimum between 30 and 42°C (Bacon and Sofos, 2003). *Escherichia coli* O157:H7 require a water activity (a_w) of at least 0.95 and are able to grow in the presence of 6.5% sodium chloride. Although they grow best at pH 6.0 to 7.0, *E. coli* O157:H7 can grow at pH 4.4 to 9.0 and unlike most foodborne pathogens, they are tolerant to acidic environments and have demonstrated resistance to acetic, citric and lactic acids. Illness

resulting from *E. coli* O157:H7 is usually associated with fecal-oral transmission by contaminated hands, water or food of animal origin with the majority of outbreaks involving the consumption of undercooked bovine meat. Between 1993 and 1998, most (72%) of the *E. coli* O157:H7 outbreaks were foodborne and of the foods implicated in the outbreaks, beef was responsible for most (45%) of the cases and 90% of the time the beef product was ground. Following ingestion ($>10^1$ cells) and a 3 to 9 day incubation period, *E. coli* O157:H7 can cause a wide range of symptoms including mild diarrhea or severe bloody diarrhea (hemorrhagic colitis), HUS and thrombotic thrombocytopenic purpura (TTP) (Bacon and Sofos, 2003).

B.I.e. Enteroaggregative *E. coli* (EAggEC)

Escherichia coli with different types of adherence patterns are described as Localized Adherent *E. coli* (LAEC), belonging to certain serotypes which form adherent microcolonies on HEp-2 cells and are associated with non-bloody acute diarrhea in children, Diffusely Adhering *E. coli* (DAEC) and Enteroaggregative *E. coli* (EAggEC) as they demonstrate enteroaggregative adherence (Germani et al., 1996; Hicks et al., 1996). Enteroaggregative *E. coli* align themselves in parallel rows to tissue and this aggregation is described as "stacked brick-like" (Hicks et al., 1996). The EAggEC are mainly associated with persistent childhood diarrhea in developing countries and can cause episodes that last more than 14 d (Cobeljic et al., 1996). These strains do not produce enterotoxins LT or ST but do appear to produce a heat-labile toxin antigenically related to hemolysin but not hemolytic and a plasmid encoded heat stable toxin (EAST1) unrelated to enterotoxin ST (Hicks et al., 1996; Wai et al., 1996).

B.I.f. Uropathogenic *E. coli* (UPEC)

Escherichia coli is the bacterium most commonly responsible for urinary tract infections (UTI) (Yamamoto et al., 1996). While the same serotypes are often found in the feces and the urine, it is apparent that UPEC possesses virulence factors which enhance their ability to cause UTI (Yamamoto et al., 1996). A very limited number of O groups (O1, O2, O4, O6, O7, O18 and O75) and a small number of K antigens (K1, K2, K3, K5, K12 and K13) are associated with UTI among the UPEC (Tay et al., 1996; Yamamoto et al., 1996). One of the more important virulence factors is a certain type of pili (fimbriae), known as the p-pili, as they bind specifically to the P-antigen (one of the blood grouping antigens) (Garcia-Martinez et al., 1996). P-piliated *E. coli* binds with significantly greater efficacy to the uroepithelial cells of persons with the P or P2 phenotype (Garcia-Martinez et al., 1996). The production of α-hemolysin is commonly associated with UPEC as well as the production of aerobactin (Swenson et al., 1996; Tay et al., 1996).

B.I.g. Neonatal Meningitis *E. coli* (NMEC)

Neonatal meningitis is reported in about 1 in 2500 live births and it is estimated that up to 80% of the cases are due to infections caused by *E. coli* (Doyle et al., 1997). An important occurrence is that approximately 80% of the *E. coli* isolates implicated in neonatal meningitis possess the K1 capsular antigen (Achtman et al., 1983; Silver et al., 1980). The K1 antigen is a 2.8 α-linked homopolymer of N-acetylneuraminic or sialic acid and is chemically and immunologically identical to the β-acidic polysaccharide of *Neisseria meningitidis* (Achtman et al., 1983). The K1 antigen masks the underlying structures of the bacterial cell surface and prevents specific antibody responses and the activation of the alternate complement system (Achtman et al., 1983; Silver et al., 1980). Additionally, the K1 antigen is a poor immunogen (possibly a result of resembling

extracellular matrix proteins) and reduces the serum sensitivity of *E. coli* (Achtman et al., 1983). Similar serotypes are isolated from meningitis cases found in the maternal feces, indicating these as a potential source of infection occurring at birth (Achtman et al., 1983).

B.II. Historical perspective and origins of *E. coli* O157:H7

Escherichia coli O157:H7 was first identified as a human pathogen following two hemorrhagic colitis outbreaks in the U.S. in 1982 involving undercooked hamburger patties (Riley et al., 1983). The first outbreak occurred in Oregon (26 cases and 19 hospitilizations) and, three months later, the second followed in Michigan (21 cases and 14 hospitilizations). Subsequent to this outbreak, another similar outbreak involving undercooking of hamburger patties contaminated with *E. coli* O157:H7 occurred with much more extensive consequences, resulting in hundreds of illnesses and the death of four children (Bell et al., 1994). Most *E. coli* strains as previously mentioned are harmless natural colonizers of the gastrointestinal tract of humans and other warm-blooded animals, however, pathogenic *E. coli* strains exist and are associated with syndromes of diarrheal illness (Doyle et al., 1997). Those strains that produce Shiga-like toxins (SLT) or verotoxins (VT) and are associated with hemorrhagic colitis and hemolytic uremic syndrome in humans are regarded as EHEC (Kaper, 1994). The predominant EHEC serotype associated with foodborne illness is *E. coli* O157:H7, however, non-O157 serotypes, especially O26, O111, O128 and O103, have been implicated in sporadic cases of gastrointestinal illness and possess the potential for an outbreak (Johnson et al., 1996; Bettelheim, 2000).

It is proposed that *E. coli* O157:H7 evolved from *E. coli* O55:H7, an enteropathogenic strain of *E. coli* associated with infant diarrhea (Whittam et al., 1993; Mead and Griffin, 1998). *Escherichia coli* O157:H7, much like other EHEC, can adhere to epithelial cells and produce attaching and effacing (AE) lesions. It is proposed that *E. coli* O55:H7, which possesses a mechanism for adherence to intestinal cells, acquired SLT genes from *Shigella* and the O157 antigen through horizontal gene transfer and recombination to constitute *E. coli* O157:H7 (Mead and Griffin, 1998). With the complete sequencing of the *E. coli* O157:H7 genome, *E. coli* K-12 MG1655 (K-12) has been established as its closest relative (Perna et al., 2001). From the distance of genetic differences between the two strains, it is estimated that *E. coli* O157:H7 and *E. coli* K-12 shared a common ancestor approximately 4.5 million years ago (Perna et al., 2001).

C. Microbial contamination of beef carcasses at slaughter

It can be assumed that some level of microbial contamination of beef carcasses will occur at slaughter (Gill et al., 1998). Jordan (1998) proposed the following trend of contamination from on-farm to carcasses: *E. coli* O157:H7 contamination frequency should be minimum on the farm and should increase during transportation to slaughter plants and in the holding period prior to slaughter (due to cross-infection when animals from different sources are mixed) and finally, depending on the hygiene and accordance with Good Manufacturing Practices (GMPs) and Sanitation Standard Operating Procedures (SSOPs), the contamination should be reduced significantly after evisceration (as the gut is removed from the carcass). The probability of *E. coli* O157:H7

contamination and the level of contamination for incoming live animals are variable depending on the animal and the environmental factors described in the previous section. The hide and gastrointestinal (GI) tract are likely to be principal sources of bacterial contamination during slaughter, however, contamination can occur via many other sources including aerosols generated in the slaughter and dressing process (Biss and Hathaway, 1996; Gill et al., 1998). Cross-contamination may also be introduced via worker's clothes, hands or equipment, other carcasses, plant equipment, plant environment (i.e., water or air) and accidental spillage of body fluids during skinning and evisceration (Lundeen, 2000).

Contamination of the carcass can occur in different forms (Gannon, 1999):

(a) accidental, random, visible contamination of carcasses (fecal contamination of carcasses following knife puncture);

(b) systematic visible contamination (defined locations of the carcass with higher demerit scores than others [Jericho et al., 1993, 1994]);

(c) accidental or random invisible contamination from aerosols, dusts and sprays; and,

(d) systematic invisible contamination from contact, aerosols, dusts or sprays (carcasses have areas with characteristically high and low bacterial populations [Jericho et al., 1993; Gill et al., 1996]).

It is thought that much of bacterial contamination on meat occurs during hide removal (Gill et al., 1998). These organisms can originate directly from the feces in the anal region which will explain why the rump region is one of the spots or locations with the highest *E. coli* and coliform counts (Gill et al., 1996, 1998). The neck, brisket and legs are also areas of high contamination with *E. coli* (Bell, 1997). This occurrence can be

related to contact between these areas of the carcass and the outside surface of the hide during the skinning process. It was shown (Bell, 1997) that points on the carcass touched by the inside of the hide had *E. coli* counts <2.0 log CFU/cm^2, while sites in direct contact with the outer surface of the hide had *E. coli* counts >2.0 log CFU/cm^2.

C.I. Microbial contamination of beef carcasses during slaughter

In 1996, the USDA-FSIS implemented new Meat and Poultry Inspection Regulations in the U.S. and part of the regulations required microbiological performance criteria for *E. coli* (USDA-FSIS, 1996). The microbiological performance criteria and standards were based on data from national baseline carcass contamination studies conducted by FSIS which tested carcasses of steers and heifers in 1992 to 1993 and carcasses of cows and bulls in 1993 to 1994 (Sofos et al., 1999a). In the first study conducted by FSIS, only 4 of 2,081 (0.2%) randomly sampled post-processing beef carcasses were contaminated with EHEC O157 (USDA-FSIS, 1994). It is possible that at this point in time, fecal, hide and carcass prevalence during processing may have been underestimated due to the lack of sensitive and specific methods for the isolation of EHEC O157 (Elder et al., 2000). A study (Sofos et al., 1999a) was conducted to determine microbiological loads of beef carcasses at different stages during the slaughtering to chilling process in seven (four steer/heifer and three cow/bull) plants during the periods of November through January ("wet" season) and May through June ("dry" season). The results showed that depending on plant and season, aerobic plate counts (APC), total coliform counts (TCC) and *E. coli* counts (ECC) were ≤4.0, ≤2.0 and ≤1.0 log CFU/cm^2 in 46.7 to 93.3, 50.0 to 100.0, and 74.7 to 100.0% of the samples, respectively (Sofos et al., 1999a). The total coliform

counts (TCC) were found to exceed 3.0 log CFU/cm^2 in 2.5% (wet season) and 1.5% (dry season) of the samples (Sofos et al., 1999a). The ECC were found to exceed 2.0 log CFU/cm^2 in 8.7%, 0.3% and 1.5% of the preevisceration, final carcass-washing and 24-h carcass-chilling samples, respectively, during the wet season while the corresponding numbers during the dry season were 3.5%, 2.2% and 3.0%, respectively (Sofos et al., 1999a).

The origins and rate at which EHEC O157 carcass contamination occurs has not been well established. To allow confident conclusions and the effectiveness of programs to control foodborne pathogens (i.e., HACCP), adequate microbiological data are required as to the prevalence of EHEC O157 on carcasses (Elder et al., 2000). Studies have been conducted to determine the prevalence of EHEC O157 in cattle feces, hides and carcasses during slaughter processes in the U.S. (Bacon et al., 2000a; Elder et al., 2000). Bacon et al. (2000a) showed that 3.6% (of hide samples, 0.4% of side samples prior to carcass washing and 0% of side samples after carcass decontamination interventions were positive for *E. coli* O157:H7 on samples taken from 12 packing plants. In addition, samples of beef trimmings tested in 6 of the 12 packing plants revealed that 0% were positive for *E. coli* O157:H7. Across the complete population, the data showed that 1 of 28 cattle entering the plant had confirmed positives for *E. coli* O157:H7 on their hide (total of 2245 sampled) and 1 of 227 carcass side samples tested (total of 2248 sampled) prior to carcass washing had confirmed positive tests for the pathogen; however, none of the carcass side samples tested (total of 2248 sampled) after application of carcass decontamination interventions was positive and none of the beef trimming samples tested was positive (Bacon et al., 2000a). A study was conducted by Elder et al.

(2000) to estimate the frequency of EHEC O157 in feces and on hides within groups of fed cattle from single sources (lots) presented for slaughter in the U.S. Of 29 lots sampled, the prevalence of EHEC O157 positives in fecal samples was 72% and 38% in hide samples. Overall, EHEC O157 prevalence in feces and on hides was 28% (91 of 327) and 11% (38 of 355). In addition, 27 of 30 lots (90%) were positive for at least one carcass within a lot (Elder et al., 2000). Of 29 lots sampled, 26 (87%), 17 (57%) and 5 (17%) were positive for EHEC O157 preevisceration, post-evisceration and post-processing, respectively. Carcass samples were taken at three points during processing: preevisceration, postevisceration before antimicrobial intervention, and postprocessing after carcasses entered the cooler. In the three post-processing sample points the prevalence of EHEC O157 was 43% (148 of 341), 18% (59 of 332), and 2% (6 of 330), respectively. In establishing a relationship between fecal or hide prevalence and carcass contamination, it was observed that 19 of 21 lots (90%) with positive fecal samples also had positive preevisceration samples and of the remaining two fecal-positive lots, one had a positive post-evisceration sample (Elder et al., 2000). It was established that all 11 lots that had positive hide samples also had positive preevsiceration samples (only 42% of all positive preevisceration samples). In summary, the data show that almost half (45.5%) of the carcasses tested positive for EHEC O157 (particularly in the posterior region of the carcass) and there was little or no association between hide prevalence and carcass contamination (Elder et al., 2000). In contrast, work done by Barkocy-Gallagher et al. (2003) demonstrated that hides were a significant and major source of beef carcass contamination. Ransom et al. (2002) found that 36.7, 13.3, and 0.0% of lots contained at least one *E. coli* O157:H7-positive hide, fecal, and carcass sample, respectively.

In modern high-speed packing plants, it is typical that over 300 head are killed and processed every hour, allowing only a 12 s or 9 s gap between animals operating at 300 and 400 head/hour, respectively (Gannon, 1999). Carcasses from modern high-speed plants are no dirtier (visual contamination), nor do they have higher microbial contamination than facilities that are smaller or operate at a lower pace (Jericho et al., 1994). However, the fast line-speeds in the plants do place restrictions on decontamination procedures, especially manual decontamination (i.e., knife-trimming, steam-vacuuming, etc.). It is vital that every step of the slaughter process be considered when evaluating the safety of carcasses, to identify which steps are more likely to introduce contamination and verify that decontamination is operating successfully. The following is a sequential outline of a generic beef slaughter model adapted from various sources (Gracey and Collins, 1992; Gill et al., 1996; USDA-FSIS, 1999): receiving, stunning and shackling of cattle; sticking, bleeding, hide opening and shank removal; free and bag the bung, rod and tie the weasand, skinning, hide removal and head removal; decontamination to remove visible fecal contamination; evisceration, splitting and trimming; final interventions, water washing, steam-pasteurization organic and acid application; chilling and storage of carcass sides; and finally, fabrication of sides into primals and sub-primals.

D. Decontamination of fresh beef

The increasing prevalence of *E. coli* O157:H7 on animals prior to and after slaughter in recent years necessitates the employment of interventions for their control (Sofos et al.,

2003). In an effort to promote control of the incidence of this pathogen in meat, the USDA-FSIS has been enforcing a zero tolerance policy for visible soil on carcasses during slaughter and has declared the pathogen an adulterant in fresh ground beef and other non-intact fresh beef cuts (http://www.fsis.usda.gov). Furthermore, to emphasize the importance of the issue of *E. coli* O157:H7 in fresh beef and to take steps to improve the safety of such products, the USDA-FSIS has issued directives, notices and guidances to meat operations to consider the pathogen as a hazard reasonably likely to occur in fresh beef (http://www.fsis.usda.gov). These regulatory mandates include re-evaluation of HACCP plans and established plant-validated control measures (USDA-FSIS Directive 10,0101.1/February 1, 1998; USDA-FSIS Notice 44-02/November 4, 2002; Proposed USDA-FSIS Directives in Federal Register October 7, 2002/Volume 67, Number 194, Pages 62325-62334; USDA-FSIS Guidance for minimizing the risk of *Escherichia coli* O157:H7 and *Salmonella* in beef slaughter operations; USDA-FSIS Guidance for beef grinders and suppliers of boneless beef and trim products).

In order to enhance the safety of beef, a comprehensive strategy is needed to address hazards in different sectors from "farm-to-fork". The meat industry has been working on intervention methods for microbial decontamination of carcasses and effective risk assessment techniques to better evaluate risks, provision of information dealing with hazard identification and characterization, the occurrence of hazards and ways to control the risks associated with the hazards. It has become an ever increasing challenge to ensure the safety of raw beef products, especially, with the constantly changing food-processing operations, emerging foodborne pathogens and demand by consumers for more varied and convenient foods (affecting processing operations and

control measures). There has been much research performed to evaluate the efficacy of various decontamination treatments, including the use of live animal washings, chemical dehairing, knife-trimming, carcass washing, spraying or steam-vacuuming, steam pasteurization, or multiple decontamination treatments in sequence (AMIF, 2000b; Bacon et al., 2000b; Cutter et al., 1997; Delmore et al., 2000; Dickson et al., 1997; Dorsa et al., 1997; Graves Delmore et al., 1997a; Hardin et al., 1995; Kochevar et al., 1997; Ransom et al., 2003a; Sofos and Smith, 1998; Sofos et al., 1999a). Choice of decontamination techniques by companies/institutions will differ depending on cost, need for decontamination, facilities available, resources availability, and product destination (Sofos and Smith, 1998). To have a better understanding for the antimicrobial interventions applied by the industry, the following is a review of currently used interventions. Although the review mainly deals with harvest/post-harvest interventions, it is worth mentioning that preharvest interventions (i.e., management factors affecting pathogens in live animals, feed strategies to combat pathogens, use of probiotics, etc.) lay the ground stone for the success of decontamination procedures as they may be responsible for controlling the prevalence of *E. coli* O157:H7 on the live animal and subsequently the level of contamination present in the slaughter plant.

D.I. Current decontamination technologies applied in the meat industry in the U.S.

D.I.a. Live animal washing

Live animals are often highly contaminated and usually asymptomatic carriers of pathogenic bacteria, including *E. coli* O157:H7 (Letellier et al., 1999). Live animal washing may help to reduce sources of carcass contamination during subsequent

slaughter, although the efficacy and viability is dependent on the type of animal, climate, availability of facilities, degree of soil, length and pressure of application, etc. Preslaughter washing of sheep has been done in New Zealand (Biss and Hathaway, 1996) and washing of cattle has been practiced in the U.S. (Sofos et al., 1999b). A potential constraint for the washing of cattle in the U.S. is the regulatory guidelines which require that cattle be dry and not dripping when they are slaughtered (Reed, 1996). Up to now, pre-slaughter washing of animals has not been researched sufficiently to draw conclusions regarding efficacy. Byrne et al. (2000) showed that pre-slaughter washing for three minutes did not statistically reduce the number of bacteria transferred from the hide of the cattle to the carcass during slaughter. However, it was found that microbial populations were present on fewer areas of the carcass, and that pre-slaughter washing may be a suitable method of decontaminating animal hides. The importance of presenting clean animals at slaughter is clearly an advantage as it ensures a reduced source of contamination. Nevertheless, poor hygiene, sanitation, and manufacturing practices during slaughter, fabrication, and processing can lead to excessive contamination even when processing clean animals (Bacon et al., 2000b; Graves Delmore et al., 1997a; Sofos and Smith, 1998).

D.I.b. Knife-trimming

Knife-trimming is a process by which visual contamination of beef carcasses is removed by physically cutting/removing visible contaminants from tissue rather that washing it away. FSIS requires proper removal of fecal, ingesta, hair and milk contamination from beef carcasses during slaughter (USDA-FSIS, 1996). Knife-trimming has been shown to be an effective means of removing visual contamination and any accompanying

microbial contamination from beef carcasses (USDA-FSIS, 1995). Research has shown knife-trimming to be effective in reducing ($P < 0.05$) bacterial aerobic plate counts (APC) of beef tissue from inoculated, untreated controls (Gorman et al., 1995; Prasai et al., 1995; Reagan et al., 1996). Kochevar et al. (1997) showed a reduction in bacterial counts achieved after knife-trimming of carcasses.

In the industry, under beef slaughtering conditions, it cannot be assumed that trimming alone will remove the physical and reduce the microbiological contamination from the carcass. Trimming is a variable process affected by the competence of the trimmer, the equipment, and the sanitation (USDA-FSIS, 1995). Bacteria of fecal origin are not necessarily limited to areas of visible contamination and transfer and cross-contamination of pathogens by employees, equipment, and inspections may occur. This suggests that decontamination of the carcass aided by knife-trimming is more effective when followed by another decontamination treatment (Dorsa et al., 1997; Hardin et al., 1995).

D.I.c. Steam vacuuming

"Steam vacuuming" is a process that involves the application of hot water, steam or a combination thereof onto the surface of a carcass and is followed by the uptake of the residual moisture (USDA-FSIS, 1995; Sofos and Smith, 1998). The object of the steam vacuuming process is to initially kill and loosen bacterial contamination on the carcass surface and then remove the residual moisture and associated contamination via vacuum applications as a means for spot decontamination of areas less than 2.54 cm^2 (USDA-FSIS, 1995; Sofos and Smith, 1998). Vacuuming of beef carcasses combined with hot water or steam application was approved by FSIS in April, 1996 as an alternative to

knife-trimming (USDA-FSIS, 1996). Studies have concluded that application of steam vacuuming effectively reduces microbial populations on beef carcass surfaces (Dorsa et al., 1997; Kochevar et al., 1997). According to Phebus et al. (1997), hot water/steam vacuuming was as effective as knife-trimming and steam pasteurization in reducing pathogen populations. In addition, decontamination washings with combinations of treatments other than steam vacuuming were found to be less effective in reducing contamination than combination treatments using steam vacuuming (Phebus et al., 1997).

Steam is sprayed concurrently above and below the vacuum head to continuously sanitize the vacuum head as it comes into contact with the carcass surface, doing so to avoid cross contamination between carcasses (USDA-FSIS, 1996). Steam and water temperatures should exceed 82°C and should be applied at a pressure of 0.34-1.03 bar, while vacuum pressure should be at -0.00903 bar for maximum effectiveness (Kochevar et al., 1997). It should be noted that both knife-trimming and steam vacuuming, irrespective of decontamination efficacies, are only applied to selective locations on the carcass thought to have heavy loads of contamination (Sofos and Smith, 1998).

D.I.d. Steam pasteurization

"Steam pasteurization" is the use of superheated steam, from potable water which is a natural and non-toxic substance used to decontaminate the surface of animal carcass and meat trimmings and is a process involving three phases. In the first phase, carcasses/trimmings are de-watered to remove any pools or residual water protecting bacteria from the potentially lethal effects of condensing steam. This is followed by exposure to nearly saturated steam (96-100°C) in a pressurized chamber for 6-8 s. Lastly, the carcass sides/trimmings are sprayed with warm water (44°C/40 kPa) for surface

chilling before entering the drip cooler (Nutsch et al., 1997). This "steam pasteurization" process has been shown to significantly reduce microbial counts of beef carcasses as compared to the no-steam pasteurization control (Phebus et al., 1997; Nutsch et al., 1997). A study by Nutsch et al. (1998) looked at the effect of steam pasteurization in plants and found there to be approximately 3.5 log CFU/cm^2 reduction in APC in five locations on the surface of carcasses.

D.I.e. Chemical dehairing

The process of chemical dehairing was developed and patented by Bowling and Clayton (1992) and is designed to remove hair, dirt, and fecal contamination from the hides of beef carcasses to reduce the amount of contamination brought into the plant (Bacon et al., 2000b; Graves Delmore et al., 1997b; Schnell et al., 1995). Chemical dehairing is a relatively new technology, which has been shown to play a role in reducing carcass contamination (Schnell et al., 1995; Sofos and Smith, 1998). Chemical dehairing is a four-step process beginning with a pre-rinse of the hides using water. This is followed by two applications of sodium sulfate (10%) for 90 and 60 second exposure times, respectively. The third step is neutralization with water and hydrogen peroxide (3%) rinses. Finally, a water rinse is applied (Bowling and Clayton, 1992). Schnell et al. (1995) found that dehairing animals did not reduce the bacterial load from that of conventionally slaughtered carcasses, but they found that chemically dehaired carcasses had fewer ($P < 0.05$) visible specks, hairs, and total carcass defects prior to final knife-trimming than non dehaired carcasses. However, Graves Delmore et al. (1997b) found that the dehairing process resulted in bacterial injury and it was demonstrated (Castillo et al., 1998) on hide samples in laboratory settings that the process resulted in significant

reductions of *E. coli* O157:H7. Thus, it is possible that the number of interventions used to minimize visual contamination could be lessened and the amount of knife-trimming required to achieve zero tolerance specifications, could be reduced (Schnell et al., 1995). A recent study (Nou et al., 2003) indicated that chemical dehairing of cattle hides is an effective intervention to reduce the incidence of hide-to-carcass contamination with pathogens.

D.I.f. Spray-washing

The spray-washing of carcasses with water of different temperatures/pressures and rinses of organic acids has been researched extensively (Berry and Cutter, 2000; Dorsa et al., 1997; Hardin et al., 1995; Prasai et al., 1995; Ransom et al., 2003a; Reagan et al., 1996; Sofos and Smith, 1998). It has been shown that although washing has the potential to spread or dilute contamination from one area to another on the carcass, there are significant reductions in contamination attributed to carcass washing (Castillo et al., 1998; Dickson and Anderson, 1992; Prasai et al., 1995; Reagan et al., 1996). The spray-washing process may owe its efficacy to the fact that removal of contamination occurs soon after hide removal and thus has a rinsing effect on bacteria associated with the surface but not necessarily attached (or attached in minimal numbers) (Sofos and Smith, 1998). It has been shown that reducing microbiological contamination on carcass tissue is enhanced by increased water temperature (Cabedo et al., 1996; Gorman et al., 1995; Graves Delmore et al., 1997a; Kochevar et al., 1997; Siragusa, 1995; Sofos and Smith, 1998). Thermal decontamination uses water of at least 73.8°C (165°C) and is applied to the carcass surfaces in a spray application (Barkate et al., 1993). Exposure of animal

tissues to hot water (>70°C) was found to result in 1.0-3.0 log CFU/cm^2 reductions of *E. coli* O157:H7 (Castillo et al., 1998).

In recent years, several organic acids have been used in an attempt to reduce the microbial contamination of animal carcasses and include the use of acetic, ascorbic, citric, formic, lactic and propionic acid. Spray-washing with organic acids such as acetic acid, lactic acid, and citric acid has yielded mixed results. The efficacy of the treatment relies on the undissociated form of the acid inhibiting microbial growth (Hardin et al., 1995). The level of application for acetic, citric and lactic acids has been approved by the FSIS at concentrations between 1.5-2.5% (USDA-FSIS, 1996). Acetic and lactic acids are the most commonly used sanitizers and both are generally recognized as safe (GRAS) compounds. It is estimated that carcass misting using these organic acids is applied in approximately 15% of beef processing plants (AMIF, 2000a). The conclusion drawn is that the overall efficacy of the organic acids as decontamination treatments is dependent on the temperature of application, pressure of application, type of tissue, type of organism, sensitivity of organism, concentration of acid, exposure time, and the point at which application is done in the plant (Dickson and Anderson, 1992; Hardin et al., 1995). Ransom et al. (2003a) found that lactic acid (2%, 55°C) was more effective than acetic acid (2%, 55°C) in reducing *E. coli* O157:H7 on the surface of lean beef tissue. This result was also confirmed by Stopforth et al. (2003a,b), who found death of *E. coli* O157:H7 was more pronounced in lactic acid washings as compared with acetic acid washings. The greater effectiveness of lactic acid compared with acetic in inactivating bacterial populations may be due to its lower pH in solution, a different mode of action or possibly the faster dissipation of acetic acid compared to lactic acid in the washings over

time (Stopforth et al., 2003a,b). Especially for *E. coli* O157:H7, its increased resistance to acetate-based meat decontamination treatments may be associated with its inherent ability to resist the toxicity of acetic acid by increasing its intracellular pH (Diez-Gonzalez and Russell, 1997). Because acetic acid is more capable than lactic acid of penetrating the cellular membrane in undissociated form to eventually acidify the cytoplasm (Diez-Gonzalez and Russell, 1997), the natural resistance of *E. coli* O157:H7 to acetic acid may enhance the development of acetate-stressed strains.

D.I.g. Multiple decontamination treatments

The use of two or more of the previously mentioned individual interventions used together may have an additive or synergistic effect on reducing microbial contamination (Sofos et al., 1999b). The use of multiple decontamination treatments as sequential hurdles may result in the disruption of the homeostatic state of microorganisms from several directions (pH, a_w, temperature, atmospheric, chemical), presenting the microorganisms with many stresses to which they must adapt in order to survive (Leistner, 1995).

Dorsa et al. (1997) showed that spray-washing with water of different temperatures, washing combined with steam applications, washing and vacuuming applications, all effectively reduce bacterial contamination as compared to individual treatments. Phebus et al. (1997) found that "steam-pasteurization", knife-trimming, warm water washing, hot water/steam vacuuming, organic acid rinses when used in combinations result in a greater reduction of bacteria than individual treatments. ConAgra, Inc. devised a "Chain of Beef Safety™" system in which they applied sequentially the following decontamination treatments: (a) steam-vacuuming, (b) preevisceration washing with 32°C water and

rinsing with 2% acetic acid in 49°C water, (c) thermal pasteurization with 74°C water and (d) final-carcass washing with 32°C water and rinsing with 2% acetic or lactic acid in 51.7°C water (Graves Delmore et al., 1998; Smith, 2000). The conclusion reached by several researchers is consistent in showing that sequential combinations of decontamination treatments are more effective than individual treatments in reducing bacterial contamination (Bacon et al., 2000b; Dorsa et al., 1997; Graves Delmore et al., 1998; Hardin et al., 1995; Phebus et al., 1997).

D.II. Novel chemicals proposed for decontamination and other approved solutions

In addition to the organic acids previously mentioned, other chemical interventions have been proposed for use in decontamination of red meat. Some of the proposed chemicals have been approved for use while others are still being tested or awaiting approval. Intervention technologies that have received increased intention recently include: ozone, peroxyacetic acid, cetylpyridinium chloride, sodium chloride, acidified sodium chlorite, trisodium phosphate and lactoferrin/lactoferricin B/activated lactoferrin.

D.II.a. Ozone

Ozone (O_3) is a water-soluble and unstable gas that is commercially produced by passing electric charges or ionizing radiation through air or oxygen. Ozone is a powerful compound used to combat bacteria due to its strong oxidizing properties and has been shown to be effective in reducing bacteria including *E. coli* O157:H7 on meat (Cabedo et al., 1996; Gorman et al., 1995; Reagan et al., 1996). This compound has been approved for use in fresh meat and poultry products (21 CFR 173.368); however, there is limited application in the meat industry due to the fact that high concentrations are required for

efficacy and may pose an unacceptable safety risk to personnel (CDC, 2002 – Available at: http://www.cdc.gov/niosh/docs/stateprof/pdfs/nebraska.pdf).

D.II.b. Peroxyacetic acid and hydrogen peroxide

Peroxyacetic acid (PAA) and hydrogen peroxide are strong oxidizing agents that can kill organisms rapidly, however, uses as a direct food additive is limited due to their bleaching effects and oxidizing effects on food constituents (Farrell et al., 1998; Ransom et al., 2003a). Hydrogen peroxide has GRAS status (21 CFR 184.1366), and is approved by the FDA for packaging and surface sterilization in the food industry (21 CFR 178.1005). Currently, a PAA-based process (21 CFR 173.370) is approved (USDA-FDA, 2003) for washing, rinsing, cooling, or for otherwise processing fresh beef carcasses. This approved process involves use of a mixture of compounds with no more than 0.022% peroxyacetic acid and 0.0075% hydrogen peroxide delivered at a maximum pressure of 250 psi and temperature of 50°C (Inspexx 200™, Ecolab, St. Paul, MN). A study conducted by Gill and Landers (2003) evaluated the effect of the commercial PAA-based product applied in a sequence of carcass decontaminations at a packing plant and found that total aerobic counts were reduced from 3.07 log CFU/cm^2, to 1.25 log CFU/cm^2.

D.II.c. Cetylpyridinium chloride

Cetylpyridinium chloride (CPC) is a water soluble, colorless and odorless compound with a neutral pH (Cutter et al., 2000). The compound is an active ingredient in mouthwashes due its ability to reduce bacterial attachment on tooth surfaces and has been shown to have good antimicrobial activity (Breen et al., 1995; Cutter et al., 2000; Kim and Slavik, 1996). Recent research has shown that CPC was able to reduce *E. coli* O157:H7 inoculated (5.8 log CFU/cm^2) onto beef carcass surfaces by 2.1-4.8 log CFU/cm^2 and was

found to be comparatively more effective than lactic acid (Ransom et al., 2003a). Lim and Mustapha (2004) reported similar reductions in pathogens although; CPC was most effective against *L. monocytogenes* and *S. aureus* compared with *E. coli* O157:H7. This result is most likely due to the fact that the hydrophobic molecules of CPC can readily interact with the surface of gram-positive microorganisms which are more hydrophobic than gram-negative surfaces (Kim and Slavik, 1996; Maeda et al., 1996).

D.II.d. Sodium chloride

Sodium chloride (NaCl) is a GRAS antimicrobial and has been shown to inhibit the growth of a number of microorganisms (Jay, 2000; Sofos, 1984; Stein, 2000). Foodborne pathogenic bacteria are generally inhibited at a salt concentration of 13% (w/v) or a a_w = 0.92 (Jay, 2000). Sodium chloride has not been used to decontaminate fresh beef carcasses but there is sufficient rationale for its use as a preservative in fermented meat products (Stein, 2000).

D.II.e. Acidified sodium chlorite

Chlorite, stabilized in acids (i.e., acidified sodium chlorite) has been shown to be an excellent solution for carcass decontamination resulting from a combination of anti-microbial activities due to the acidic pH of the spray and the antimicrobial properties of chlorine (Castillo et al., 1999; Sofos and Smith, 1998). Acidified sodium chlorite is formed from the reaction of sodium chlorite with an organic acid (usually citric acid) and is a powerful oxidant that is approved as a direct food additive to be used for decontamination of poultry and red meat carcasses (USDA-FDA, 1998). Acidified sodium chlorite has been approved for application on poultry carcasses and parts; meat carcasses, parts, and organs; processed, comminuted, or formed meat food products at a

concentration of 500 to 1200 ppm in combination with any GRAS acid at a level sufficient to achieve a pH of 2.3 to 2.9 in accordance with 21 CFR 173.325 (FSIS Directive 7120.1, Amendment 1 – Available at: http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7120.1Amend1.htm). Castillo et al. (1999) showed that *E. coli* O157:H7 inoculated (5.5 log CFU/cm^2) on carcass surfaces is reduced by 3.8 log CFU/cm^2 after treatment with citric-activated acidified sodium chlorite. Ransom et al. (2003a) showed that *E. coli* O157:H7 inoculated (5.8 log CFU/cm^2) on beef carcass tissue was reduced by 1.8 log CFU/cm^2 with lactic-activated acidified sodium chlorite. The order of effectiveness of ASC on pathogens attached to beef surfaces was shown to decrease in the order *E. coli* O157:H7 > *L. monocytogenes* > *S. aureus* (Lim and Mustapha, 2004).

D.II.f. Trisodium phosphate

Trisodium phosphate (TSP) is an antimicrobial treatment that was approved for application on beef and poultry carcasses in the U.S. for application before chilling (24-48 h before fabrication) (USDA-FSIS, 1996). Research (Cabedo et. al., 1996; Gorman et al., 1995, 1997) has shown that spray-washing with TSP reduced contamination of beef brisket tissue with both pathogenic and nonpathogenic bacteria. Graves Delmore et al. (1998) showed that spraying with TSP resulted in 2.0 log CFU/cm^2 reduction in bacterial populations on beef carcass surfaces. Recent amendments have limited the use of trisodium phosphate to raw unchilled poultry carcasses and giblets (FSIS Directive 7120.1, Amendment 1 – Available at: http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7120.1Amend1.htm). Spraying or

dipping of giblets is regulated for up to 30 s while carcasses are regulated by spraying or dipping in a temperature range of 18.3 to 29.4°C for up to 15 s (per 21 CFR 182.1778).

D.II.g. Lactoferrin, lactoferricin B and activated lactoferrin

Lactoferrin (LF), also termed lactotransferrin, or lactosiderophilin (Nagasawa et al., 1972) is an iron-binding, bioactive glycoprotein of the transferin family that contributes to the control of iron in biological fluids. The functional properties, including antimicrobial activity, of LF are mainly associated with its ability to bind two Fe^{3+} in combination with two CO_3^{2-} ions. It is found mainly in milk, the mucosal surfaces (e.g., intestinal epithelial cells) and exocrine secretions of mammals such as saliva, tears, and seminal fluid, as well as in the secondary granules (vesicles) of polymorphonuclear neutrophills or lymphocytes (Levy, 1996; Lönnerdal and Iyer, 1995; Naidu, 2000; Van der Strate et al., 2001). Lactoferrin possesses antioxidant properties and has a broad antimicrobial spectrum including antibacterial, antifungal, antiprotozoal, antiviral, and antitumor properties (Naidu, 2000). Lactoferricin (LFcin) is an active peptide which is derived from peptic hydrolysis of human and bovine LF close to the N-terminus (Jones et al., 1994; Tomita et al., 1994). It exhibits marked antimicrobial effects, which in the majority of studies has been found stronger than those of LF. Activated LF (ALF) is an immobilized form of LF on edible substrates and has been presented as a deterrent to pathogenic bacteria that may be present on meat surfaces (Naidu et al., 2003). Specifically, ALF is presented to act as a blocking agent, which interferes with microbial adhesion/colonization, detaches live or dead microorganisms from biological surfaces, inhibits microbial growth/multiplication, and neutralizes the activity of endotoxins (Naidu, 2002). It is considered a GRAS compound by the Food and Drug Administration

(21 CFR.170.36[f]), since it is a derivative of the naturally occurring LF in milk, which in turn is isolated from cheese-whey and skim milk of bovine origin. Use of ALF on fresh beef was recently approved by the USDA to assist in controlling bacterial contamination during processing (Naidu et al., 2003).

E. Chilling beef carcasses to reduce or minimize microbial contamination

E.I. Importance of carcass chilling

The intact tissues of healthy animals are essentially sterile; however, in the conversion of live animals into meat, it can be expected that some level of microbial contamination of carcasses will occur at slaughter (Chung et al., 1989; Gill et al., 1998). During slaughter, carcasses may be cross-contaminated via workers' clothes, hands or equipment, other carcasses, plant equipment, plant environment (i.e., water or air) and accidental spillage of body fluids during skinning and evisceration (Gill et al., 1998; Lundeen, 2000). Carcass contamination occurs during processing despite the efforts by the meat industry to incorporate control strategies such as animal washing prior to slaughter, treatments to decontaminate carcasses, and prerequisite programs to keep the processing environment clean. The inevitability of carcass contamination presents one of the most critical quality and safety challenges to the meat processing industry. Most of the microorganisms transferred to carcasses during slaughter are nonpathogenic spoilage bacteria; however, the potential for introducing pathogens like *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter* spp., *Yersinia* spp., *Bacillus cereus* and *Clostridium botulinum* exists and is of great concern from a public health standpoint

(Narasimha Rao et al., 1998). Pathogen contamination of raw meat and poultry products entering the food supply may result in severe economic losses and more importantly in foodborne illness and even death among consumers. Reduction of such pathogens in meat products could save up to $12 billion annually in the United States in medical costs, lost productivity, recalls, legal fees, and loss of business (Buzby et al., 1996), and more importantly relieve human suffering and loss. In order to effectively reduce microbial contamination on carcasses, it is essential to control their proliferation on the carcass surface and as such it is imperative to maintain an adequate chilling process.

Due to metabolic activity in pre-rigor muscle of carcasses, the average carcass temperature may increase to about 40°C immediately following slaughter (Gill, 2000). Carcasses are typically cooled before being further processed; however, the period of time it takes carcasses to cool from temperatures as high as 40°C to chiller temperatures (e.g., 7°C) provides an opportunity for proliferation of spoilage and pathogenic bacteria, especially psychrotrophic organisms (Gill, 1998). For the safety of the product it would be most ideal to cool the carcass as rapidly as possible and as such minimize the growth of the microflora on the carcass surface and, although, psychrotrophic growth may be expected on chilled carcasses, the extent to which it occurs must be controlled by ensuring that the chilling process is under control.

The important criteria for chilling carcasses are (Mallikarjunan and Mittal, 1995): (i) meeting regulations; (ii) minimizing carcass mass loss; (iii) avoiding cold-shortening of muscles; and, (iv) minimizing chilling time to increase throughput. Considering the inherent interaction of these criteria, it is essential for an establishment to find a balance that will optimize their chilling process to achieve the required throughput while

minimizing carcass mass loss and avoiding cold-shortening but most importantly preventing microbial proliferation.

Regulatory authorities in the United States and European Union require that the center of the thickest portion of the carcass, in the deep leg at the proximal part of the thigh, be no more than 10 or 7°C, respectively, before removal from the cooler for transport or further processing (James and Bailey, 1990). This usually translates into reduction of deep muscle temperature to the required endpoint within 12 and 24 h, and, although, this is attainable for the comparatively smaller lamb and pork carcasses, it is insufficient time for the larger beef carcasses (Nottingham, 1982). Thus, large beef carcasses, which are intrinsically slow to cool, are often processed before they have been reduced to a temperature of 7-10°C that is generally regarded as the maximum temperature for preventing the proliferation of mesophilic, enteric pathogens (Smith, 1985). The USDA-FSIS recommends deep muscle (approximately 15 cm) chilling to 10°C within 24 h and to 7.2°C within 36 h and that the surface be chilled to 10°C in 5 h and below 4.4°C in 24 h with periodic overhead water spraying (NACMCF, 1993; USDA-FSIS, 1995). Processing facilities in the U.S. are typically allowing carcasses to chill for 36-48 h with periodic (10 min every hour for the first 12 h of the chilling process) overhead water spraying prior to further processing or transport (Stopforth et al., 2003b). The throughput of any chilling system decreases when the dwell time in the cooler increases; however, this may be necessary to allow plants processing large carcasses (and especially older, heavier animals) that require more time to meet the specified temperature.

To minimize carcass mass loss and prevent microbial proliferation, the carcass surface temperature should be lowered as fast as possible (Mallikarjunan and Mittal, 1995). While the specific effects of chilling or freezing on bacteria are not completely understood, it is known that low temperatures extend the lag phase of these organisms and in so doing reduce the overall microbial load on carcasses (Vanderzant et al., 1985). Furthermore, the reduction of temperature to near freezing can damage and kill bacteria by: (i) formation of ice crystals that kill bacteria when they penetrate the cell membrane with consequential release of cellular constituents (Mazur, 1966); or, (ii) causing chemical changes in the lipid bilayer that result in permanent physical damage of the cells (Jay, 2000; Smith, 1995). The problem faced with rapid chilling of beef carcasses is that cooling muscle to chiller temperatures prior to the development of rigor results in a change called "cold shortening" wherein the contraction of muscle fibers results in permanent toughening of the tissue (Locker and Hagyard, 1963; Tornberg, 1996). In addition, the protein denaturation brought about by "cold shortening" will result in tissue damage and consequently produce a large amount of drip loss (Offer et al., 1988).

Although, the rapid chilling and freezing of muscle tissue, to prevent drip loss from carcasses, suppress bacterial growth and alleviate problems with product throughput appears to be the ideal practice, there are some major problems associated with such methods. Firstly, there is the problem of cold shortening and "thaw rigor" that is encountered when rapidly chilling pre-rigor muscle. Cold shortening (contraction during chilling) occurs when the meat temperature drops to below 10°C but does not freeze before fuel for contraction, that is, the adenosine triphosphate (ATP) is expended (Locker and Hagyard, 1963). Meat that is frozen rapidly will not experience cold shortening;

however, upon thawing, a more severe shortening, thaw rigor, will occur (Bendall, 1974). Cold-shortening of pre-rigor muscle by rapid chilling may be overcome by promoting the rapid onset of rigor through electrical stimulation of carcasses following dressing (Locker, 1985; Joseph, 1996). Secondly, the chemical changes that occur after slaughter result in lower pH values of 5.5-5.7 in muscle tissue compared with the normal pH of living muscle tissue of 7.0-7.2 (Voyle, 1974). The drop in pH results in increased permeability of the sarcolemma that allows sarcoplasmic proteins and water to pass out of the cell (Voyle, 1974). Chilling post-rigor muscle tissue to its freezing point very rapidly will result in crystallization of the intracellular water within muscle and the resulting ice crystals will damage the cell membrane resulting in cellular leakage upon thawing (Voyle, 1974). To achieve a reduction in loss of carcass mass due to drip without concurrent cold shortening, carcass chilling needs to be conducted in a multi-stage scheme (Mallikarjunan and Mittal, 1995). Such chilling methods will be discussed in the following sections.

E.II. Conventional beef carcass chilling

Conventional carcass chilling involves the use of cool, unsaturated air flowing through chill rooms that generally operate under two or more temperature conditions during the chilling cycle (Gill, 2002). Conventional carcass chilling may be done in batches or continuously (Mirade and Picgirard, 2001).

With batch chilling, the coolers are filled over a period of time and are operated while it is being filled (Gill, 2000). Carcasses may enter the cooler throughout the course of a day and, once filled, the coolers continue to operate (Gill, 2000). Carcass batches

may be unloaded the day after their entry into the cooler; however, the chilling process usually extends over two days to allow adequate chilling of the carcasses (Gill, 2000). The difference in air velocity at various locations within the chiller is the major source of variation in chilling time among carcasses (Mirade and Picgirard, 2001). The difference in carcass chilling rates is exacerbated with large carcasses placed in low, compared with high, velocity areas; however, the location within the chiller is as important as the carcass weight (Wooton, 1986). Drumm et al. (1992) indicated that carcasses on rows closer to the cooling fans were subjected to higher air velocities and lower temperatures than those further away on the row. Unloading a cooler is often faster than the loading thereof and as such carcasses may be subjected to chilling for different times (Gill and Jones, 1997). Moreover, a partially full batch chiller may lead to process control problems; that is, more space between carcasses will lead to higher air velocities and consequently more rapid chilling rates (Mirade and Picgirard, 2001). It is thus, essential to control the airflow inside chilling rooms as it determines the efficiency and homogeneity of carcass chilling (Mirade and Picgirard, 2001).

Continuous chillers operate by applying the same average air velocity over each carcass and the existence of poorly ventilated areas is much more of a problem for process efficiency than the homogeneity of airflow (Daudin and Van Gerwen, 1996; Mirade and Picgirard, 2001). Most meat processing establishments use batch chilling which requires longer chill periods and consequently larger cooler space meaning that any increase in production must be accompanied by a corresponding increase in cooler capacity (James, 1987; Mallikarjunan and Mittal, 1996).

Data from beef chilling at a commercial facility indicate that chilling in air at 0 and 4°C resulted in times of 20.3 and 27.7h, respectively, to reduce the temperature of the deep leg in a small (100 kg) beef carcass side to 7°C (Bailey and Cox, 1976). In a beef carcass side approximately twice the size (220 kg), the time taken to reduce the deep leg temperature to 7°C increased to 45.9 h (Bailey and Cox, 1976). Such data indicate that it is almost impossible to achieve the specified temperature of deep leg muscle by conventional chilling at 0 to 4°C within 24 h. Beef is typically chilled using batch chillers applying near-zero temperatures for a nominal 36 to 48 h to ensure adequate chilling of deep muscle tissue from almost 40, to 7°C (James, 1987). Research (James and Bailey, 1990) has shown that a 48 h chilling period using a mean chill temperature of 1.5°C during the first 24 h is required to chill beef carcasses to within 7°C. A strategy for optimum beef chilling within 35 h without causing cold-shortening and reducing carcass mass loss was proposed by Mallikarjunan and Mittal (1995). The strategy encompassed a three-stage (pre-, during-, and post-rigor) system that applied air temperature of -4°C for 2 h at the pre-rigor stage followed by 8.5°C during rigor and -1.2°C for the remaining post-rigor chilling period.

E.III. Spray-chilling of beef carcasses

One of the major challenges in the meat processing industry remains the preservation of the fresh state of the product; however, there needs to be a balance between reducing microbial proliferation as well as shrink or drip loss of carcasses during primary chilling. During the chilling process, while the carcass surface is warm and moist, there is ample opportunity for microbial proliferation (Gill and Landers, 2003). The microbiological

condition of carcasses during chilling is influenced by the extent to which the carcass surface dries during chilling as well as the rate of temperature decline in the surrounding air (Nottingham, 1982; Scott and Vickery, 1939). With such knowledge, most regulatory authorities still require that the carcass surface be adequately dried by air alone during chilling to assure the safety and shelf-life of the meat (Bailey, 1986). The drawback of conventional chilling using air alone is the evaporation of moisture from the drying tissues and the consequential economically undesirable carcass mass loss (Gigiel et al., 1989a; James and Bailey, 1989). Thus, in 1987, to effectively reduce shrink or drip loss due to evaporation during carcass chilling using relatively high air speeds, most of the major slaughter plants in North America adopted the application of water to beef carcasses during chilling (Jones and Robertson, 1988; Kastner, 1981). The process of intermittently spraying carcasses with water during the first few hours of chilling, termed spray-chilling hass rapidly gained commercial acceptance in the United States (Allen et al., 1987; Heitter, 1975; Johnson et al., 1988).

The process of spray-chilling was identified by Swift Fresh Meats Company in 1975 in order to meet three primary objectives: (i) reduce shrink loss of carcasses; (ii) reduce spoilage bacteria normally present on carcasses; and, (iii) sustain commercially acceptable product (Heiiter, 1975). The original process involved intermittent spraying of freshly dressed beef carcasses with a mild chlorine solution at predetermined intervals during the normal chilling process and the result of this process was more effective destruction of spoilage bacteria and an intervening layer of moisture for evaporation (Heitter, 1975). Common conditions for beef carcass spray-chilling involve the use of air chilling with intermittent water spraying for the first 3-12 h of chilling followed by air

chilling for the remaining period of the chilling process (Hippe et al., 1991). Spray-chilling applications using a water spray for either 60 or 120 s every 15 min for up to 17 h have been investigated (Strydom and Buys, 1995). Results from these studies revealed that spray application for 17 h with a spray duration of 120 s was the most effective treatment for reducing carcass mass loss; however, this process yielded carcasses that were too wet at the end of an 18 h chilling process which proved to be insufficient drying time.

The initial 24 h period of conventional air chilling of beef carcasses has been reported to result in evaporative mass losses of at least 2% (Brown et al., 1993; Gigiel et al., 1989b; Jones and Robertson, 1988); however, since the incorporation of spray-chilling, shrink loss has been reduced by as much as 1.5% in beef carcasses (Brown et al., 1993; Gigiel et al., 1989b; Allen et al., 1987; Greer et al., 1990).

The USDA-FSIS issued a final rule on January 9, 2001, limiting the amount of water retained by raw, single-ingredient, meat and poultry products as a result of post-evisceration processing such as carcass washing and chilling (USDA-FSIS, 2001). According to this regulation, raw livestock and poultry carcasses and parts derived thereof are not permitted to retain water resulting from post-evisceration processing unless the establishment producing such products demonstrates, with scientific data, to USDA-FSIS that any water retained in the products is an inevitable consequence of processes used to meet applicable food safety requirements (USDA-FSIS, 2001). Furthermore, the establishments are required to disclose on the label of the meat or poultry products, the maximum percentage of retained water in the product.

E.IV. Use of antimicrobials during spray-chilling of beef carcasses

Spray-chilling using water at specified time intervals during the initial stages of chilling has been the dominant method of carcass chilling in commercial meat processing facilities (Johnson et al., 1988; Gill and Landers, 2003). The reason for incorporating such systems has been to increase chilling rates by enhanced evaporative cooling and to reduce the carcass mass loss due to drip (Dickson and Anderson, 1992). The first application of spray-chilling made use of a mild chlorine solution for spraying the carcasses with the intent of not only reducing drip loss and increasing the chilling rate but also enhancing the microbiological status of the product to improve safety and shelf-life (Heitter, 1975). Other studies have considered the incorporation of chemicals approved for fresh beef decontamination into the spraying process during the initial stages of spray-chilling as a method of sanitizing carcasses. Hamby et al. (1987) studied the effects of spray-chilling using continuous applications of 1% acetic or lactic acid and reported significant reductions in total aerobic populations on treated compared to untreated carcasses. In similar investigations (Dickson, 1991; Dickson and Anderson, 1992), a modified spray-chilling using up to 2% acetic acid on beef tissue inoculated with pathogens proved to be more effective in reducing the pathogenic contamination compared to spray-chilling using water.

Bacterial attachment and consequential penetration through meat surfaces and into the interior is a serious concern during slaughter and further processing of animal carcasses (Woody et al., 2000). Bacteria may use various methods to attach to meat surfaces (Butler et al., 1979; Beachey, 1981; Selgas et al., 1993) and, although attachment post-mortem may be non-specific, many researchers believe that

polysaccharide-containing components on the cell wall such as flagella and pili (Butler et al., 1979), cell surface charge (Dickson and Koohmaraie, 1989) and hydrophobicity of bacteria (Dickson and Koohmaraie, 1989; Benito et al., 1997), and specific binding sites or receptors on animal cell membrane (Beachey, 1981) are responsible for adhesion. Bacterial attachment to meat during chilling is generally considered a surface phenomenon; however, it has been suggested (Gill and Penney, 1977) that bacteria can penetrate the surface due to breakdown of connective tissue by bacterial proteolytic enzymes. Furthermore, shrinkage of muscle fibers and subsequent formation of gap regions during the development of rigor may facilitate bacterial penetration (Gill et al., 1984). These findings highlight the importance of incorporating antimicrobial substances into the process during spray-chilling of animal carcasses to not only reduce the level of bacterial contamination on the surface of the carcasses but more importantly prevent attachment and penetration of the microorganisms during the chilling period. The use of antimicrobials such as activated lactoferrin, an immobilized form of lactoferrin, has been presented as a deterrent to pathogenic bacteria that may be present on meat surfaces (Naidu et al., 2003). Specifically, activated lactoferrin, a GRAS compound, is thought to act as a blocking agent, which interferes with microbial adhesion/colonization, detaches live or dead microorganisms from biological surfaces, inhibits microbial growth/multiplication, and neutralizes the activity of endotoxins (Naidu, 2002).

F. Cooking beef to inactivate microorganisms

There are several methods for cooking meat and although the main objective is to achieve a specific internal temperature, the type of product, the rate and type of heat, the type of equipment used and several other factors affect the cooking process (Aberle et al., 2001). Numerous foodborne outbreaks involving infection with *E. coli* O157:H7 are thought to have originated with the consumption of hamburger meat and other beef products that were cooked inadequately while in other cases, the purge of raw beef contacting other food products is implicated (CDC, 2000a, 2001; Kassenborg et al., 1998). Although it may be assumed that organisms survive cooking and result in illness, there is usually no direct evidence that the organism survived the cooking process but rather epidemiological evidence of relationships of food consumed and associated illness (Gannon, 1999). With the multistate outbreak involving hamburgers contaminated with *E. coli* O157:H7, 86% (398 of 501) of cases reported eating at the foodservice facility implicated, while 92% (366 of 398) of these reporting that hamburgers appeared "regular" (Bell et al., 1994). Upon investigation, it was revealed that 63% (10 of 16) of "regular" hamburgers cooked according to the restaurants protocol were below 60°C (Bell et al., 1994). Sporadic cases of *E. coli* O157:H7 infection have been linked to the consumption of undercooked ground beef (Kassenborg et al., 1998; Slutsker et al., 1998). It is hypothesized that with certain cases, cooking temperatures may have permitted survival of *E. coli* O157:H7 and that the characteristic low infectious dose associated with this organism result in foodborne illness. Juneja et al. (1997) reported that ground beef patties cooked to an internal temperature of 68.3°C for 40 s should inactivate at least 99.99% (4 D_{10}) of *E. coli* O157:H7 cells. Based on this, Clavero et al. (1998) recommended a temperature of 71.1°C (160°F) be used to safely cook ground beef.

The USDA regulations concerning proper cooking of beef products (with over 16 time-temperature combinations) should be used as a guideline for cooking beef and verification of the suggested temperatures should be accompanied using quick-reading thermometers to assess the internal temperature of beef products (Orta-Ramirez et al., 1997; USDA-FSIS, 2002). Another method of verifying the bacterial killing as a result of adequate time-temperature processes has been suggested by Veeramuthu et al. (1998) in which the use of triose phosphate isomerase would be a potential indicator because this heat-labile enzyme has thermal inactivation characteristics closely resembling thermal death parameters of *E. coli* O157:H7. Using this method, determination of D and zeta values are determined for bacterial counts in meat exposed to thermal treatment and correlated with values obtained for residual ezyme activity or protein concentration of triose phosphate isomerase from the same sample of meat.

Since the laws of thermodynamics are relative to mass of the meat it is important to match the time of cooking to reach the desired temperature with the size of the meat (Gannon, 1999). Ground beef is thought to be heated as a result of steam-vapor flow rather than conduction of heat and once a given temperature is reached it tends to be maintained for several seconds and thus, a sufficiently high internal temperature is reached to allow optimal kill without specifying holding times (Gannon, 1999). It is also important to note that several factors, especially fat percentage, may increase the thermal resistance of organisms (Jay, 2000), strengthening the concerns that pathogenic organisms may survive cooking and subsequently recover and grow as sublethally injured pathogens (Clavero et al., 1998).

G. *Listeria monocytogenes*

In the past, *L. monocytogenes* was classified by *Bergey's Manual of Determinative Bacteriology* to be in the family *Corynebacteriaceae* (Stuart and Pease, 1972). The pathogen was first described by Murray et al. (1926) as the etiologic agent of an infection in laboratory rabbits for which it was named *Bacterium monocytogenes*. In 1927, Pirie (1927) discovered an organism he named *Listerella hepatolytica* which was rejected in 1939 by the Judicial Commission of the International Committee on Systematic Bacteriology only to later be named *Listeria* (Pirie, 1940). In the latest edition of *Bergey's*, it is listed together with *Lactobacillus*, *Erysipelothrix*, *Brochothrix*, and other genera, in a section entitled "Regular, Nonsporing, Gram-Positive Rods" (Seeliger and Jones, 1987). The genus *Listeria* is comprised of six species namely: *L. monocytogenes*, *L. innocua*, *L. seelegeri*, *L. welshimeri*, *L. ivanovii*, and *L. grayi* of *which L. monocytogenes* is the most important pathogen (Rocourt et al., 1992).

Listeria monocytogenes is a gram-positive, non-sporeforming, facultatively anaerobic rod able to grow between –0.4 and 50°C (Junttila et al., 1988). The rod-shaped cells are relatively small ranging from 0.4 to 0.5 μm in diameter and 0.5 to 2.0 μm in length and are slightly rounded at the ends (Peel et al., 1988). *Listeria monocytogenes* possesses peritrichous flagella responsible for the characteristic tumbling motility displayed by this organism (Palumbo, 1991). The organism displays a tumbling motility when cultured at 20-25°C, due to the formation of a few peritrichous flagella; however, when cultured at 37°C it is non-motile or weakly motile as the organism produces reduced amounts of flagellin (Peel et al., 1988). The pathogen is generally catalase-

positive although catalase-negative strains have been observed (Rocourt, 1999) and, indeed, isolated from foods (Hagen et al., 1998). Furthermore, the pathogen is oxidase-negative and exhibits ß-hemolysis, producing zones of clearing on blood agar although; there are a few non-hemolytic isolates (Kathariou and Pine, 1991). The hemolysin activity is synergistic with the ß-hemolysis of *Staphylococcus aureus* on sheep erythrocytes; the agent responsible for this effect is identified to be the CAMP (Christie, Atkins and Munch-Petersen) factor (Christie et al., 1944). The organism is mainly facultatively anaerobic but can grow aerobically in the presence of glucose, lactose, rhamnose and maltose, while anaerobically, only hexoses and pentoses support growth (Pine et al., 1989).

Listeria monocytogenes is a well-adapted microorganism able to survive adverse conditions and even proliferate. *Listeria monocytogenes* is a psychrotrophic organism and can grow at temperatures ranging from as low as –0.4°C to 45°C (optimum of 30 to 37°C) (Bacon and Sofos, 2003; Parish and Higgins, 1989). Growth of *L. monocytogenes* occurs in environments of pH 4.4 to 9.4, at a_w levels above 0.92 and survives at sodium chloride levels of up to 30% and at currently approved nitrite levels for foods (Bacon and Sofos, 2003; Parish and Higgins, 1989). The infectious dose of listeriosis may be as low as 100 cells/g of food and the illness usually has an incubation period of a few days to 2 to 3 months (depending on host susceptibility) (Bacon and Sofos, 2003). *Listeria monocytogenes* can grow at temperatures as high as 45°C (Bacon and Sofos, 2003) and its heat resistance is greater than other non-sporeforming foodborne pathogens including *Salmonella* and *Campylobacter* (Brown, 1991; Masters et al., 1994).

G.I. Distribution and disease

Listeria monocytogenes is ubiquitous in the environment including soil, plant material, and water (Weis and Seeliger, 1975). Furthermore, the pathogen may be isolated from sewage, silage, animal feces, milk of animals, and the intestinal tract of animals and humans (Farber and Peterkin, 1991, 1999; McCarthy, 1990; Wesley, 1999). Although, the annual occurrence of listeriosis in the U.S. is low (estimated to be approximately 2,500 cases) compared to infections from other foodborne pathogens such as *Campylobacter jejuni* and *Salmonella*, the relative rate of mortality, approximately 20%, is the highest among these pathogens (Mead et al., 1999). Despite the fact that many individuals are exposed to the pathogen, only a small proportion develops listeriosis. The disease is mainly an invasive illness in susceptible individuals. The most cases of human listeriosis occur in individuals that are immunocompromised and/or pregnant woman and their fetuses (El-Kest and Marth, 1988; Slutsker and Schuchat, 1999). *Listeria monocytogenes* appears to be a normal resident of the intestinal tract in humans, partially explaining the common existence of antibodies to *Listeria* spp. in healthy people (Ryser and Marth, 1999). The underlying condition in immunocompromised individuals leads to suppression of their T-cell-mediated immunity. There are instances in which healthy individuals become ill with listeriosis in foodborne epidemics (Schlech et al., 1983) and sporadic cases (Azadian et al., 1989). The clinical manifestations associated with the invasive disease include meningitic or encephalitic infections, primary bacteremia, and endocarditis (Farber and Peterkin, 1991). Listeriosis in pregnant women manifests as a mild, self-limiting influenza-like illness with associated fever, headache, myalgia and gastrointestinal symptoms but inevitably leads to fetus infection through transplacental

transmission resulting in spontaneous abortion, stillbirth, early labor or early onset of neonatal infection (Bacon and Sofos, 2003; Slutsker and Schuchat, 1999). The highest rate of listeriosis incidence is seen in neonates and the elderly (McLauchlin, 1990). Pregnant women are 17 times more likely to contract listeriosis than the healthy general population and account for 27% of all listeriosis cases (Lorber, 1997). Those over the age of 65 have a 7.5 times higher risk of listeriosis than healthy adults younger than 65 (Goulet and Marchetti, 1996). Human listeriosis may be caused by all 13 serovars of *L. monocytogenes*, although, serovars 1/2a, 1/2b, and especially 4b cause most of the cases (Gellin and Broome, 1989; Rocourt et al., 2000). There has not been any direct link made between particular forms of listeriosis and certain serovars, however there is an epidemiological association between perinatal listeriosis and serovars 1/2b, 3b, and 4b (Gellin and Broome, 1989).

Although, *L. monocytogenes* was the etiologic agent of human disease for more than 70 years, it was not until the 1980s that the primary route of infection was identified as foods (Schlech et al., 1985). Transmission of *L. monocytogenes* to humans is primarily through foods that are contaminated either directly from the environment or from surface contact in processing facilities (Richmond, 1990). Foodborne transmission of *L. monocytogenes* has been implicated in numerous outbreaks of listeriosis attributed to various food products including milk, soft cheeses, fruits, vegetables, fresh or frozen meat, poultry, fish, seafood and various processed, meat, dairy, fish and seafood products (Doyle, 1991; Jay, 2000). Since *L. monocytogenes* gained prominence in the 1980s, 15 outbreaks have occurred in the U.S. and nine of these implicated food that resulted in 362 cases of listeriosis and 105 deaths (FDA/FSIS, 2001). The largest outbreak of listeriosis

occurred in 1985 due to consumption of soft cheese which resulted in 142 cases and 48 deaths (Linnan et al., 1988). In 1998 to 1999, a multistate outbreak associated with the consumption of hot dogs and deli meats resulted in 101 cases and 21 deaths (CDC, 1999). This was followed by another multistate outbreak in 2000 involving deli turkey meat products that resulted in 29 cases and 7 deaths (CDC, 2000b). More recently, a multistate outbreak involving deli poultry products resulted in 46 cases and 10 deaths (CDC, 2002). Between 2000 and 2003, there were at least 98 reported recalls for RTE meat and poultry products suspected of *L. monocytogenes* contamination (http://www.fsis.usda.gov/OA/recalls/rec_pr2.htm). Results from a USDA-FSIS monitoring program (1990-1999) for RTE meat and poultry products, conducted in approximately 1,800 federally inspected establishments, showed that *L. monocytogenes* was present in 5.2% of sliced ham and luncheon meats, 3.6% of small cooked sausages, 3.3% of fermented sausages and 3.1% of cooked roast corned beef (Levine et al., 2001). More recently, in a survey on the prevalence of *L. monocytogenes* in refrigerated vacuum-packaged frankfurters, 532 (1.65%) of 32,800 products of different sizes and formulations from 12 commercial manufacturers were found positive (Wallace et al., 2003). In an additional survey (Gombas et al., 2003), 31,705 samples of various RTE foods obtained from retail markets in Maryland and California were tested to determine prevalence of *L. monocytogenes*; 0.89% of the sliced luncheon meat samples were found positive. The levels of *L. monocytogenes* in the positive samples ranged from 0.04 to 10^4 CFU/g, with the majority of samples (i.e., 42 out of 82) having levels in the range of 0.04 to 0.1 CFU/g, and a fraction of the samples having levels in the range of $>10^2$ to 10^3 CFU/g (i.e., 7 out of 82) and $>10^3$ to 10^4 CFU/g (1 out of 82). Results also showed that

in-store packaged luncheon meats, deli salads and seafood salads had higher frequencies of *L. monocytogenes* than the same products when manufacturer-packaged, highlighting the additional risk of product contamination as a result of extra handling at the retail level. Of greater concern is that although the prevalence of *L. monocytogenes* tended to be higher in in-store-packaged products, levels of *L. monocytogenes* tended to be higher in manufacturer-packaged products. Specifically, of the 21 samples with counts greater than 10^2 CFU/g, 16 were manufacturer-packaged. The extent (cell density) of *L. monocytogenes* contamination appeared to be low, with only a fraction of the contaminated products containing high levels of *L. monocytogenes* (Gombas et al., 2003); it is likely that these latter products are more likely to cause listeriosis outbreaks. In fact, combining the data of Gombas et al. (2003) with additional epidemiological data, Chen et al. (2003) derived a dose-response model by which it was concluded that risk of disease increases with levels of the pathogen consumed. They suggested that an alternative to the zero tolerance strategy would be to implement a management strategy that focuses on limiting the concentration of *L. monocytogenes* cells rather than its presence or absence (Chen et al., 2003). These events illustrate the major threat listeriosis poses to public health and the economy in the U.S. and as such there is a definite need for better approaches to control the pathogen in food and especially RTE meat and poultry products.

H. Control of *L. monocytogenes* in RTE meat and poultry products

The severity of listeriosis has led regulatory agencies since the late 1980s to recommend or require industry to establish control strategies to minimize the presence, survival, and growth of *L. monocytogenes* in foods. In 1991, the National Advisory Committee for Microbiological Criteria in Foods (NACMCF) recommended control strategies such as development of an effective surveillance system for listeriosis, and use of HACCP–based programs to ensure the safety of foods (NACMCF, 1991). Additionally, the USDA-FSIS established a 'zero tolerance' (negative 25 g samples) in RTE meat and poultry products and initiated microbiological testing for *L. monocytogenes* (USDA-FSIS, 1990).

Contamination of RTE products occurs primarily during post-lethality (during peeling, slicing, repackaging, etc.) exposure to the environment. For this reason, USDA-FSIS proposed a rule on performance standards for the production of processed meat and poultry products (USDA-FSIS, 2001). Moreover, in order to identify the relative risk of death posed by RTE foods, the Food and Drug Administration (FDA) and USDA-FSIS published a preliminary draft of risk, ranking models for 20 categories of RTE products (FDA/FSIS, 2001). This assessment indicated that deli meats posed the greatest health risk for listeriosis of all RTE foods, being responsible for 80% of all deaths and cases. More research data, however, on growth responses of *L. monocytogenes* in different RTE products was identified as a crucial issue for assessment of relative risks. Considering the results of the preliminary risk ranking, the outbreaks of listeriosis during the past four years (CDC, 1998; 2000a; 2002), as well as the numerous recalls of RTE products since 1996 due to potential presence of *L. monocytogenes*, USDA-FSIS established an interim final rule to control *L. monocytogenes* in RTE meat and poultry products (USDA-FSIS, 2003a). In particular, establishments that produce RTE products that are exposed to the

environment after lethality treatments and that support growth of *L. monocytogenes* are required to have, in their HACCP plans, or in their standard operating procedures or other prerequisite programs, controls that prevent product adulteration by *L. monocytogenes*. Three alternatives to control *L. monocytogenes* during post-lethality exposure of products are proposed: (i) Alternative 1 requires application of a post-lethality pathogen reduction treatment (that may include a chemical) and an inhibitory antimicrobial agent or process to control *L. monocytogenes*; (ii) Alternative 2 requires application of either a post-lethality treatment or an antimicrobial agent or process; and, (iii) Alternative 3 requires no application of a post-lethality treatment, but instead requires the combination of a sanitation program with microbiological testing of food contact surfaces and holding of products when positive testing results occur (USDA-FSIS, 2003a). Moreover, USDA-FSIS modified the Directive for microbiological sampling for *L. monocytogenes* (USDA-FSIS, 2003b), based on the three alternatives of the final rule, to assist in the verification of the control strategies implemented by the establishments (USDA-FSIS, 2003a).

H.I. Strategies to control *L. monocytogenes*

Considering that *L. monocytogenes* contamination occurs primarily during post-lethality exposure or processing, the most effective methods to reduce, control, or inactivate the pathogen in RTE meat and poultry products is to incorporate antimicrobials as an ingredient in the formulation of the products or use post-lethality antimicrobial applications or treatments such as steam pasteurization. Antimicrobial agents, processes or the combination thereof may be the best strategy for controlling this pathogen in RTE

meat and poultry products. The following section illustrates the effectiveness of such strategies to control *L. monoctyogenes* contamination.

H.I.a. Antimicrobial treatments

The USDA-FSIS has increased the permissible levels of sodium acetate (as a flavor enhancer) and sodium diacetate (as a flavor enhancer and pathogen inhibitor) to 0.25% in meat and poultry products, and has permitted the use of sodium lactate and potassium lactate at a level of 3% (corresponding to a 4.8% of the 60% commercial product), in the same products, to act as inhibitors of growth of certain pathogens (USDA-FSIS, 2000). Numerous studies showing the antilisterial effect of chemical preservatives added to the formulation of RTE meat and poultry products have been published (Bedie et al., 2001; Blom et al., 1997; Buncic et al., 1995; Choi and Chin, 2003; Deumier and Collignan, 2003; Glass et al., 2002; Mbandi and Shelef, 2001, 2002; Porto et al., 2002; Qvist et al., 1994; Samelis et al., 2002c; Schlyter et al., 1993a,b; Seman et al., 2002; Shelef, 1994; Shelef and Addala, 1993; Stekelenburg, 2003; Weaver and Shelef, 1993; Wederquist et al., 1994). Wederquist et al. (1994) found that *L. monocytogenes* growth was significantly inhibited in turkey bologna formulations containing 0.5% sodium acetate, followed by 2% sodium lactate, or 0.26% potassium sorbate. Blom et al. (1997) found that a combination of 2.5% sodium lactate and 0.25% sodium acetate inhibited growth of *L. monocytogenes* in RTE meat products during storage for 5 weeks at 4°C. In a study with pork frankfurters, Bedie et al. (2001) reported that 6% sodium lactate or 0.5% sodium diacetate were listeriostatic, or even listeriocidal, throughout the 120-day storage period at 4°C. Using 3% sodium lactate, the pathogen was inhibited for at least 70 days, whereas 0.25% sodium diacetate inhibited growth of the pathogen for 50 days (Bedie et al., 2001).

Samelis et al. (2002c) reported that a combination of sodium lactate (1.8%) with 0.25% sodium acetate, sodium diacetate or glucono-δ-lactone added to the formulation of frankfurters prevented *L. monocytogenes* growth in pork frankfurters for 120 days at 4°C. Similarly, combinations of 0.1 and 0.2% of sodium diacetate or sodium acetate with 1.8% and 2.5% of sodium lactate were more effective than the single additives in meat emulsion inoculated with *L. monocytogenes* and stored at 5 or 10°C for 30 and 20 days, respectively (Mbandi and Shelef, 2001). Additionally, Mbandi and Shelef (2002) showed that sodium lactate (2.5%) and sodium diacetate (0.2%), used individually, delayed growth of *L. monocytogenes* on beef bologna during storage at 5°C, whereas their combination resulted in listeriocidal effects. Chemical preservatives have also been tested as spray (Islam et al., 2002b) or dipping (Palumbo and Williams, 1994; Samelis et al., 2001c; Glass et al., 2002, Islam et al., 2002a) solutions. As can be seen from the above-mentioned studies, a wide variety of additives with GRAS status have been tested for their antimicrobial activity against *L. monocytogenes*. The most widely used additives in the industry are sodium lactate and sodium diacetate, used alone or in combination, at levels of 1.5-2% sodium lactate (on a dry weight basis) and 0.1 to 0.15% sodium diacetate (Tompkin, 2002).

Predictive modeling has been used to quantitatively express the effect of GRAS chemical preservatives on growth kinetics of *L. monocytogenes*, and develop predictive models for growth of the pathogen in RTE products formulated with different levels and types of antimicrobials (Houtsma et al., 1996, Seman et al., 2002). These models provide useful data for risk assessment purposes, as well as for evaluation of effectiveness of potential new formulations, whereas many effective antimicrobial formulations tested in

broth (Yoon et al., 2003) and foods (Qvist et al., 1994; Schlyter et al., 1993a,b; Blom et al., 1997; Mbandi and Shelef, 2001) are available for validation of existing models and/or forming the basis of new ones.

In recent years, there has been increased interest in biopreservation systems, specifically bacteriocinogenic lactic acid bacteria and/or their bacteriocins, to control *L. monocytogenes* (Muriana, 1996; Gill and Holley, 2000). Antagonism towards *Listeria* spp. in wiener sausages (Yousef et al., 1991), frankfurters (Berry et al., 1991), German-style fresh sausages 'Mettwurst' (Schillinger et al., 1991), American-style fermented sausage and dry fermented sausage (Berry et al., 1990; Foegeding et al., 1992; Hugas et al., 1995), Italian salami (Campanini et al., 1993), turkey summer sausage (Luchansky et al., 1992) and turkey slurries (Schlyter et al., 1993b) have been reported. Although strains of *L. monocytogenes* may be sensitive to numerous bacteriocins (nisin, sakacin and pediocin PA-1) (Katla et al., 2003), their activity is affected by a number of factors (e.g., strain variation, inactivation by food components) (Muriana, 1996).

A combination of acetic and citric acid (2.5% each) applied as dipping solution, was found to restrict growth of *L. monocytogenes* on surface-inoculated frankfurters for 90 days at 5°C (Palumbo and Williams, 1994). It was, however, found that at 12°C storage, none of the acid treatments was effective; therefore, the antilisterial effect of the acid dips could not counter temperature abuse of the product. In another study, Samelis et al. (2001c,d) examined the antilisterial activity of organic acids, also applied as dipping solutions, to slices of pork bologna and showed that samples dipped in 2.5 or 5% acetic acid did not permit significant growth of the pathogen during the 120-day storage period at 4°C.

H.I.b. Post-lethality treatments

Post-lethality physical treatments may be applied pre-packaging, including radiant heating (Gande and Muriana, 2003) and flash steam heating (Cygnarowicz-Provost et al., 1994), or post packaging, including steam pasteurization (Murphy et al., 2001), immersion in hot water (Cooksey et al., 1993a,b; Hardin et al., 1993; Roering et al., 1998; Muriana et al., 2002; Murphy et al., 2002; 2003a,b,c; Samelis et al., 2002d), ionizing radiation (Sommers et al., 2002; although not approved yet), cycles of vacuum-steam (Kozempel et al., 2000; Sommers et al., 2002), and high hydrostatic pressure (Simpson and Gilmour, 1997). Combined application of pre- and post-packaging treatments has also been reported (Gande and Muriana, 2003). Of the pre-packaging post-lethality treatments, radiant heating for 60 to 120 s at 475°F (246°C) to 750°F (399°C) using a radiant oven ('Infrared Grill', Unitherm Foodsystems) reduced *L. monocytogenes* in several RTE meat and poultry products by 1.5 to 3.5 logs (Gande and Muriana, 2003). Post-packaging steam pasteurization (Murphy et al., 2001) and hot water pasteurization (Murphy et al., 2002; Roering et al., 1998) have been shown to be efficacious in reducing *L. monocytogenes* on RTE meats. Ionizing radiation and steam pasteurization through vacuum-steam cycles have resulted in significant reductions of *L. monocytogenes* (Sommers et al., 2002); however, there are limitations associated with these two interventions in that high radiation levels (kGy), or excessive numbers of steam-vacuum cycles may have negative organoleptic effects on the product (Kozempel et al., 2000; Sommers et al., 2002). Recently, application of high hydrostatic pressure (87,000 psi; 3 min) to inoculated retail-packaged sliced ham, turkey and roast beef allowed no recovery

of *L. monocytogenes* after 61 days of storage at 1.1°C (http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/97-013F/CompGuidelines.pdf).

H.I.c. Combination of post-lethality treatments and antimicrobials

Combining antimicrobials with post-lethality treatments may have an additive or synergistic effect on elimination or control of growth of *L. monocytogenes* contamination in RTE meat and poultry products, and is the essence of hurdle technology (Leistner and Gorris, 1995; Leistner, 2000). This approach is regarded as more effective than the application of single treatments in that it enables the use of hurdles of lower intensity, and in so doing, it has less of an adverse effect on product quality (Leistner and Gorris, 1995). To our knowledge, only one study has been published where antimicrobial and post-lethality treatments were combined to control *L. monocytogenes* in a RTE product (Samelis et al., 2002c,d). In this study, frankfurters were formulated with chemical preservatives (1.8% sodium lactate added singly or in combination with 0.25% sodium acetate, sodium diacetate or glucono-δ-lactone), inoculated with *L. monocytogenes,* then subjected to a post-packaging thermal pasteurization treatment of hot water before storage at 4°C (Samelis et al., 2002c,d). Results showed that post-packaging pasteurization (80°C, 60s) enhanced the antilisterial effect of the least effective treatment (1.8% sodium lactate used singly). Other antimicrobial treatments were more effective in controlling *L. monocytogenes* than 1.8% sodium lactate and, thus, the contribution of post-packaging pasteurization could not be determined.

I. The digestive tract as a last line of defense against microorganisms

Ingested pathogens entering the body via contaminated food do so through the gastrointestinal (GI) tract. The body does have defenses to combat these pathogens; however, an overwhelming dose of pathogens or their toxic products and a weak immune system (i.e., young, elderly and immunocompromised individuals) can lead to foodborne illness. The threshold point for illness differs from person-to-person; for pathogens that cause infections this point is termed infective dose while the point for pathogens causing foodborne intoxications is termed toxic dose (Jay, 2000). The severity of illness may be different among people eating contaminated food and this variability may be due to several factors including the virulence of the pathogen, the health status of the individual and the concentration or quantity of the pathogen (the minimum infectious dose differs among pathogens).

I.I. The gastrointestinal tract

Food digestion begins in the mouth, where food is masticated and mixed with enzyme-containing saliva (Chang et al., 1996; Johnson and Gerwin, 2001; Magee and Dalley, 1986). The enzymes secreted in the mouth are salivary amylases which initiate carbohydrate digestion and are secreted by three pairs of salivary glands. The salivary glands also secrete saliva, which keeps the mouth moist and aids in forming food into a mass called a bolus to make swallowing easier (Chang et al., 1996; Johnson and Gerwin, 2001; Magee and Dalley, 1986). Food travels from the mouth down the esophagus into the stomach. Within the stomach lining, there are various types of cells which perform different functions. The parietal cells produce hydrochloric acid which kills many bacteria and aids in the digestion of protein (Johnson and Gerwin, 2001). Hydrochloric

acid (HCl) converts pepsinogen, which is inactive, to pepsin, which initiates the digestion of proteins by breaking the bonds that link amino acids together, leaving smaller chains of amino acids called polypeptides. The gastrin secreted by the gastric glands stimulates the secretion of HCl which has a major role in converting pepsinogen to pepsin (as mentioned previously) but also in lowering the pH of the stomach environment (Chang et al., 1996; Ruiz Chavez, 1996). There are also glands, cardiac and pyloric, that secrete mucus that protects the stomach lining. The stomach also mechanically mashes food so that the digestive juices are distributed thoroughly. The food then passes through into the small intestine where most digestion and absorption of food occurs (Johnson and Gerwin, 2001). Gastric juice contains HCl, salts, pepsin, mucus, water, intrinsic factor and bicarbonate all of which form chyme and as the production of chyme increases, the hydrogen ion (H^+) concentration increases, further lowering the pH (Chang et al., 1996; Gardner et al., 2002, Ruiz Chavez, 1996). The large molecules of proteins, fats and carbohydrates cannot be digested in the stomach but are digested into smaller molecules in the small intestine. The bile duct (tube) from the liver and the pancreatic duct from the pancreas merge and empty into the duodenum (the first 25cm of the small intestine) where they secrete pancreatic juice, which contains digestive enzymes. Most of the enzymes that act in the small intestine originate in the pancreas; they act on carbohydrates, proteins, and fats. The surface of the tissue of the small intestine is covered with villi, which are very small, finger-like projections of the cell membrane (Chang et al., 1996; Johnson and Gerwin, 2001; Magee and Dalley, 1986). The two major types of cells that line the small intestine are the goblet cells, which are more numerous and secrete mucus, and the absorptive cells, which aid in absorption. Digested

food matter passes through the cecum, through the transverse colon and then through the descending colon before exiting through the rectum and almost no digestion occurs in the large intestine, but absorption of sodium, chloride, and water does occur (Chang et al., 1996; Johnson and Gerwin, 2001; Magee and Dalley, 1986).

I.II. The gastrointestinal tract as a defense system against pathogens

The body possesses a broad array of defense mechanisms for fighting foodborne pathogens which includes: (a) stomach acid, (b) the GI tract immune system (or mucosal immune network in the gut), (c) commensal intestinal flora, and (d) bile salts and digestive enzymes (Gibson et al., 1998).

I.II.a. Stomach pH

The chyme or gastric fluid, formed and secreted in the stomach, ranges in pH from 1 to 2 (Kos et al., 2000), and many bacteria are easily killed in such a low pH before they reach the small intestine (Waterman and Small, 1998). Certain characteristics of consumables decrease the stomach acidity and can potentially protect many pathogens or their toxins from degradation, increasing their chances of reaching the small intestine and posing a risk of exposure to becoming ill. These characteristics may include: (a) the buffering capacity of the food (milk decreases acidity); (b) things with antacid properties (buffering agents which decrease acidity); (c) acid blockers (inhibiting the secretion of stomach acids); and, (d) partial or total gastrectomies (removal of stomach tissue) (Chang et al., 1996; Van Duijn et al., 1989).

I.II.b. The GI tract immune system

The GI tract immune system assists in keeping an individual healthy by reducing absorption of specific large molecules or by reducing colonization or invasion of the epithelium (cells lining the gut) by pathogens (Merrell and Camilli, 2002). In the mucosa of the gut, numerous and various kinds of immune-component cells (i.e., epithelial cells, T-cells, B-cells, dendritic cells, macrophages etc.) form part of a unique mucosal immune network which contributes to the induction of specific and protective immunity against foreign antigens (Ag) (Iijima et al., 2001). Antigen-specific secretory immunoglobulin A (S-IgA) produced in the mucosa is one of the major elements in the gut mucosal defenses (Iijima et al., 2001). Particles such as toxins become immobilized within the epithelium where they are presented for degradation by certain enzymes (Iijima et al., 2001). Another way in which the mucosa minimizes foreign body entry is by targeting particles or cells that attach to the bowel wall (Iijima et al., 2001). Although the GI immune system is quite capable of resisting invading molecules and pathogens, some microorganisms are able to change their outside surfaces in order to prevent them from being recognized as harmful (i.e., *Borrelia recurrentis* exhibiting antigenic variation).

I.II.c. Competition and exclusion by intestinal flora

There are over 400 species of bacteria (comensal intestinal flora) which inhabit the human GI tract (Merrell and Camilli, 2002). The comensal intestinal flora are able to protect the gut by resisting colonization of the intestine by pathogenic bacteria foreign to the gut (Montrose, 2001). In order to cause illness, foodborne pathogens must successfully compete against the normal flora by either colonizing the epithelium or evading the GI immune response. Pathogens produce attachment factors and have certain surface components that help them colonize the intestinal wall while others produce

enzymes, toxins or other molecules that alter the permeability or damage epithelial cells allowing them to invade (Prescott et al., 1999). Thus, the body relies on the natural gut flora to inhibit the colonization and proliferation of pathogens in the gut.

I.II.d. Digestive enzymes and bile acids

As mentioned previously, bile acids produced in the liver assist in the digestion and absorption of fat but also act to inhibit the growth of pathogenic microorganisms considering that they lower the pH to levels where the microbes are inactivated. In addition, digestive enzymes are active throughout the GI tract and may kill or inactivate pathogenic microbes (i.e., lysozyme in saliva) (Chang et al., 1996).

Other methods for protecting the GI tract from invasion by foodborne pathogens include the use of vaccines or probiotic treatment. Certain antigen-specific immune responses are not generated by oral vaccines without mucosal adjuvant and thus safe and effective adjuvants are essential for the development of mucosal vaccines protecting against infectious diseases (Iijima et al., 2001). Iijima et al. (2001) recently proposed the presence of an IgA-specific induction pathway which should be considered in the development of mucosal vaccines. While antibiotic therapy is usually effective in treating foodborne illness it may be ineffective and cause the condition to worsen by preventing the growth of the normal flora allowing pathogenic bacteria to successfully colonize and invade. In general, antibiotic therapy should be used parsimoniously, especially if the pathogen is resistant and the normal flora is sensitive to the antibiotic. Probiotics are defined as live cultures consumed for a health benefit (Hansen, 2002). Most probiotic products contain bacteria from the genera *Lactobacillus*, *Bifidobacterium*, *Escherichia*,

Enterococcus, *Bacillus* and *Saccharomyces* (Hansen, 2002; Marteau et al., 2002). The minimum requirements of a probiotic for human consumption are that they should be safe, effective and maintain their efficacy and potency until they are consumed (Hansen, 2002; Marteau et al., 2002).

As previously mentioned, the gastric environment serves as a barrier to colonization of the intestine by pathogenic bacteria due to the inhibitory actions of the low pH and enzymes (Waterman and Small, 1998). It is also inevitable that certain pathogenic bacteria may be able to survive the gastric environment and as such have a greater opportunity to colonize the intestinal mucosa and result in illness (Callaway et al., 2003). It is known (Griffin, 1998; Tilden et al., 1996) that *E. coli* O157:H7 has a low infectious dose and because such a low number of cells are capable of causing illness, the capacity of *E. coli* O157:H7 to survive gastric exposure is a direct indication of its ability to cause foodborne illness. Diez-Gonzalez et al. (1998) showed that *E. coli* recovered from cattle fed a grain diet were almost 1000-fold more resistant than those fed a hay diet when exposed to extreme acid shock simulating passage through a human stomach. Callaway et al. (2003) showed that *E. coli* isolated from cattle switched from a grain diet to a forage diet experienced a 1000-fold decrease in survival when exposed to extreme acid shock similar to human gastric environment.

There has been limited work conducted involving the exposure of pathogens on or from food sources to simulated gastric fluid (Roering et al., 1999; Uljas and Ingham, 1998; Waterman and Small, 1998). Arnold and Kaspar (1995) tested the acid tolerance of *E. coli* O157:H7 grown to stationary phase or to a state of starvation and found that such cultures were more resistant to the effects of a simulated gastric fluid. Roering et al.

(1999) found that *L. monocytogenes* was almost eliminated in apple cider (pH 3.3-3.5) within 2 days of storage at 4 or 10°C and comparatively, it was eliminated within 30 min of incubation in simulated gastric fluid (pH 1.5). It was also shown (Uljas and Ingham, 1998) that acid habituation of *E. coli* O157:H7 in apple juice sensitized the cells to subsequent exposure in simulated gastric fluid (pH 2.5). The inoculation of acid-sensitive enteric pathogens onto food surfaces has been shown to protect such cultures from extreme acid conditions (pH 2.5) representing gastric acidity (Waterman and Small, 1998). Thus, gastric shock survivability studies are important to determine the risk associated with ingesting *E. coli* O157:H7 that may have survived an extreme acid shock (i.e., animal digestive systems).

J. Resistance, adaptation, and cross-protection of microorganisms to food processing stresses

J.I. Resistance and adaptation of microorganisms to food processing stresses

Bacteria often encounter stresses that threaten their existence. Among the hostile environmental conditions confronted in nature are various starvations, oxidations (hydrogen peroxide, superoxide, nitrous oxide), heavy metals, DNA-damaging agents, osmolarities, weak acids, and temperatures or pH values outside the normal growth range (Bukau, 1993; Rowbury, 2001). Within a group of organisms, a wide range of environmental parameters may be tolerated, and the more competitive and successful an organism is at enduring these stresses, the broader its growth niche will be (Foster, 1995). The ability to adapt according to the change in environmental conditions is vital for

growth and survival of bacteria. Although, microorganisms are adapted for optimal functioning in their normal physiological environments, extreme shifts in conditions from the optimum will inflict stress on an organism (Beales, 2004). The extent of deviation from the optimum will determine the organism's response and whether it is destroyed, inhibited from growing, or experiences delayed growth (Russell et al., 1995). Microorganisms can thrive in many habitats and appear to have a large number of signal transduction systems and regulatory mechanisms that can respond to small changes in environmental stresses (Hengge-Aronis, 1999). These responses are usually observed when cells encounter a number of different stresses and are often accompanied by reduced growth rate or entry into stationary phase. Starvation, high osmolarity, low pH, adverse temperatures and other harsh conditions may trigger what is considered a general stress response (Hengge-Aronis, 1999). The physiological outcome of this response includes multiple stress resistances, the accumulation of storage compounds, changes in cell envelope composition and altered morphology (Hengge-Aronis, 1996).

Parameters such as temperature, water activity, pH, salt concentration, and antimicrobial substances are often used to inhibit or destroy microorganisms in food (Abee and Wouters, 1999; Brul and Coote, 1999; Marechal et al., 1999). The response of microorganisms to such stresses may be an immediate but temporary emergency response for short-term, extreme stress (shock) or long-term adaptation (Yousef and Courtney, 2003). In addition to the general stress response that protects cells from a variety of stresses as mentioned above, there are specific, self-protective stress responses (Yousef and Courtney, 2003). Some of the specific mechanisms are outlined in the following section.

J.I.a. Starvation as a stress

In nature, bacteria seldom encounter conditions that permit exponential growth but are rather confronted by long periods of nutritional deprivation punctuated by short periods allowing fast growth (the feast-or-famine life style) (Kolter et al., 1993; Velkov, 1999). Starvation usually refers to microbial existence in the absence of an exogenous supply of an essential nutrient and, as such, the requirement needs to be met entirely from endogenous sources (Matin, 1996). Stationary phase is a term used synonymously with this condition of starvation (Matin, 1996). Limitation of a single nutrient does not necessarily result in transition to stationary phase growth but may induce alternative scavenging systems including: cAMP/CAP system, NTR system and the PHO regulons (Kolter et al., 1993). As cells enter stationary phase they undergo a distinct physical adaptation to ensure that they can cope with physical stresses while relatively dormant (Rees et al., 1995). Changes in morphology are most obvious in states of stationary phase (i.e., *E. coli* becomes much smaller and almost spherical due to *rpoS*-mediated induction of the *bola* morphogene) (Lange and Hengge-Aronis, 1991; Rees et al., 1995). Subcellularly, the cytoplasm becomes condensed while the volume of the periplasm increases and the cell membrane composition changes (proportion of cyclopropyl derivatives increases, replacing more unsaturated fatty acids) (Rees et al., 1995). The action of DNA gyrase and the synthesis of DNA binding proteins causes an increase in the superhelicity of chromosomal DNA and allows it to be packed more densely (Dorman, 1996; Tse-Dinh et al., 1997); and acts to protect DNA in the bacteria from oxidative damage (Altuvia et al., 1994) and damage from excessive temperatures (Tse-Dinh et al., 1997). Phenotypically, bacteria in the stationary phase are more

thermotolerant (Hengge-Aronis et al., 1991), resistant to oxidative stress (Lange and Hengge-Aronis, 1991), acid-resistant (Lee et al., 1994a; Arnold and Kaspar, 1995) and more suited to survive osmotic stress and starvation (McCann et al., 1991). Sigma^{s} (σ^{s}) confers two major attributes to a starved cell: (a) enhanced ability to catabolize and scavenge and (b) increased resistance to a variety of stresses (Matin, 1996). The enhanced scavenging capacity is conferred through the production of increasing levels of "substrate-capturing" or entry enzymes (i.e., *E. coli* grown under glucose limitation exhibits a progressively increasing concentration of hexokinase with decreasing dilution rates [Harvey, 1970; Matin, 1996]) and other catabolic enzymes (*E. coli* subjected to glucose limitation increases production of enzymes like β-galactosidase and the CstA protein in order to utilize energy sources not present in the immediate environment) (Schultz and Matin, 1991; Matin, 1996). Superior stress resistance is attributed, as it is not possible for the bacteria to predict the length of the starvation conditions in the environment and thus prepare for survival in a state of complete starvation by synthesizing a class of starvation proteins (Blum et al., 1992; Tian et al., 1995). At the onset of starvation, *E. coli* experiences a temporally ordered program of gene expression involving 40-80 genes which later in the growth state yields cells with an enhanced resistance to different environmental stresses (Velkov, 1999). The identities of only some of the proteins involved in this protection are known, and several (i.e., DnaK, GroEL, HtpG) are concerned with preventing protein denaturation during starvation and renaturation when the starvation state is alleviated (Blum et al., 1992; Tian et al., 1995). Other proteins like SurA are actually thought to be involved in repairing *cis*-conversion of prolines thought to be damaged during starvation (Visick and Clarke, 1995). Two

classes of genes identified to be induced upon carbon starvation are the *cst* genes, requiring cAMP and the *pex* genes, not requiring cAMP which are proposed to protect the cellular membrane and envelope (Velkov, 1999). Other classes of proteins produced upon starvation of bacterial cells include: Pcm (thought to catalyze conversion of isoaspartate into L-aspartyl residues), PapC (pilin porin with a role in heat and osmotic resistance), KatE and KatG (hydroperoxidase II and I, respectively, responsible for destroying H_2O_2 which may cause damage to the cells DNA during starvation), XthA and PexB (repair damage done by H_2O_2 to DNA) and AidB (reversing DNA methylation) (Loewen and Hengge-Aronis, 1994; Matin, 1996).

J.I.b. Acid as a stress

Any change in environmental conditions from the optimum to an extreme may impose a stress on an organism. For the most part, bacteria are able to tolerate small changes in their environment and adapt over time to cope with the stress. Inducible tolerance to acidic and alkaline environments is an important strategy for survival of many bacteria (Foster, 1999). The use of regulatory genes, specific tolerance mechanisms and genes associated with tolerance are of particular interest in the maintenance of pH homeostasis (Foster, 1999). The mechanism cells utilize to sense changes in pH and subsequently alter transcriptional patterns is of utmost importance. A mechanism commonly employed by bacteria in response to environmental stimuli, involves two-component signal transduction systems (Miller et al., 1989; Nixon et al., 1986). According to Miller et al. (1989), these systems are usually made up a sensor/transmitter and a regulator protein. The sensor/transmitter is a transmembrane protein with cytoplasmic and extracytoplasmic domains. The extracytoplasmic domain (sensor) senses the environment and transfers the

signal through the transmembrane region to the cytoplasmic domain (transmitter) (Miller et al., 1989). Once the signal is received, the transmitter domain uses an intrinsic kinase activity to phosphorylate the amino terminus of the second protein, the transcriptional regulator. Once phosphorylated, the regulator can bind DNA through its carboxyl-terminal region and regulate gene expression (Miller et al., 1989). There must also be some form of transmembrane signaling in response to external pH fluctuations. The exact nature of the signal is unknown; however, four potential signals exist. They are external pH (pH_0), internal pH (pH_i), ΔpH ($pH_0 - pH_i$), and proton motive force (Δp) (Hall et al., 1995). A natural, often self-imposed, environmental condition endured by bacteria is acid stress, which is defined as the combined effect of low pH or high concentration of the H^+ ion and weak (organic) acids present in the environment (Bearson et al., 1997). Weak acids include organic acids like lactic acid and acetic acid, as well as volatile fatty acids (VFAs) like butyrate, propionate and acetate produced as a result of fermentation. Weak acids in their uncharged forms can diffuse across the cell membrane and dissociate inside the cell, lowering the internal pH (pH_i) in the process. The lower the external pH (pH_o), the more undissociated weak acid will be available (based upon pK_a values) to cross the membrane and affect pH_i (Bearson et al., 1997). This means that it takes less organic acid to kill a cell at pH_o 3.5 than is needed at pH_o 5.0 and that acids become more effective antimicrobials as the pH_o approaches their pKa. Intracellular accumulation of weak acids is also thought to have harmful effects on the cell beyond that of acidifying pH_i. It is important to be aware of the relationship between pH and weak acid concentration when considering cellular acid survival strategies. Organic acid stress may be different from that caused by inorganic acids (i.e. HCl) (Foster, 1999). The

reason is that weak organic acids not only acidify the pH_i of the cell but also accumulates as intracellular anions (Foster, 1999). Despite lowering the pH_i of the cell the effect of anion accumulation may affect the intracellular glutamate pool, not K^+ levels (Roe et al., 1998).

Management of pH stress by organisms could include both constitutive and inducible components (Hall et al., 1995; Foster, 1995). Constitutive, or intrinsic, pH stress survival strategies include pH homeostasis, membrane structural influences over protein permeability, internal buffering capacity, and the pH stability of essential proteins (Slonczewski and Foster, 1996). Inducible systems of pH stress survival can include systems that alter proton pumping, decrease membrane permeability, produce chaperones, repair DNA or prevent macromolecular damage (Foster, 1995). Microorganisms strive to maintain a stable internal pH over a wide range of external pH conditions (Booth, 1999). This concept, termed pH homeostasis, is achievable due to the low proton conductance of biological membranes (passive homeostasis) and the presence of proton-driven transporters (active homeostasis), which either bring protons into the cell at basic external pH or extrude them out at acidic external pH. Passive homeostasis is afforded by the low permeability of the membrane to protons and other ions which may effect large changes in pH_i (Hill et al., 1995). The lack of permeability can only be overcome by undissociated lipid-permeable weak acids or if the cell is treated with an ionophore (Hill et al., 1995). Another major factor of passive homeostasis preventing disruption of pH_i is the high buffering capacity of the cell which is a result of the protein content of the cytoplasm as well as the glutamate and polyamine pools of the cytoplasm (Hill et al., 1995). Active homeostasis depends primarily on the potassium ion and proton

circuits to maintain pH_i. Additionally, the Na^+ circuit is important in the alkaliphiles (bacteria growing optimally at pH 8-9) and has a limited role in acidophilic (growing optimally in acid pH) and neutrophilic (growing optimally in neutral pH) bacteria (Hill et al., 1995). A cell at low pH_o must exclude protons entering the cell associated with acids; however, the translocation of protons across the membrane creates a membrane potential limiting further proton exclusion and, as such, large scale exclusion can only occur if the potential created is dissipated (Hill et al., 1995). Potassium ion entry fulfills this need and generates a transmembrane pH gradient allowing constant proton pumping (Kroll and Booth, 1981). There are several major systems located in the cytoplasmic membrane influencing proton circulation, which can potentially contribute to pH homeostasis (Padan, 1999): (a) the K^+/H^+ antiport, K^+ transport and Na^+/H^+ antiport systems couple H^+ movement to the transport of K^+ and Na^+. K^+/H^+ antiport is thought to raise the internal pH of the cell in an acidic environment (Booth, 1999), while the Na^+/H^+ antiport will lower the internal pH in an alkaline environment; (b) the F_1F_0 proton-translocating ATPase activity couples H^+ movement with the synthesis and hydrolysis of ATP; (c) electron transport chains and H^+-ATPase extrude proteins and are responsible for the generation of proton motive force; and, (d) transport systems that couple proton flow with the import of other solutes.

Potential acid damage to a microorganism can be viewed as occurring in two stages. As pH_o starts to become more acidic, internal pH homeostasis mechanisms are able to maintain pH_i. This in turn creates a large pH change. As the external pH declines further, pH homeostasis mechanisms eventually fail, proton leakage increases, and pH_i is reduced with subsequent damage to internal macromolecules and acid-sensitive

metabolites (Hall et al., 1995). Acid death is thought to result from intracellular damage caused by decreasing pH_i as opposed to extracellular damage caused by acidic pH_o. Very few studies have investigated damage occurrence after the decline in internal pH levels and thus the defining lethal event has not been classified. Deoxyribonucleic acid (DNA) damage due to depurination and protein denaturation by altered ionic interactions is hypothesized as the major causes of death. Other effects reported include inhibition of substrate transport, depression of cytoplasmic pH and inhibition of macromolecular synthesis (Hall and Foster, 1996).

The central concept of inducible acid tolerance among bacteria is that growth in a moderately acid environment triggers synthesis of proteins that protect the cell from more extreme acid conditions (Foster, 1999; Heyde and Portalier, 1990). Synthesis of proteins in *E. coli* transferred from pH 6.9 to 4.3 included four well-known heat shock proteins GroEL, DnaK, HtpG and HtpM (induction was RpoH-dependent), three other proteins induced by osmolarity, aerobosis or low temperatures and seven other acid shock proteins (*asps*) (one of which the induction was RpoH-dependent while the others were RpoH-independent) (Heyde and Portalier, 1990).

J.I.c. Heat as a stress

Microorganisms in food typically encounter heat stress during processing. Heat results in damage to macromolecular cell components and as such, the major role of heat-induced stress proteins is to repair, destroy or replace damaged components to prevent disruption of cellular metabolism (Yousef and Courtney, 2003). Generally, microorganisms respond to heat by inducing the synthesis of a group of proteins called heat shock proteins (*hsps*); the most highly conserved genetic system known (Lindquist and Craig, 1988). All

organisms produce proteins encoded by the hsp70 and hsp90 gene families in response to elevated temperatures and furthermore, either the *hsps* or close relatives of this gene family are present at normal temperatures and play vital roles in normal cell function (Lindquist and Craig, 1988).

In *E. coli*, heat shock or temperature increase results in the upregulation of two groups of *hsps* that are transcribed by the $E\sigma^{32}$ and $E\sigma^{E}$ holoenzymes (Thomas and Baneyx, 1998). The signal responsible for the induction of the heat shock response is thought to be an increase in the intracellular concentration of unfolded and misfolded proteins (Gross, 1996). The σ^{32} regulon is implicated in the management of intracellular protein damage in the cytoplasm (and is negatively regulated by the heat shock proteins themselves) while those proteins transcribed by $E\sigma^{E}$ are upregulated to deal with protein damage in the periplasm or cell envelope (Gross, 1996; Thomas and Baneyx, 1998). Most of the members of the σ^{32} regulon are classified as either molecular chaperones or ATP-dependent proteases (Gross, 1996). Molecular chaperones (including Dnak-Dna-J-GrpE and GroEL-GroES systems) facilitate the proper folding of newly synthesized polypeptides and help thermally damaged proteins regain biological activity (Hartl, 1996). The DnaK-DnaJ-GrpE and GroEL-GroES systems are the best characterized molecular chaperones in *E. coli* (Thomas and Baneyx, 1998). Heat shock proteases (including ClpP, Lon and HflB) degrade misfolded proteins which cannot be rescued by the chaperone activity (Gottesman, 1996). The Clp ATPases (ClpB, Clpx and ClpY), HtpG and the small *hsps* (IbpA and IbpB) are believed to perform a "minor" chaperone activity but their actual role in heat shock is somewhat unclear (Gross, 1996; Squires and Squires, 1992). The small *hsps* IbpA and IbpB are dispensable for normal *E. coli* growth

at temperatures up to 45°C but their absence leads to growth defects at 46°C and HtpG is important in cell recovery following exposure to lethal temperatures (Thomas and Baneyx, 1998). Regulation of the proteins involved in heat shock is closely related with regulation of thermotolerance and is necessary for the maintenance of an energy-efficient growth cycle. In *E. coli*, the heat shock response is transcriptionally regulated by the cellular concentration of σ^{32} concentrations (Bukau, 1993; Lindquist and Craig, 1988; Velkov, 1999). It is suggested that σ^{32} (RNA polymerase subunit) for expression of heat shock genes is controlled at the level of its synthesis (transcription, translation and posttranslational), activity and its stability (Velkov, 1999). The σ^{32} gene, *rpoH*, is transcribed constitutively by the σ^{70}-containing polymerase (Lindquist and Craig, 1988) and the response to heat is achieved rapidly by an immediate increase in translational efficiency of the σ^{32} message, an increase in the concentration of the σ^{32} message and the increased stability of the normally unstable protein product (Lindquist and Craig, 1988). The DnaK protein is involved in the repression of σ^{32} activity and its degradation via direct physical interactions involving a binding and release cycle, dependent on ATP hydrolysis (Velkov, 1999).

Heat shock has also been found to increase σ^{s} levels by inhibition of its proteolysis due to the increased steady-state level of the induced DnaK chaperone which protects σ^{s} against degradation (Velkov, 1999). It is suggested that the σ^{s} and σ^{32} induced by heat shock and controlled by DnaK may be connected to stress response due to the fact that σ^{s} does not appear to contribute to heat tolerance (although it is induced under these conditions) but may induce cross-protection against other stresses (Muffler et al., 1997).

J.I.d. Cold as a stress

Due to the impact of temperature on all reactions of the cell in life, adaptations to fluctuations in temperature are probably the most common. In the environment, psychrophilic and psychrotrophic organisms are faced with cold temperature growth, however, even these organisms need to have adaptations to face temperatures which may fall below the optimum for growth (Berry and Foegeding, 1997). Adaptation to growth temperatures is an overall cellular phenomenon involving the maintenance of the structural integrity of macromolecules and their assemblies (proteins, ribosomes and membranes) and maintenance and regulation of systems such as protein synthesis and nutrient uptake (Hochochka and Somero, 1984; Russell, 1990). The most urgent problems of cold shocked cells are twofold: firstly, membrane fluidity decreases which hampers membrane-associated cellular functions (active transport, protein secretion, etc.) and secondly, the 2° structures of RNA and DNA are stabilized which decreases the efficacy of DNA replication and mRNA transcription and translation (Gottesman, 1996). It is established that microorganisms adjust the fatty acid composition of their membrane phospholipids in response to changes in growth temperature (Cronan and Rock, 1987; De Mendoza and Cronan, 1983). Cells in a normal growth state require fluid lipid membrane bilayers but as temperature is decreased, the fatty acid chains of the membrane lipids undergo a change of state from a fluid disordered state to a more ordered crystalline array of fatty acid chains (Cronan and Rock, 1987; De Mendoza and Cronan, 1983). Microorganisms respond to the decrease in temperature by incorporating proportionally more low-melting-point fatty acids into membrane lipids and in so doing lower the temperature for the disorder-order transition in order to maintain membrane fluidity and consequently function (Berry and Foegeding, 1997; Cronan and Rock, 1987; De

Mendoza and Cronan, 1983). Transport or synthesis of compatible solutes such as betaine and carnitine has been suggested to confer cold shock tolerance to *L. monocytogenes* (Angelidis and Smith, 2003; Ko et al., 1994). With a decrease in temperature from 37°C to 10°C, a number of proteins are induced in *E. coli* (Jones et al., 1987) and among these is CspA which has been identified as the major cold-shock protein (Goldstein et al., 1990). In *E. coli*, the proportion of *cis*-vaccenic acid ($C_{18:1}$) (unsaturated fatty acid) increases substantially as the growth temperature decreases and this process does not require the induction of proteins but is exerted by a constitutive protein active at low temperatures (Berry and Foegeding, 1997; De Mendoza and Cronan, 1983). At the levels of RNA and DNA, CspA may facilitate translation of the unsaturated fatty acids by acting as an RNA chaperone to block the secondary structures in mRNAs (Jiang et al., 1997).

The identities of many of the *E. coli* cold shock proteins are known: NusA, polynucleotide phosphorylase, initiation factors 2α and 2β, RecA, the dihydrolipoamide acetyltransferase subunit of pyruvate dehydrogenase, pyruvate dehydrogenase-lipoamide, the nucleoid-associated DNA binding protein H-NS, GyrA (the α subunit of topoisomerase DNA gyrase and Hsc66 (Hsp70 homologue; a 70 kDa heat shock protein which has 62% similarity to DnaK protein and functions as a molecular chaperone in the heat shock response) (Jones et al., 1987,1992; La Teana et al., 1991; Lelivelt and Kawula, 1995; Kandor and Goldberg, 1997). As mentioned previously, Goldstein et al. (1990) showed that when *E. coli* was shifted from 37 to 10°C, cold shock proteins were synthesized at rates 2- to 10-fold greater and that CspA (cold shock protein) was the most prominently induced protein. CspA consists of 70-amino-acid residues forming a closed

β-barrel structure with five β-strands (Newkirk et al., 1994; Schindelin et al., 1993). An important finding concerning the structure of CspA protein is the two RNA-binding motifs which are thought to contribute to the role of CspA as a chaperone because it has been shown that CspA binds both single-stranded RNA and DNA (Jiang et al., 1997). Nine independent genes have been identified on the *E. coli* genome encoding CspA homologues (Altschul et al., 1990; Goldstein et al., 1990; Lee et al., 1994b; Nakashima et al., 1996; Yamanaka et al., 1994, 1998) encoding the following proteins: CspA, CspB, CspC, CspD, CspE, CspF, CspG, CspH and CspI.

It was proposed (Goldstein et al., 1990) that CspA functioned as an antifreeze protein but it was later suggested that due to its sequence homology with the Y-box transcription factors (eukaryotic nucleic binding proteins) that it served a regulatory role (Wistow, 1990). It has been shown that CspA acts as a transcriptional activator of at least two cold shock proteins, H-NS and the DNA gyrase α-subunit (Jones et al., 1992; La Teana et al., 1991). The notion of CspA functioning as a RNA chaperone is supported by research conducted by Jones and Inouye (1994), Jiang et al. (1997) and Brandi et al. (1996). Another proposed role for CspA is that it acts as a putative repressor which binds to the cold-box sequence of the cold shock mRNAs during the acclimation phase and in so doing it blocks the transcription of the cold shock genes or destabilizes their mRNA products (Bae et al., 1997; Jiang et al., 1997). The role of the CspA and its homologues may be classified into five classes: (a) CspA, CspB and CspG for low-temperature stress, (b) CspD for nutritional stress, (c) CspC and CspE for transcriptional regulation and/or chromosome condensation, (d) CspF and CspH with as yet unknown function (potentially DNA chaperones), and (e) CspI with currently no known function (Bae et al., 1997;

Etchegaray et al., 1996; Hu et al., 1996; Nakashima et al., 1996; Schnuchel et al., 1993; Yamanaka et al., 1994; Yamanaka and Inouye, 1997). It has been shown that the CspA and protein homologues thereof are regulated at the level of transcription (Goldenberg et al., 1997; Mitta et al., 1997; Tanabe et al., 1992), at the level of mRNA stability (Brandi et al., 1996; Fang et al., 1997; Goldenberg et al., 1996) and also at the level of translation (Brandi et al., 1996; Jones and Inouye, 1996; Mitta et al., 1997).

J.I.e. Antimicrobial substances as a stress

Responses of microorganisms faced with sufficiently high concentrations of antimicrobials may be: inherent, apparent, or acquired resistance (Davidson and Harrison, 2002). Inherent resistance to a particular stress is controlled by the genetic makeup of the microorganism and the innate resistances vary among microorganisms (Davidson and Harrison, 2002). Some of the inherent mechanisms that may offer a microorganism protection to antimicrobial substances include: cellular barriers such as the outer membrane in Gram-negative bacteria and the techoic acids in Gram-positive bacteria; cellular efflux or related mechanisms that pump compounds in and out of cells to maintain homeostatic balance; lack of biochemical targets; and, inactivation of antimicrobial substances (Bower and Daeschel, 1999). Apparent resistance is related to the conditions of application; that is, usually in a food substrate, the presence of interacting stress conditions may increase or decrease the resistance of a microorganism (Davidson and Harrison, 2002). One of the best examples is the interaction of the most common food antimicrobials, weak acids, with the actual pH of the food. Weak organic acids can more effectively penetrate the cytoplasmic membrane of microorganisms when the acid is in its protonated or undissociated form (Davidson, 2001). Thus, the use of

weak organic acids is more effective in low pH foods and, hence, certain microorganisms may develop a resistance to weak acids in a food that dissociates the acid and renders it relatively ineffective. Acquired resistance is the result of genetic changes in the microbial cell due to mutation, acquisition of genetic material from plasmids, or altered protein regulation (Berry and Foegeding, 1997; Russell, 1991).

J.II. Cross-protection of microorganisms to food processing stresses

Cross-protection is a term referring to microorganisms which are subjected to a non-lethal stress and subsequently exhibit significantly greater resistance when either the same or a seemingly unrelated stress is reapplied (Rowe and Kirk, 1999). Growth restriction of microorganisms due to environmental stress(es) has caused them to develop survival strategies to survive the duration of the stress or the subsequent exposure of another stress. The survival strategies of microorganisms can be divided into two main classes (Pichereau et al., 2000): (a) survival as a result of specific systems induced by sublethal doses of a particular stress which permits survival against a challenge of the same agent or cross-protects it against another non-homologous stress, and (b) survival due to general systems induced in the stationary phase of growth under starvation conditions. As mentioned previously, microorganisms respond to environmental stresses by inducing specific sets of proteins to protect against damage, and the proteins within each set and the genes that encode them make up a stimulon (VanBogelen et al., 1987). With certain stimulons, induction of stress proteins by exposure to nonlethal levels of a stress has been shown to confer protection to subsequent exposure of lethal levels of the same stress (Christman et al., 1985; Yamamori and Yura, 1982). In some cases, the

proteins associated with a certain stimulon may possibly be induced during exposure to other stresses (Jenkins et al., 1988). It is evident (Leyer and Johnson, 1993) that exposure to a low level of stress protects microorganisms against more lethal levels of the same stress or to different stresses.

J.II.a. Acid-induced cross-protection

Bacterial cells that have undergone acid adaptation (or acid shock) may exhibit cross-protection to other environmental stresses such as heat (Farber and Pagotto, 1992; Leyer and Johnson, 1993), osmotic stress (Csonka, 1989; Leyer and Johnson, 1993) and sodium chloride and sodium lactate exposure (Garren et al., 1998). It was observed that rates of inactivation of acid-adapted *E. coli* O157:H7 cells at 37°C are slower than those nonadapted cells in broth acidified with organic acids (Ryu et al., 1999). Goodson and Rowbury (1989) revealed that acid-habituated *E. coli* cells grown at pH 5.0 are able to survive in acid environments representing foods better than those cells grown in a pH environment of 7.0 while, Leyer et al. (1995) and Ryu et al. (1998) demonstrated that acid adaptation of *E. coli* O157:H7 increases its survival in acidic food as compared with the nonadapted counterparts. It was also demonstrated that acid-habituated *E. coli* experienced decreased DNA damage by acid and had increased repair of acid-damaged DNA as compared with nonhabituated cells (Raja et al., 1991). Conner and Kotrola (1995) show that *E. coli* O157:H7 grown in the presence of acid had the ability to survive acidic conditions better than did those cells not grown in the presence of acid. The acid shock responses of *E. coli* O157:H7 and non-O157:H7 was shown to increase the survival rate of these cells when exposed to acid as compared to those cells which were acid-adapted (Garren et al., 1997). Nonadapted cells of *E. coli* O157:H7 were more

sensitive when exposed to organic acids than those cells which were acid-adapted prior to organic acid exposure (Ryu et al., 1999). Adaptation to acidic conditions by *E. coli* O157:H7 were found to negatively influence the effectiveness of 2% acetic acid spray washing to reduce contamination on carcass surfaces (Berry and Cutter, 2000). Rowe and Kirk (1999) showed that cross-protection using acid as an initial stress is induced against subsequent heat (56°C) and salt (20% v/v) stresses. Research indicated that pH-dependent acid resistance provided *E. coli* O157:H7 with cross-protection against heat treatments (Buchanan and Edelson, 1999). This was supported by the finding that acid-adapted *E. coli* O157:H7 cells were more resistant to heat treatments than were those cells nonadapted or acid-shocked (Ryu and Beuchat, 1999). Riordan et al. (2000) and Duffy et al. (2000) show *that E. coli* O157:H7 grown at low pH or previously acid-adapted confers cross-protection to the cells against heating processes. The heat resistance of *E. coli* O157:H7 and *L. monocytogenes* was shown to increase if cells were previously acid-adapted versus those that were previously nonadapted (Mazzotta, 2001). It was demonstrated that prior growth under conditions that induce a pH-dependent stationary phase is able to cross protect *E. coli* against radiation inactivation (Buchanan et al., 1999). Acid-adapted cells of *E. coli* O157:H7 showed a significantly higher survival than the nonadapted cells when stressed with bile salt (0.85%) (Cheng et al., 2002). *Listeria monocytogenes* was found to be more resistant to acidified skim milk at pH 3.5 and 4.0 when previously acid-adapted to pH 5.5 with lactic acid (Ravishankar and Harrison, 1999). O'Driscoll et al. (1996) also revealed that acid-adapted *L. monocytogenes* was more resistant to heat, cold salt, selected surface active agents, and ethanol. On the other

hand, alkaline stress enhanced the resistance of *L. monocytogenes* to subsequent heat (56 and 59°C) treatments (Taormina and Beuchat, 2001).

J.II.b. Heat-induced cross-protection

Heat shock (42°C for 5 min) resulted in increased survival of *E. coli* O157:H7 cells to a subsequent heat treatment as compared with those cells that were not heat shocked (Murano and Pierson, 1992). The effect of acid adaptation and low temperature storage enhanced the survival of *E. coli* O157:H7 in acidic condiments (Tsai and Ingham, 1997). Sublethal heat treatment (10 min at 48°C) of *E. coli* O157:H7 cells substantially increases their tolerance to acidic (pH 2.5) conditions (Wang and Doyle, 1998).

J.II.c. Starvation-induced cross-protection

Glucose- or nitrogen-starved cultures of *E. coli* exhibited enhanced resistance to exposure to heat (57°C) or H_2O_2 (15 mM) compared with exponentially growing cells (Jenkins et al., 1988). Stationary phase *E. coli* cultures displayed increased osmotic resistance compared with cultures in the mid-logarithmic phase or those preadapted to osmotic stress (Jenkins et al., 1990). Starvation of log-phase *E. coli* O157:H7 cells and those in stationary phase growth had higher acid tolerance than those in the mid-logarithmic phase of growth (Arnold and Kaspar, 1995). Nutrient stress generated by growing *E. coli* O157:H7 in their stationary phase until starvation significantly increased heat resistance of the pathogen (Rowe and Kirk, 1999).

J.II.d. Growth atmosphere as an initial stress to induce cross-protection

Escherichia coli O157:H7 grown under anaerobic conditions were significantly more heat resistant compared to those cells grown under aerobic conditions (Murano and Pierson, 1992; Gadzella and Ingham, 1994). Brudzinski and Harrison (1998) showed that

cultures of *E. coli* O157:H7 subjected to no agitation (more anaerobic) were more resistant to acetic acid than those cultures agitated (more aerobic). Anaerobic *E. coli* O157:H7 cultures were more resistant to extreme acid shock than aerobic cultures if the glucose concentration in the growth medium was high (Diez-Gonzalez and Russell, 1999).

J.II.e. Osmolarity as an initial stress to induce cross-protection

Escherichia coli grown in low salt broth are more acid resistant than those not grown in the presence of salt for periods less than 30 min after which sensitization to acid occurs (Lazim et al., 1996). Salt-induced organisms are indeed more sensitive to acid damage as a function of the high internal sodium ion concentration (Rowbury et al., 1996).

CHAPTER III

EFFECTIVENESS OF CHEMICALS AS SINGLE AND SEQUENTIAL DECONTAMINATION INTERVENTIONS TO REDUCE *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* TYPHIMURIUM ON BEEF TISSUE

ABSTRACT

This study evaluated chemicals as single and sequential interventions to reduce *E. coli* O157:H7 and *Salmonella* Typhimurium populations on beef tissue. Samples (5x2.5x1 cm) of boneless beef plates were inoculated (approximately 10^5 CFU/cm^2) with four-strain composites of *E. coli* O157:H7 or *S.* Typhimurium and dipped (5 pieces in 1.5 L for 30 s at 23°C, unless otherwise indicated) either singly or in all possible combinations of two of the following solutions: acidic oxidative water (0.005%, AOW – pH 2.67); basic oxidative water (BOW – pH 11.21); lactic acid (2.5%, LA - pH 2.12 at 55°C); distilled water (W, pH 7.01), ammonium hydroxide (0.1%, AH - pH 10.89); or, sodium metasilicate (4%, SM – pH 12.35 at 82°C) to evaluate the effectiveness of such solutions in reducing contamination. In another experiment, an approach incorporating sequential treatments that may be applied in multiple-hurdles systems was evaluated. The multiple-hurdles system included sequential dipping in 1% SM (82°C) followed by hot (82°C) water followed by 5% LA (55°C) and lastly by 5% LA (55°C) after 48 h (at -3°C for 10 h and 1°C for 38 h) of spray-chilling by overhead misting every 30 min for the first 10 h using a handheld sprayer. Additional systems ranged from no dipping steps to four

sequential dipping steps using combinations of 1% SM (82°C), 5% LA (55°C), warm (55°C) or hot (82°C) water. Individual treatments or combinations among W, AOW, BOW or AH had minimal (0.1-0.4 log CFU/cm^2) decontaminating effects compared to the higher reductions achieved with individual applications or combinations of treatments using 2.5% LA at 55°C and especially 4% SM at 82°C. In general, it appeared that pathogen reductions by the multiple-hurdles system were separated into two groups, based on efficacy, in increasing order: those treatments with one or more hot (82°C) application or a single 5% LA application < those combining two 5% LA applications or at least one hot (82°C) and one 5% LA application.

INTRODUCTION

In the conversion of beef cattle into carcasses and raw beef products, the underlying tissue, which is essentially sterile, may become contaminated with bacterial flora from the environment which may include foodborne pathogenic bacteria. Carcasses may become contaminated from various sources including fecal material, gastrointestinal content, or hide, as well as from cross-contamination introduced via workers' clothes, hands or equipment, other carcasses, plant equipment, plant environment and accidental spillage of body fluids during skinning and evisceration (Gill et al., 1998). Contamination of raw beef products entering the food supply may have adverse economic implications for the industry and, more importantly result in illness and even death among consumers (Huffman, 2002). Thus, bacterial reduction and, in particular, pathogen reduction on beef carcasses has been a constant challenge to the meat processing industry. In order to

improve the microbiological state of fresh beef, the USDA-FSIS established the HACCP systems final rule in which it was required that slaughter establishments apply at least one antimicrobial treatment to carcasses prior to chilling (USDA-FSIS, 1996). The principle of hurdle technology (Leistner, 2002) when applied to carcass decontamination, implies that if the initial microbial load is significantly reduced by decontamination procedures, the surviving microorganisms are readily inhibited in subsequent processing steps. This principle has been demonstrated experimentally (Castillo et al., 1998; Graves Delmore et al., 1998) and in actual plant settings (Bacon et al., 2000b).

Interventions previously determined to result in reduction of contamination on beef tissue have included: (i) spraying with hot or cold water (Castillo et al., 1998; Graves Delmore et al., 1997a; Sofos and Smith, 1998); (ii) steam pasteurization (Nutsch et al., 1997; Phebus et al., 1997); (iii) hot water/steam vacuuming (Graves Delmore et al., 1997a; Phebus et al., 1997); and, (iv) sanitizing with solutions such as organic acids (Castillo et al., 2001a,b; Dorsa et al., 1998a,b; Gill and Landers, 2003; Gill and Badoni, 2004; Ikeda et al., 2003; Ransom et al., 2003a), acidified sodium chlorite (Castillo et al., 1999; Gill and Badoni, 2004; Ransom et al., 2003a), peroxyacetic acid (Gill and Badoni, 2004; Ransom et al., 2003a), and cetylpyridinium chloride (Cutter et al., 2000; Ransom et al., 2003a). Acidic and basic electrolyzed water are GRAS compounds for beef and have been reported as effective against pathogenic bacteria in cell suspensions (Fabrizio and Cutter, 2003; Kim et al., 2000; Venkitaranayanan et al., 1999a), on various food contact surfaces (Park et al., 2002a; Russell, 2003; Venkitaranayanan et al., 1999b), on seeds and produce (Deza et al., 2003; Koseki et al., 2003; Stan and Daeschel, 2003; Sharma and Demirci, 2003), and on poultry (Fabrizio et al., 2002; Park et al., 2002b). Although, there

is no published scientific research supporting the use of sodium metasilicate on beef, it is approved for use on raw beef carcasses, subprimals and trimmings at a maximum concentration of 4% (FSIS Directive 7120.1, Amendment 1).

The objectives of this study were: (i) to evaluate the use of acidic and basic electrolyzed oxidative water, lactic acid, ammonium hydroxide, water and sodium metasilicate as single and sequential decontamination interventions applied to beef tissue inoculated with *E. coli* O157: H7 and *S.* Typhimurium; and, (ii) to evaluate the efficacy of simulated multiple-hurdle systems incorporating sodium metasilicate, lactic acid and water applied to beef tissue inoculated with *E. coli* O157:H7 and *S.* Typhimurium.

MATERIALS AND METHODS

Preparation and inoculation of beef tissue

Fresh boneless beef plates were obtained from a local commercial beef processing plant, stored at 4°C and used within 72 h postmortem. For use in the studies, meat was cut into 5x2.5x1 cm (total surface area of 40 cm^2) pieces. The inoculum used in this study was a composite of either *E. coli* O157:H7 strains ATCC43895, ATCC43894, ATCC43890 and ATCC43889 (isolated from venison jerky), or *S.* Typhimurium DT104 strains ATCC 700408, ATCC14028, DT104 var. Copenhagen (isolated from beef animal hides) and UK1 (isolated from horse wound). These strains were available as frozen cultures (-70°C; tryptic soy broth [TSB; Difco, Becton Dickinson Co., Sparks, MD] with 25% glycerol) and were activated by streaking onto tryptic soy agar (TSA) (Difco) and incubating at 35°C for 48 h. For each strain, a single colony was picked after incubation

(35°C for 48 h) and subsequently subcultured (35°C, 24 h) by inoculating 10 ml of TSB (TSB; 10 ml containing 0.25% glucose) with 100 μl of the activated stock cultures. The overnight cultures were then mixed to form a 40 ml composite culture which was centrifuged at 4,628 x g (Eppendorf, model 5810 R; Brinkmann Instruments. Inc., Westbury, NY) for 15 min at 4°C. For *E. coli* O157:H7 and *Salmonella*, the resulting pellet was washed in sterile phosphate-buffered saline (PBS, pH 7.4, Sigma Chemical Company, St. Louis, MO) centrifuged for a second time and the final pellet was resuspended in 20 or 40 ml PBS, respectively, for further use in the experiment. Use of different volumes for resuspension was based on preliminary investigation that indicated higher yields of *Salmonella* compared with *E. coli* O157:H7 cells after overnight incubation. Individual meat samples were inoculated (*ca.* 10^7 CFU/ml) with 0.2 ml of each composite culture, which was subsequently spread over all surfaces of the sample. Samples were kept at 25°C for 30 min to allow for bacterial attachment.

Preparation and application of decontamination solutions

Lactic acid (2.5 and 5%, LA - pH 2.12 and 2.04, respectively) solutions were prepared with 88% lactic acid (Purac® FCC 88; Purac America, Lincolnshire, IL) and completed to a final volume of 30 L with distilled water. Ammonium hydroxide (0.1%, AH - pH 10.89) solutions were prepared using a 28-30% ammonium hydroxide solution (Mallinckrodt AR® [ACS]; Mallinckrodt and Baker, Inc., Paris, KY) and completed to 30 L with sterile distilled water. Sodium metasilicate (1 and 4% wt/wt, SM – pH 12.27 and 12.35, respectively) solutions were prepared with anhydrous sodium metasilicate (Avgard® XP, Rhodia Inc.; Rhodia Food Ingredients, Cranbury, NJ). Acidified oxidized

water (AOW) was generated using an electrolyzed oxidized (EO) water generator (Primacide® P-5000) instrument (Primacide, Inc., Salt Lake City, UT) that yielded 0.005% AOW (pH 2.67/oxidation-reduction potential [ORP] 1107 mV) and as a byproduct, basic oxidized water (BOW – pH 11.21/ORP -805 mV). For water (W) treatments, distilled water (pH 7.01) was used. Properties (pH and ORP readings) of the treatment solutions were measured immediately after preparation using a dual-mode digital pH meter (*Ultra*Basic, UB-10; Denver Instrument, Denver, CO) with a glass pH/ORP electrode (pH/ATC Electrode #300729.1; Denver Instrument). The residual chlorine concentration in the AOW solution was determined with a chlorine test kit (Hach Co., Ames, IA).

Experimental design

In phase I, single treatments (five samples in each of two replicates for each pathogen) included: (1) nonntreated control (NT); (2) W (23°C); (3) 2.5% LA (pH 2.12, 55°C); (4) 0.1% AH (pH 10.89, 23°C); (5) 0.005% AOW (pH 2.67, 23°C); (6) BOW (pH 11.21, 23°C); (7) 4% SM (pH 12.35, 82°C). Sequential (combination of two) treatments were applied by combining each of the above-mentioned single treatments with each of the remaining treatments. The effect of temperature (4, 23, 55 or 82°C) and concentration (1 or 4% wt/wt) of SM was also evaluated on populations of *E. coli* O157:H7 attached to beef tissue. The following treatments (five observations per treatment) were applied to evaluate the effect of temperature: (1) nontreated control; (2) SM (4%) at 4°C; (3) SM (4%) at 23°C; (4) SM (4%) at 55°C; and, (5) SM (4%) at 82°C. In addition, the following treatments (with n=5 per treatment) were applied to determine the effect of concentration-

temperature combinations of sodium metasilicate: (1) nontreated control; (2) SM (1%) at 82°C; (3) SM (4%) at 82°C; (4) SM (1%) at 55°C; and, (5) SM (4%) at 55°C.

An additional experiment (phase II) was designed to simulate multiple-hurdles systems that may be applied in the industry to decontaminate fresh beef. The sequence of applications was chosen to simulate a packinghouse scenario with dipping (30 s) in sodium metasilicate (1% at 82°C) applied first (representing a pre-evisceration application), followed by hot (82°C) water after 20 min (representing a post-evisceration application), and then 5% lactic acid (55°C) after 10 min before the samples were spray-chilled (at -3°C for 10 h, with intermittent [every 30 min] spraying using water cooled to 4°C, followed by storage for 38 h at 1°C) for a total of 48 h (representing a pre-chill application) and lastly followed by the application of 5% lactic acid (55°C) (representing a post-chill application). Treatment of beef tissue during the chilling process was achieved by overhead (20 cm from tissue surface) spraying (simulated misting) of samples suspended on a wire mesh using a 1-L Envirokind® All-purpose Sprayer (Delta Industries, N. Hollywood, CA). Additional treatment systems studied were comprised of up to four sequential dipping steps combining 1% SM (82°C), 5% LA (55°C), warm (55°C) or hot (82°C) water (Table III.1). Inoculated samples (five observations for each of two replicates for each organism) were left nontreated (control) or dipped (30 s in 1.5 L) in solutions according to the treatment systems outlined in Table III.1. After exposure, samples were placed into an 18-oz Whirl-Pak® filter sterile plastic bag (Nasco, Fort Atkinson, Wisconsin) containing 40 ml sterilized maximal recovery diluent (MRD; 1.0 g Bacto™ Peptone [Difco] and 8.5 g sodium chloride [Fisher Scientific, Houston, TX] in 1

L distilled water) and homogenized (Masticator, IUL Instruments, Barcelona, Spain) for 2 min for microbiological analysis.

Table III.1: Design of treatment systems

Description of treatment system	Sequential dipping treatments prior to spray-chilling*			Dipping after spray-chilling
	Application #1	Application #2	Application #3	Application #4
Control using no treatments	0	0	0	0
Water treatments	0	H	0	0
	H	H	W	W
Main test system	S	H	L	L
Main test system with single omissions	0	H	L	L
	S	0	L	L
	S	H	0	L
	S	H	L	0
Alternative systems with four applications	H	H	L	L
	S	H	W	W
5% lactic acid treatments	0	0	L	0
	0	0	0	L
	0	0	L	L
Single water and 5% lactic acid treatment	0	H	0	L

*Spray-chilling involving storage at -3°C for 10 h, with intermittent (every 30 min) spraying using water cooled to 4°C, followed by storage for 38 h at 1°C
0: nondipped
H: dipped in hot (82°C) water
L: dipped in 5% lactic acid (55°C)
S: dipped in 1% sodium metasilicate (82°C)
W: dipped in warm (55°C) water

Microbiological analysis

For microbiological analysis, 1 ml of the homogenized sample was serially diluted in 9 ml of sterile 0.1% buffered peptone water (BPW; Difco, pH 7.2) and appropriate dilutions were plated using a Spiral Plater™ (Spiral System®, Spiral Sytems, Inc., Cincinnati, OH) onto TSA (Difco) for enumeration of *E. coli* O157:H7 and *Salmonella*

populations. Colonies formed on plates were automatically counted (CASBA™ 4, Spiral Biotech, Inc., Norwood, MA) after incubation at 35°C for 48 h. The detection limit of the microbiological analysis was 1.26 log CFU/cm^2 for cells attached to the beef tissue. The pH values of the homogenized samples were measured after microbiological analysis using a digital pH meter (*Ultra*Basic, UB-10; Denver Instrument) with a glass pH electrode (pH/ATC Electrode #300729.1; Denver Instrument).

Statistical analysis

Populations of bacteria were expressed as mean log CFU/cm^2 with associated standard deviations. Values for the mean log and standard deviation of each set of bacterial counts were calculated on the assumption of a log-normal distribution of microorganisms. In phase I, two replicate experiments were conducted with 10 samples per treatment for each organism. Preliminary analysis of fixed effects using the GLM procedure of SAS® v.8e (SAS, 2002) indicated that log CFU/cm^2 populations were dependent on pathogen and/or treatment. In phase II, two replicate experiments were conducted with 10 samples per treatment for each pathogen. Preliminary analysis of fixed effects using the GLM procedure of SAS® v.8e (SAS, 2002) indicated that log CFU/cm^2 populations were dependent on pathogen and treatment. The viable population data were separated by pathogen and evaluated using nonparametric one-way ANOVA procedures of SAS® v.8e (SAS, 2002) to test treatment differences between least squares means. All differences were reported at a significance level of alpha = 0.05.

RESULTS AND DISCUSSION

Phase I

Reductions in *E. coli* O157:H7 and *S.* Typhimurium populations on beef tissue exposed to decontamination treatments were similar (Table III.2). Overall, individual applications of W, AOW, BOW or AH and sequential applications of two of these solutions for 30 s at ambient temperature (23°C) resulted in $\leq$ 0.6 log CFU/cm^2 pathogen reductions (Table III.2). Generally, treatments that combined W, AOW, BOW or AH with LA or SM resulted in significantly ($P<0.05$) greater reductions in pathogen populations on beef tissue compared to single applications or combinations of W, AOW, BOW or AH treatments (Table III.2). Sequential treatments combining LA or SM with other chemicals did not have additional effects beyond those of the individual treatments but treatments that used SM as the second intervention tended to cause higher bacterial reductions, indicating that a residual bactericidal effect may be occurring (Table III.2). In contrast, however, it appeared that the use of SM alone resulted in higher pathogen reductions than those combination treatments applying SM first (especially SM/LA, SM/AOW and SM/AH) (Table III.2). This suggests that there was a potential neutralization or "quenching" of SM or more specifically the basic pH when it was followed by a subsequent intervention, in particular one with a lower pH. It should be noted that, the effectiveness of LA and especially SM treatments may have been a function of the temperatures of application which were 55 and 82°C, respectively.

Studies (Deza et al., 2003; Fabrizio and Cutter, 2003; Fabrizio et al., 2002; Kim et al., 2000; Koseki et al., 2003; Park et al., 2002a,b; Russell, 2003; Sharma and Demirci,

2003; Stan and Daeschel, 2003; Venkitaranayanan et al., 1999a,b) have shown the effectiveness of electrolyzed oxidizing water against *E. coli* O157:H7 and *Salmonella* in or on various liquid and solid surfaces, respectively. However, in this study, AOW and BOW treatments were not effective at reducing pathogen populations on beef tissue. Application time may affect the efficacy of electrolyzed oxidizing water treatments in reducing bacterial populations; Venkitanarayanan et al. (1999a,b) used long application times (>5 min) compared to the 30 s used in the present study. Additionally, the organic material associated with beef tissue may inactivate the chlorine or other oxidizing compounds during the decontamination process, reducing the efficacy of such treatments on beef carcasses. In the beef industry, the length of time needed to apply a treatment is important and a 5 min application time is too long at current line speeds; additionally, the presence of fecal contamination on beef carcasses may increase the organic load and result in greater inactivation of an electrolyzed oxidizing water treatment, thereby rendering it ineffective as a decontaminant.

Table III.2. Least squares means (± standard deviations) and pooled (mean values for samples from both pathogens) pH of samples indicating survival and reduction of *Escherichia coli* O157:H7 and *Salmonella* on beef tissue after dipping in decontamination solutions (1.5 L) for 30 s.

Treatment group (n=10/pathogen)	*Escherichia coli* O157:H7		*Salmonella*		
	Survival (log CFU/cm^2)	Log Reduction	Survival (log CFU/cm^2)	Log Reduction	Pooled sample pH
Nontreated (NT)	5.5a (0.1)	-	5.2a (0.4)	-	5.73g (0.1)
Water (W, 23°C)	5.2bc (0)	0.3	4.9ab (0.2)	0.3	5.73g (0.1)
Acidified oxidized water (AOW, 23°C)	5.4ab (0.2)	0.1	4.9ab (0)	0.3	5.63g (0.1)
Basic oxidized water (BOW, 23°C)	5.2bc (0.1)	0.3	4.9ab (0)	0.3	5.83g (0.1)
0.1% Ammonium hydroxide (AH, 23°C)	5.3abc (0.1)	0.2	4.8bc (0.1)	0.4	7.60de (0.8)
2.5% Lactic acid (LA, 55°C)	4.4e (0.2)	1.1	4.6cd (0.1)	0.6	4.51j (0.3)
4% Sodium metasilicate (SM, 82°C)	3.6f (0.4)	1.9	3.9fg (0.1)	1.3	9.30ab (0.8)
W / AOW	5.2bc (0.1)	0.3	4.7bc (0.1)	0.5	5.63g (0.1)
W / BOW	5.1bc (0.1)	0.4	4.6cd (0.6)	0.6	5.83g (0.1)
W / AH	5.0cd (0.1)	0.5	4.7bc (0.1)	0.5	8.50c (0.6)
W / LA	4.4e (0.2)	1.1	4.2ef (0.1)	1.0	4.51j (0.1)
W / SM	3.8f (0.3)	1.7	3.6h (0.4)	1.6	9.60ab (0.4)
AOW / W	5.2bc (0.1)	0.3	4.7bc (0.1)	0.5	5.62g (0.1)
AOW / BOW	5.2bc (0.1)	0.3	4.8bc (0.1)	0.4	5.82g (0.2)
AOW / AH	5.2bc (0.1)	0.3	4.8bc (0.1)	0.4	8.04d (0.7)
AOW / LA	4.3e (0.2)	1.2	4.9ab (0.1)	0.3	4.33j (0.1)
AOW / SM	3.7f (0.6)	1.8	3.9fg (0.2)	1.3	9.43ab (0.3)
BOW / W	5.2bc (0.1)	0.3	4.6cd (0.1)	0.6	5.75g (0.1)
BOW / AOW	5.1bc (0.3)	0.4	4.7bc (0.1)	0.5	5.50g (0.1)
BOW / AH	5.2bc (0.1)	0.3	4.6cd (0.1)	0.6	8.41c (0.8)
BOW / LA	4.4e (0.2)	1.1	4.0fg (0.3)	1.2	4.44j (0.1)
BOW / SM	3.3g (0.3)	2.2	3.9fg (0.1)	1.3	9.74a (0.3)
AH / W	5.2bc (0.1)	0.3	4.7bc (0.1)	0.5	7.22e (0.8)
AH / AOW	4.8d (0.2)	0.7	4.7bc (0.1)	0.5	6.72f (0.4)
AH / BOW	5.1bc (0.2)	0.4	4.8bc (0.1)	0.4	7.33e (0.6)
AH / LA	4.7d (0.1)	0.8	4.5cd (0.1)	0.7	5.32gh (0.5)
AH / SM	4.2e (0.3)	1.3	3.8fg (0.1)	1.4	9.61ab (0.2)
LA / W	4.7d (0.1)	0.8	4.4de (0.1)	0.8	4.90hi (0.1)
LA / AOW	4.7d (0.1)	0.8	4.4de (02)	0.8	4.73i (0.1)
LA / BOW	4.4e (0.2)	1.1	5.0ab (0.7)	0.2	4.74i (0.1)
LA / AH	4.8d (0.1)	0.7	4.5cd (0.1)	0.7	7.32e (0.7)
LA / SM	3.8f (0.2)	1.7	3.5h (0.3)	1.7	9.31ab (0.6)
SM / W	4.2e (0.2)	1.3	3.8fg (0.1)	1.4	9.01b (0.4)
SM / AOW	4.4e (0.1)	1.1	3.7gh (0.1)	1.5	8.50c (0.7)
SM / BOW	4.2e (0.2)	1.3	3.8fg (0.3)	1.4	8.60c (0.7)
SM / AH	4.1ef (0.1)	1.4	3.8fg (0.2)	1.4	9.60ab (0.2)
SM / LA	4.3e (0.2)	1.2	3.7gh (0.2)	1.5	6.60f (0.3)

abcdefghij - means within the same column with different letters are different ($P<0.05$)

Treatments involving high pH such as ammonium hydroxide, trisodium phosphate, or sodium metasilicate are thought to be more effective against Gram-negative bacteria, because of their thinner cell walls compared to Gram-positive bacteria, as a result of disruptions in the cytoplasmic membrane (Mendonca et al., 1994). In this study, treatment of beef tissue with AH did not result in significant ($P \geq 0.05$) reductions in pathogen populations. Ammonia has been used to treat beef trimmings (for use in production of lean finely textured beef) to reduce microbial populations (Niebuhr and Dickson, 2003). For ammonia treatments to have an immediate bactericidal (6 log reduction) effect on pathogens in the latter application, the pH of the meat must be >9.0 (Niebuhr and Dickson, 2003). In that study, the ammonium hydroxide solution had a pH of 10.89 which may be expected to cause destruction or at least injury of cells; however, considering that the beef tissue used contained a high proportion of adipose tissue (approximately pH 5.7), AH was only able to raise the pH of the tissue to 7.6, which may not have been high enough to adversely affect the pathogens.

Sodium metasilicate (pH 12.3) had a greater effect than did other treatments on pathogen populations in this study (Table III.2). Different application temperatures (4, 23, 55 or 82°C) of 4% SM did not cause any difference ($P \geq 0.05$) in pathogen population reduction (Table III.3). Thus, it appeared that temperature of application did not influence the antimicrobial effect of SM; however, it is possible that a concentration of 4% had a limit of antimicrobial activity not enhanced by temperature. When SM was evaluated with different concentration and temperature combinations (1% at 55°C, 1% at 82°C, 4% at 55°C and 4% at 82°C) the effect of SM concentration was less at the higher application temperature (Table III.4). Thus, it appears that the efficacy of SM applied at 1% may be

more dependent upon the temperature of application than would be the efficacy of SM applied at 4%. Treatment of beef tissue with SM resulted in adverse visual appearance of product including a grayish coloring and wrinkling effect on the adipose tissue. Additional studies need to be conducted with differing parameters (temperature, concentration and pressure of application) to evaluate the effect of such parameters on the efficacy of SM in reducing pathogen populations and on the resulting sensory properties of beef tissues.

Table III.3. Least squares means (± standard deviations) indicating survival and reduction of *Escherichia coli* O157:H7 on beef carcass tissue after exposure to sodium metasilicate (SM; 4% [wt/wt]) at different temperatures.

Temperature of SM application (°C)	Survival (log CFU/cm^2)
Nontreated control	6.3a (0.2)
4	3.9b (0.2)
23	4.1b (0.3)
55	3.9b (0.4)
82	4.0b (0.3)

ab - means within the same column with different superscript letters are different (P<0.05)

Table III.4. Least squares means (± standard deviations) indicating survival and reduction of *Escherichia coli* O157:H7 on beef carcass tissue after exposure to sodium metasilicate (SM) at different temperature-concentration combinations.

Temperature (°C)	Concentration (%)	Survival (log CFU/cm^2)
Nontreated control		5.6a (0.3)
55	1	4.2bc (0.2)
	4	3.9c (0.4)
82	1	3.6c (0.4)
	4	3.7c (0.3)

abc - means within the same column with different letters are different (P<0.05)

Phase II

The adverse effect of the sodium metasilicate treatment on the beef tissue described above was reversed when spray-chilling was included in the process. *Escherichia coli* O157:H7 and *Salmonella* had similar survival trends in response to the application of

interventions in the multiple-hurdles systems. In general, it appeared that the multiple-hurdles systems were separated into two groups based on efficacy to decontaminate beef tissue. The first group of treatment systems included those using one or more thermal (82°C) applications with no 5% LA or a single 5% LA application that resulted in 1.7 to 2.1 log/CFU/cm^2 pathogen reductions (Table III.5). The second and more effective group of treatment systems included those combining two 5% LA applications or a thermal (82°C) and 5% LA application that resulted in 2.3 to 3.3 log CFU/cm^2 pathogen reductions (Table III.5). The use of a single hot (82°C) water treatment did not generate results different ($P \geq 0.05$) than did applying two hot treatments and two warm (55°C) water treatments (Table III.5). This would imply that there is a limit in pathogen reduction brought about by hot (82°C) solutions that is not enhanced by further thermal (hot [82°C] or warm [55°C]) treatments and suggests that a potential resistant pathogenic sub-population exists, which, after selection due to certain interventions, may be tolerant to subsequent interventions. Reduction caused by treatment systems comprising one or more thermal treatments and no 5% LA application were similar ($P \geq 0.05$) to that resulting from single application of 5% LA. Thus, hot (82°C) water and 5% LA may have similar ($P \geq 0.05$) decontaminating effects on beef tissue; however, it appeared that the reduction caused by 5% LA was further enhanced by subsequent 5% LA applications. This may be due to the 48-h time lapse between the pre-chill application of 5% LA and the post-chill application of the acid which may have provided a stressful environment for the mesophilic pathogen, and sensitized the cells to the subsequent acid treatment. Under the conditions of this study, hot (82°C) treatment followed by the use of 5% LA or the exclusive use of two 5% LA treatments appeared to enhance pathogen reduction and

should be considered when selecting interventions for incorporation in a multiple-hurdles system. The use of multiple-hurdles systems increases the chance of beef carcasses meeting established regulatory requirements for *E. coli* O157:H7 and *Salmonella.* The intervention systems evaluated in the present study could easily be incorporated in a multiple-hurdles system in most U.S. beef packing plants and furthermore, the alternative approaches identified in this study that result in comparable reductions may provide options for the industry to select systems that best fit the scenario in individual plant settings.

CONCLUSIONS

Results of this study demonstrated that individual treatments or combinations among W, AOW, BOW or AH had minimal decontaminating effects on fresh beef tissue, while individual applications or combinations using 2.5% LA at 55°C and especially 4% SM at 82°C resulted in higher pathogen reductions due to the additive effects of the chemical and temperature of application. Under the conditions of this study, it appeared that the temperature of application (4, 23, 55, or 82°C) did not affect the antimicrobial activity of 4% SM while use of 1% SM applied at 82°C appeared to be more effective than when applied at 55°C . Although, electrolyzed oxidative water has been an effective decontaminant of pathogens on food contact surfaces, produce and poultry (applied as a submersion bath), the organic matter loads on beef tissue may result in sufficient inactivation of the decontaminating effects of chlorine or other oxidizing to be ineffective when applied to beef carcasses. Results indicate that there was a limit in pathogen

reduction brought about by a single hot (82°C) treatment that was not enhanced by further hot or warm (55°C) treatments. This suggests the potential existence of a resistant pathogenic sub-population, which may be tolerant to certain interventions and, as such, might be selected to dominate product surfaces and resist subsequent similar interventions. Conversely, it was apparent that the reduction caused by 5% LA may be further enhanced by a subsequent 5% LA treatment possibly due to the effects of cold stress placed on the pathogen between acid applications.

Overall, under the conditions of this study, the most effective pathogen reductions may be achieved with multiple-hurdle systems combining two 5% LA applications or a hot (82°C) water/SM application and 5% LA application followed by those systems with one or more hot (82°C) applications or a single 5% LA application. Specifically, results of this study suggest that use of one hot (82°C) water application followed by one 5% LA application is sufficient to achieve the maximum reductions with the chemicals tested. Currently, the most commonly applied decontamination interventions in the industry include the combination of a hot water treatment followed by a lactic acid application, and results of this study indicate that additional applications of hot water or lactic acid do not enhance the effectiveness of such programs. Results of the present study suggest that, for beef processing plants with limited opportunity to incorporate several decontamination interventions there are options for applying one or two interventions that may result in comparable pathogen reductions.

Table III.5. Least squares means (± standard deviations) and pooled (mean values for samples from both pathogens) pH of samples indicating survival and reduction of *Escherichia coli* O157:H7 and *Salmonella* on beef tissue not dipped (control) or after dipping (for 30 s in 1.5 L) in various treatment systems.

Organism	Treatment system	Survival (log CFU/cm^2)	Reduction (log CFU/cm^2)	Pooled sample pH
E. coli O157:H7	Control	5.7a (0.2)	-	5.54b (0.09)
	0-H-0-0	4.0b (0.4)	1.7	5.62b (0.12)
	S-H-W-W	3.9b (0.3)	1.8	6.02a (0.23)
	H-H-W-W	3.8b (0.7)	1.9	5.69b (0.12)
	0-0-0-L	3.7b (0.3)	2.0	4.46c (0.15)
	0-0-L-0	3.6b (0.3)	2.1	3.80e (0.20)
	0-H-L-L	2.8c (0.3)	2.9	4.08cd (0.10)
	0-H-L-0	2.7c (0.2)	3.0	4.21c (0.43)
	S-H-0-L	2.7c (0.4)	3.0	3.90de (0.20)
	0-0-L-L	2.6c (0.4)	3.1	3.70e (0.40)
	S-H-L-0	2.6c (0.2)	3.1	4.00cde (0.10)
	H-H-L-L	2.5c (0.4)	3.2	4.10cd (0.09)
	S-H-L-L	2.5c (0.5)	3.2	4.24c (0.07)
	S-0-L-L	2.4c (0.6)	3.3	3.90de (0.10)
Salmonella	Control	5.6a (0.3)	-	5.54b (0.09)
	0-0-0-L	3.7b (0.5)	1.9	5.62b (0.12)
	0-H-0-0	3.7b (0.3)	1.9	6.02a (0.23)
	H-H-W-W	3.6b (0.3)	2.0	5.69b (0.12)
	0-0-L-0	3.5b (0.4)	2.1	4.46c (0.15)
	S-H-W-W	3.3b (0.4)	2.3	3.80e (0.20)
	S-H-0-L	3.2bc (0.2)	2.4	4.08cd (0.10)
	0-0-L-L	2.9c (0.2)	2.7	4.21c (0.43)
	0-H-L-0	2.9c (0.5)	2.7	3.90de (0.20)
	0-H-L-L	2.8cd (0.5)	2.8	3.70e (0.40)
	S-H-L-0	2.8cd (0.4)	2.8	4.00cde (0.10)
	H-H-L-L	2.8cd (0.4)	2.8	4.10cd (0.09)
	S-0-L-L	2.5d (0.4)	3.1	4.24c (0.07)
	S-H-L-L	2.3d (0.4)	3.3	3.90de (0.10)

abcde - means within the same column for each pathogen with different letters are different ($P<0.05$).
0: nondipped
H: dipped in hot (82°C) water
L: dipped in 5% lactic acid (55°C)
S: dipped in 1% sodium metasilicate (82°C)
W: dipped in warm (55°C) water

CHAPTER IV

EFFECT OF SIMULATED SPRAY-CHILLING WITH CHEMICAL SOLUTIONS ON ACID-HABITUATED AND NONACID-HABITUATED *ESCHERICHIA COLI* O157:H7 CELLS ATTACHED TO BEEF CARCASS TISSUE

ABSTRACT

Samples (10x20x2.5 cm) of beef carcass tissue were inoculated (10^4-10^5 CFU/cm^2) with *Escherichia coli* O157:H7 which was either nonacid-habituated (prepared by incubating at 15°C for 48 h in inoculated filter-sterilized composite [1:1] of hot and cold water meat decontamination runoff fluids – W; pH 6.05) or acid-habituated (prepared in inoculated W fluids mixed with filter-sterilized 2% lactic acid runoff fluids in a proportion of 1/99 [vol/vol] – LA/W; pH 4.12). The inoculated surfaces were exposed to conditions simulating carcass chilling (-3°C for 10 h followed by 38 h at 1°C). Treatments applied to samples (between 0 and 10 h) during chilling included: (i) no spraying (NT), or spraying (for 30 s every 30 min) with (ii) water (W), (iii) cetylpyridinium chloride (CPC; 0.1 or 0.5%), (iv) ammonium hydroxide (AH; 0.05%), (v) lactic acid (LA; 2%), (vi) acidified sodium chlorite (ASC; 0.12%), (vii) peroxyacetic acid (PAA; 0.02%), (viii) sodium hydroxide (SH; 0.01%), or (ix) sodium hypochlorite (SC; 0.005%) solutions of 4°C. Samples were taken at 0, 10, 24, 36 and 48 h of the chilling process to determine changes in *E. coli* O157:H7 populations. Phase I tested W, SH, PAA, LA and 0.5% CPC on meat inoculated with nonacid-habituated pathogen populations, while phase II tested

W, SC, AH, ASC, LA and 0.1% CPC on meat inoculated with acid- and nonacid-habituated populations. Reductions in nonacid-habituated *E. coli* O157:H7 populations from phase I increased in the order NT = W = SH < PAA < LA < CPC. Reductions from phase II for acid-habituated cells increased in the order NT=W= SC < ASC=LA= AH < CPC, while on nonacid-habituated cells the order observed was NT=W=SC < AH=ASC < LA < CPC. Previous acid-habituation of *E. coli* O157:H7 inocula rendered the cells more resistant to the effects of spray-chilling, especially with acid; however, the trend of reduction remained spray-chilling with water = nonspray-chilling < spray-chilling with chemical solutions.

INTRODUCTION

Most of the contamination introduced on beef carcasses during the slaughtering and dressing process is nonpathogenic; however, pathogens may be present and pose a significant public health threat. In an attempt to comply with regulatory criteria (USDA-FSIS, 1996) established by the USDA-FSIS, the beef industry focuses primarily on meat decontamination through application of steam-vacuuming as well as pre- and post-evisceration spraying with hot water and/or organic acid solutions or exposure to steam (Bacon et al., 2000b; Sofos and Smith, 1998). The decontamination strategies applied to fresh beef are intended to reduce levels of spoilage and pathogenic microorganisms; however, they may create potentially stressful sublethal environments that may induce tolerances or resistances over time and subsequently may allow presence of resistant survivors (Stopforth et al., 2003b).

Although drying of the surface during carcass cooling may contribute to microbiological control, it may also result in loss of carcass weight, which is economically undesirable (Gill, 1998). Thus, spray-chilling has been adopted and implemented in most slaughtering plants in North America to increase chilling rates by evaporative cooling, reduce water losses of carcasses (Dickson and Anderson, 1992; Gill, 1998), and facilitate achieving the recommended chilling of deep muscle (15 cm) to 10.0°C within 24 h and to 7.2°C within 36 h (NACMCF, 1993). In a typical U.S. beef packing plant, carcasses are initially exposed to an environment of approximately -3°C for up to 12 h, then to approximately 1°C for the remainder of the chilling time (typically 26-38 h) (Dickson, 1991). During the first 12 h of chilling, carcasses may be exposed intermittently (typically for up to 2 min every 30 min) to a fine mist of 2°C water or chlorine (up to 50 ppm) solution. Decontamination treatments are applied most commonly as a final process on beef carcasses while they are warm and before they are chilled; however, application of spray-chilling with sanitizers (i.e., chlorine) has been considered as potentially contributing to the control of bacterial populations (Dickson and Anderson, 1992). Fluctuations in chiller temperatures, inadequate chilling time and contact between carcasses may result in failure to adequately chill carcasses and may allow growth of bacteria, including pathogens. Mesophilic and psychrotrophic bacteria have the potential to grow on carcasses exposed to spray-chilling using water (Gill and Landers, 2003). Specifically, research has indicated that *E. coli* (Gill and Bryant, 1997; Gill and Landers, 2003), and furthermore *E. coli* O157:H7 (Dorsa et al., 1997), have the ability to grow on carcasses in the chiller. Solutions of peroxyacetic acid, acidified sodium chlorite, trisodium phosphate, activated lactoferrin and organic acids (i.e., acetic

or lactic acid) are approved for application on warm beef carcasses in U.S. packing plants (Bjerklie, 2001). In addition to the use of chlorine (Swift and Company, 1973), studies have evaluated the use of acetic or lactic acid (Dickson, 1991; Hamby et al., 1987) incorporated into the spray-chilling process as a method of sanitizing beef carcasses and found significant reduction in total aerobic and pathogen populations. Additional studies are needed to evaluate the effect of approved or proposed chemicals incorporated into the spray-chilling process on survival of pathogens contaminating carcass surfaces.

Bacteria may become resistant to sublethal antimicrobial hurdles which may potentially lead to cross-protection from other environmental food-related stresses (Samelis and Sofos, 2003). Studies evaluating stress responses of pathogenic microorganisms have mostly been conducted with laboratory media; however, considering the variable reactions of pathogens in natural systems, it is imperative to study *in situ* stress-adaptation or adaptation induced within a specific food environment like meat decontamination runoff fluids (washings) (Samelis et al., 2001a,b, 2002a,b; Stopforth et al., 2002, 2003a,b). This is, to the best of our knowledge, the first published study simulating the effect of previous stress-adaptation (specifically acid-habituation) in a natural system of decontamination fluids on subsequent survival of *E. coli* O157:H7 inoculated on fresh beef tissue exposed to simulated spray-chilling.

The objective of this study was to investigate the effectiveness of simulated chilling without spraying and spray-chilling using water and chemical solutions in reducing *E. coli* O157:H7 contamination on beef carcass tissue. In addition, this study also evaluated the effect of previous acid-habituation (in sublethal acidic meat decontamination runoff fluids) or nonacid-habituation (in water meat decontamination

runoff fluids) on subsequent responses of *E. coli* O157:H7 cells on beef carcass tissue exposed to spray-chilling treatments. This study involved two phases: phase I was conducted to evaluate antimicrobial effects of selected chemicals on nonacid-habituated *E. coli* O157:H7, while phase II evaluated the two most effective treatments from phase I against additional chemicals approved for fresh beef decontamination that may enhance microbial reduction of both acid- and nonacid-habituated *E. coli* O157:H7.

MATERIALS AND METHODS

Preparation of fresh beef decontamination runoff fluids

Spray-washing runoff fluids from decontamination of beef carcasses at a commercial slaughtering plant using water (84°C at spraying) or lactic acid (2.5% in runoff) solutions (55°C at spraying) were collected and stored in 10-L sterile bottles (Nalgene, Nalge Co., Rochester, NY) for ≤30 d at -30°C. The thawed (4°C overnight) washings were passed through four layers of cheesecloth three times to remove large beef tissue particles. Following initial filtering, the washings were passed through Fisherbrand® filter paper (Qualitative P8; Fisher Scientific, Houston, TX) four times using a Buchner funnel under vacuum to remove remaining small beef particles. The resulting fluid was filter-sterilized through 500-ml Millipore™ Stericups (0.22 μm GV Durapore Membrane; Millipore Corporation, Bedford, MA) under vacuum. After preparation, the meat washings were tested microbiologically to ensure sterility by plating onto TSA. The pH of the washings was measured before storage and on each day of analysis with a digital pH meter

(*Ultra*Basic, UB-10; Denver Instrument Co., Denver, CO) and a glass pH electrode (pH/ATC Electrode #300729.1; Denver Instrument).

Bacterial strains and acid-habituation of *E. coli* O157:H7

Five rifampicin (Rif) resistant (100 μg/ml) derivatives of *E. coli* O157:H7 (ATCC 43895, ATCC 43889, ATCC 51658; JKS111; EO139) developed in our laboratory (using methods described by Hardin et al., 1995) were used in this study. The Rif-resistant strains were used to selectively detect the pathogen inoculum on TSA (Difco) with Rif (100 μg/ml; Sigma Chemical Company, St. Louis, MO) (TSA+Rif) in the presence of the natural microbial flora associated with beef carcass tissue. The strains were stored as frozen (-70°C) cultures in TSB (Difco) with 25% glycerol and were activated by streaking onto TSA+Rif and incubating at 35°C. After overnight incubation, a single colony was used to inoculate TSB (10 ml containing 0.25% glucose), which was incubated statically at 35°C for 18-24 h.

The cultures were used to individually inoculate (*ca.* 5 log CFU/ml) either filter-sterilized water beef washings (W; pH 6.05) for preparation of nonacid-habituated cultures or W washings mixed with filter-sterilized 2.5% lactic acid washings (LA) in a proportion of 1/99 (vol/vol; LA/W, pH 4.12) for preparation of acid-habituated cultures. The inoculated washings were subsequently incubated for 48 h at 15°C (a reasonable beef processing plant abuse temperature) to allow the pathogen to become habituated in the environment of washings. The five individual cultures of *E. coli* O157:H7 from each type of washings were combined (50 ml) after the 48 h habituation period and centrifuged at 4,629 x g (Eppendorf, model 5810 R; Brinkmann Instruments. Inc., Westbury, NY) for

15 min at 4°C. The resulting W and LA/W pellets were washed in sterile phosphate-buffered saline (PBS, approximately pH 7.4; Sigma) centrifuged for a second time and the final pellet was resuspended in 50 or 5 ml PBS, respectively, for further use in the studies. Different volumes were used for resuspension of W and LA/W cultures to maximize recovery of pathogen populations harvested from LA/W washings as they were lower than those from W washings during the habituation period.

Acid tolerance response (ATR) of inocula

The nonacid-habituated inoculum was serially diluted in 0.1% BPW (approximately pH 7.2; Difco) to yield approximately 5.5 log CFU/ml, while the acid-habituated culture was used directly for subsequent acid tolerance tests. A 1-ml volume of each composite inoculum was inoculated in 9-ml BPW for control samples (time-0) and 9 ml TSB with 0.6% yeast extract (TSBYE) acidified to pH 3.5 with lactic acid (Purac® FCC 88; Purac America, Lincolnshire, IL) for 120 min to evaluate acid tolerance. Samples (1 ml) were taken at 30 min intervals and inoculated in 9-ml 0.1% BPW to neutralize the pH effect and act as diluent for determination of surviving populations by plating appropriate dilutions (in 0.1% BPW) of samples onto TSA agar.

Preparation of spray-chilling solutions

Distilled water (pH 7.01) was used for water spray-chilling treatments and for preparation of the following solutions in volumes of 2 L each: (i) sodium hypochlorite (0.005%, SC - pH 9.18) from a commercial sodium hypochlorite preparation (6.2% NaOCl, Chlorox® Ultra; The Clorox Co., Oakland, CA); (ii) lactic acid (2%, LA - pH 2.24) from 88% lactic

acid preparation (Purac); (iii) ammonium hydroxide (0.05%, AH - pH 10.77) from a 28-30% ammonium hydroxide solution (Mallinckrodt; Mallinckrodt and Baker, Inc., Paris, KY); (iv) cetylpyridinium chloride (0.1 or 0.5%, CPC - pH 6.56-6.86) from a 40% cetylpyridinium chloride solution (Cecure®; Safe Foods Corporation, Little Rock, AR); (v) acidified sodium chlorite (0.12%, ASC - pH 2.90) from a 7.5% sodium chlorite solution (Dioxy-Chlor; Birko Corporation, Westminster, CO) acidified with citric acid (USP-FCC Anhydrous; Humco, Texarkana, TX); (vi) sodium hydroxide (0.01%, SH - pH 11.0) from anhydrous sodium hydroxide pellets (Mallinckrodt); and, (vii) peroxyacetic acid (0.02%, PAA - pH 3.37 from a commercial peroxyacetic acid solution (5% peracetic acid, Vigorox®; Birko). Although it is known that SC adjusted to a pH 6.8 may be more effective than when unadjusted (Stopforth et al., 2002), use of pH-unadjusted SC in this experiment was selected to represent current application in the industry.

Spray-chilling treatments

The following treatments (three samples for each of the five sampling times in each of two replicates) were evaluated during phase I with nonacid-habituated inocula: (i) NT; (ii) W; (iii) LA; (iv) PAA; (v) CPC (0.1%); and, (vi) SH. The two most effective treatments (LA and CPC) were selected from phase I to be evaluated in a more comprehensive treatment comparison in phase II. The maximum number of treatments that was technically feasible to be conducted simultaneously was limited to seven. Thus, chemical treatments approved for fresh beef decontamination were selected for evaluation of their effects against acid- or nonacid-habituated *E. coli* O157:H7 cells. In phase II of the study the following treatments (three samples for each of the five

sampling times in each of two replicates) were evaluated for acid- and nonacid-habituated inocula: (i) NT; (ii) W; (iii) LA; (iv) ASC; (v) SC; (vi) AH; and, (vii) CPC (0.5%). After phase I, two concentrations (0.1 and 0.5%) of CPC were tested in a preliminary study to determine the concentration that would be used in phase II. The level of 0.1% CPC reduced *E. coli* O157:H7 by 3.2 logs while the use of 0.5% CPC rendered the pathogen undetectable (<0 log CFU/cm^2) (initial population of 4.2 logs). Based on these results, 0.5% CPC was chosen for evaluation of its effect on acid-habituated *E. coli* O157:H7 populations in phase II of the study.

Inoculation and spray-chilling of beef carcass tissue

Fresh decontaminated (approximately 82°C water) beef brisket adipose tissue was obtained from a commercial beef processing plant and used $\leq$ 6 h postmortem to represent the outside surface of a beef carcass. The adipose tissue samples to be inoculated were cut to measure 200 cm^2 in total (10x20x2.5 cm on the outside surface of the adipose tissue) and were placed on a wire mesh grid suspended over a tray for inoculation and subsequent application of spray-chilling solutions. Samples were inoculated with 1 ml/200 cm^2 (4-5 log CFU/cm^2) of either acid-habituated or nonacid-habituated cultures and allowed to attach for 45 min at ambient (23-25°C) temperature. After attachment, a 40 cm^2 (4x10x0.5 cm) layer was removed from all pieces by surface excision and analyzed microbiologically, as described below, for time-0 samples. The remaining adipose tissue samples were exposed to conditions of beef carcass chilling at -3°C for 10 h in a refrigerated incubator (VWR™ Refrigerated Incubator Model # 2020, Shell Lab, Sheldin Manufacturing, Inc., Cornelius, OR), without or with intermittent

spraying (every 30 min) of the various chemical solutions followed by 38 h of holding at 1°C.

Treatment of adipose tissue during the chilling process was achieved by overhead (20 cm from tissue surface) spraying (simulating misting) of samples suspended on a wire mesh using a 1-L Envirokind® All-purpose Sprayer (Delta Industries, N. Hollywood, CA). Inoculated samples were left nontreated (NT) or treated by spraying (manual depression of spray mechanism repeated three times/sample) with the appropriate treatment solution (approximately 4°C). Following completion of treatments (every 30 min during initial 10 h), a surface portion (40 cm^2) of the treated samples was excised and analyzed microbiologically and for pH. The remaining tissue was further chilled at 1°C for an additional 38 h and analyzed, as above, at 24, 36 and 48 h of the chilling process.

Microbiological and physical analysis of samples

Tissue samples were placed into an 18-oz Whirl-Pak® filter sterile plastic bag (Nasco, Fort Atkinson, Wisconsin) containing 40 ml of sterilized MRD and homogenized (Masticator, IUL Instruments, Barcelona, Spain) for 2 min. A portion (1 ml) of the homogenized sample was serially diluted in 9 ml of sterile 0.1% BPW and appropriate dilutions were plated onto TSA for determination of total bacterial counts and onto TSA+Rif or sorbitol MacConkey agar (SMAC; Difco) supplemented with tellurite and cefixime (CT-supplement; Dynal Ltd., Lake Success, NY) (SMAC-CT) for the selective enumeration of inoculated rifampicin-resistant *E. coli* O157:H7 populations. Colonies were counted after incubation at 35°C for 48 h. The detection limit was considered to be 0

log CFU/cm^2 based on the assumption that plating 1 ml of sample would represent 1 cm^2 of product. If this yielded at least 1 CFU on the agar plate it would give an estimate of the detection limit (0 log CFU/cm^2). However, if we only consider as acceptable a minimum of 25 CFU per plate, then the detection limit would be 1.4 log CFU/cm^2. The pH of the homogenized samples was measured after microbiological analysis as indicated above.

Statistical analysis

Two replicate experiments were conducted for each phase of the study, with three samples per treatment in each replicate. Microbiological data were converted to log CFU/cm^2 before being analyzed. Values for the mean log and standard deviation of each set of bacterial counts were calculated on the assumption of a log-normal distribution of microorganisms. For phase I data, preliminary analysis of fixed effects using the GLM procedure of SAS® v 8.2 (SAS, 2002) indicated that log CFU/cm^2 populations were dependent on type of media (F-statistic=128.06; P<0.0001), treatment (F-statistic=290.16; P<0.0001) and time of analysis (F-statistic=425.66; P<0.0001). The data were evaluated using a 3 x 6 x 5 (media x treatment x time of analysis) factorial design. For phase II, preliminary analysis of fixed effects using the GLM procedure of SAS® v 8.2 (SAS, 2002) indicated that log CFU/cm^2 populations were not dependent on the type of culture media (F-statistic=0.01; P=0.9346) nor on the replicate (F-statistic=0.54; P=0.4609). The data for each agar medium were evaluated using a 7 x 5 x 2 (treatment x time of analysis x type of inoculum, respectively) factorial design. For each culture medium, individual fixed effects and up to three-way interactions were evaluated with ANOVA using the model $y = a + x_1 + x_2 + x_3 + x_1x_2 + x_1x_3 + x_2x_3 + x_1x_2x_3$ in the GLM

procedures of SAS® (SAS, 2002); where x_1 represents treatment (NT, W, SC, LA, CPC, ASC or AH), x_2 represents time of analysis (0, 10, 24, 36 or 48 h) and x_3 represents type of inoculum (acid-habituated vs. nonacid-habituated). Least-squares means were separated using a protected pairwise t-test of SAS® (SAS, 2002). All differences were reported at a significance level of alpha = 0.05.

RESULTS AND DISCUSSION

Determination of ATR of acid- and nonacid-habituated *E. coli* O157:H7 inoculum

The acid-habituated (incubated in LA/W; pH 4.12) *E. coli* O157:H7 cells were more acid resistant ($P < 0.05$) than the nonacid-habituated cells (W; pH 6.05), experiencing reductions of 0.7 and 1.4 (from 4.0 and 4.2 log CFU/ml, respectively) log CFU/ml, respectively, after 120 min of exposure to lactic acid (pH 3.5) with a tailing effect observed between 30 and 120 min of exposure to acid.

Effect of spray-chilling treatments on nonacid-habituated cells

The initial pH of the beef tissue samples (time-0) prior to treatment was 6.80±0.04 which is expectedly higher than the normal pH of postmortem meat due to the higher water content of adipose tissue and because of its pre-rigor state (Table IV.1). Generally, nontreated samples (NT) and those treated with W or CPC maintained a pH near neutrality throughout storage while samples treated with SH and PAA became more basic (pH 8.96) and acidic (pH 3.48), respectively, after spraying (during the initial 10-h), before equilibrating to a near-neutral pH by 24 h, which remained constant throughout

storage. However, LA-treated samples experienced a decrease in pH to 4.27 within the 10-h spray period increasing gradually to pH 5.72 throughout refrigerated storage (Table IV.1).

The antimicrobial effect of the different spray-chilling solutions (phase I) increased in the order NT=W=SH < PAA < LA < CPC, with the last treatment rendering pathogen populations (2.9 to 3.3 log CFU/cm^2 initial) undetectable (<0 log CFU/cm^2) after the 10-h spray-chilling process (Table IV.2). There was a significant ($P < 0.05$) difference between culture media in bacterial recovery, with SMAC-CT allowing less recovery of potentially injured cells during spray-chilling as compared to TSA and TSA+Rif which permitted similar levels of recovery ($P \geq 0.05$) (Table IV.2). The difference in cell numbers considered as indicative of injury was significant ($P < 0.05$) for all treatments with detectable levels of populations but greater in acidic treatments, especially LA, as compared to the higher pH treatments (W and SH) (Table IV.2). The LA residues deposited on the carcass tissue as evidenced by lower pH values, may have caused a residual antimicrobial effect (Dorsa et al., 1997; Goddard et al., 1996; Ikeda et al., 2003). Cetylpyridinium chloride was the most effective treatment, reducing pathogen populations (by approximately 3.0 log CFU/cm^2) to undetectable levels (<0 log CFU/cm^2) after the 10 h of spray-chilling, followed by LA which appeared to have residual injuring effects. However, to the best of our knowledge, CPC has not been approved at this time for use in beef decontamination, while the efficacy of LA is not unexpected as it is one of the most widely studied and used compounds in beef carcass decontamination (Castillo et al., 1998, 2001b; Conner et al., 1997; Dorsa et al., 1997; Ransom et al., 2002).

Table IV.1: Mean (standard deviation) pH values of beef carcass adipose tissue samples inoculated with *Escherichia coli* O157:H7 and exposed to various treatments during the simulated spray-chilling process.

Experimental phase	Treatment	Time during spray-chilling (h)				
		0 (Before spraying)	10	24	36	48
Phase I	NT	6.80az (0.04)	6.94ay (0.08)	6.93az (0.15)	6.96az (0.10)	6.94az (0.08)
	W	6.80bz (0.04)	6.68by (0.26)	7.10az (0.14)	6.98az (0.12)	7.12az (0.14)
	SH	6.80cz (0.04)	8.96az (0.28)	7.06bz (0.07)	7.13bz (0.10)	6.87cz (0.09)
	PAA	6.80az (0.04)	3.48cw (0.45)	6.63by (0.42)	7.08az (0.06)	6.88az (0.24)
	0.1% CPC	6.80az (0.04)	6.82ay (0.32)	6.89azy (0.23)	7.01az (0.07)	6.86az (0.19)
	LA	6.80az (0.04)	4.27cx (0.42)	4.87cx (0.41)	5.43by (0.33)	5.72by (0.33)
Phase II	NT	7.10az (0.07)	7.14ax (0.12)	7.49az (0.21)	7.34az (0.08)	7.00ay (0.30)
	W	7.10az (0.07)	7.32ax (0.14)	7.40az (0.20)	7.35az (0.28)	7.10azy (0.10)
	SC	7.10bz (0.07)	8.50ay (0.24)	7.36bz (0.05)	7.37bz (0.23)	7.28bz (0.10)
	AH	7.10bz (0.07)	9.60az (0.60)	7.36bz (0.09)	7.20bz (0.07)	7.27bz (0.11)
	0.5% CPC	7.10az (0.07)	7.22ax (0.05)	7.41az (0.07)	7.32az (0.09)	7.22az (0.06)
	LA	7.10az (0.07)	4.61dw (0.25)	4.95cy (0.19)	5.07bcy (0.49)	6.86ay (0.25)
	ASC	7.10az (0.07)	7.30ax (0.19)	7.26az (0.11)	7.22az (0.27)	7.25az (0.08)

abc - means in the same row with different letters are different ($P<0.05$)
zyxw - means in the same column with different letters are different ($P<0.05$)
Phase I: Carcass tissue samples (n=6) inoculated with 2.9-3.3 log CFU/cm^2 of nonacid-habituated *E. coli* O157:H7
Phase II: Carcass tissue samples (n=12) inoculated with 5.1-5.3 log CFU/cm^2 of acid- and nonacid-habituated *E. coli* O157:H7
pH values obtained by homogenizing excised tissue and thus, represent the pH at or near the treated surface of the tissue
NT- nontreated; W - water; SH – 0.01% sodium hydroxide; PAA - 0.02% peroxyacetic acid; CPC - 0.1 or 0.5 % cetylpyridinium chloride; LA - 2% lactic acid; SC - 0.005% sodium hypochlorite; AH – 0.1% ammonium hydroxide; ASC – 0.12% acidified sodium chlorite

Table IV.2: Mean (standard deviation) surviving bacterial populations on beef carcass adipose tissue samples inoculated with nonacid-habituated *Escherichia coli* O157:H7 and sprayed at 30 min intervals with various chemical solutions during chilling at -3°C for 10 h and then stored at 1°C for 38 h

Agar media	Spray treatment	Spray application every 30 min for 10 h at -3°C		Duration (h) of refrigerated storage at 1°C		
		0 (Before spraying)	10	24	36	48
TSA	NT	3.3az (0.5)	3.0abz (0.2)	2.8bcz (0.2)	2.6cz (0.3)	2.4cz (0.3)
	W	3.1az (0.3)	3.0az (0.2)	2.8abz (0.2)	2.6bz (0.3)	2.4bz (0.3)
	SH	3.4az (0.4)	2.9bz (0.1)	2.7bcz (0.2)	2.4cdz (0.4)	2.0dz (0.4)
	PAA	3.2az (0.3)	2.1by (0.5)	1.7bcy (0.4)	1.6bcy (0.4)	1.4cy (0.7)
	LA	3.0az (0.2)	1.3bx (0.8)	1.3by (0.4)	0.7bcx (0.7)	0.6cx (0.4)
	0.1% CPC	3.0az (0.3)	<0*bw	<0bx	<0bw	<0bw
TSA+Rif	NT	3.3az (0.5)	3.0abz (0.2)	2.8bcz (0.2)	2.6cz (0.2)	2.4cz (0.4)
	W	3.1az (0.3)	2.8bz (0.2)	2.7bz (0.3)	2.3bcz (0.5)	1.9cz (0.5)
	SH	3.4az (0.4)	2.9bz (0.1)	2.7bcz (0.2)	2.4cdz (0.4)	1.9dzy (0.4)
	PAA	3.2az (0.3)	2.1by (0.5)	1.7bcy (0.4)	1.5cy (0.5)	1.3cy (0.6)
	LA	3.0az (0.2)	1.2bx (0.8)	0.9bcx (0.8)	0.6cx (0.7)	0.4cx (0.4)
	0.1% CPC	3.0az (0.3)	<0bw	<0bw	<0bw	<0bw
SMAC-CT	NT	3.1az (0.4)	2.5bz (0.2)	1.9cz (0.4)	1.8cz (0.5)	1.6cz (0.4)
	W	2.9az (0.3)	2.5az (0.3)	1.7bz (0.5)	1.6bz (0.8)	1.3bzy (0.6)
	SH	3.2az (0.4)	2.5bz (0.2)	2.0cdz (0.3)	1.7dz (0.4)	1.3ezy (0.6)
	PAA	3.1az (0.3)	1.7by (0.5)	0.7cy (0.7)	0.8cy (0.6)	0.7cy (0.6)
	LA	2.9az (0.3)	0.1bx (0.4)	0.1by (0.2)	<0cx	<0cx
	0.1% CPC	2.9az (0.3)	<0bw	<0bx	<0bx	<0bx

TSA: tryptic soy agar; TSA+Rif: TSA supplemented with 100μg/ml rifampicin; SMAC-CT: Sorbitol MacConkey agar with tellurite and cefixime
*: below 0 log CFU/cm^2, the analysis detection limit for cells attached to the adipose tissue
abcde - means in the same row with different letters are different (P<0.05)
zyxw - means in the same column for each agar medium with different letters are different (P<0.05)
NT- nontreated; W - water; SH – 0.01% sodium hydroxide; PAA - 0.02% peroxyacetic acid; CPC - 0.1% cetylpyridinium chloride; LA - 2% lactic acid

Under the conditions of this study, PAA reduced populations of *E. coli* O157:H7 by approximately 1.5 logs (Table IV.2) after the spray-chilling process was completed. Currently, a PAA-based process (21 CFR 173.370) is approved (USDA-FDA, 2003) for washing, rinsing, cooling, or for otherwise processing fresh beef carcasses. This approved process involves use of a mixture of compounds with no more than 0.022% peroxyacetic acid and 0.0075% hydrogen peroxide delivered at a maximum pressure of 250 psi and temperature of 50°C (Inspexx 200™, Ecolab, St. Paul, MN). Gill and Landers (2003)

evaluated the effect of the commercial PAA-based product applied in a sequence of carcass decontaminations at a packing plant and found that total aerobic counts were reduced from 3.07 log CFU/cm^2 to 1.25 log CFU/cm^2. In the present study, use was made of a PAA sanitizer for equipment surfaces and not the commercial PAA-based beef decontamination product and, thus, the efficacy of the commercial product under these conditions is unknown.

Effect of chemical spray-chilling treatments on acid- and nonacid-habituated *E. coli* O157:H7 on beef tissue

The pH of samples inoculated with acid-habituated *E. coli* O157:H7 cells did not differ ($P > 0.05$) from that of the nonacid-habituated counterparts and, as such, the data were pooled to generate mean pH values of adipose samples (Table IV.1). The initial pH of the samples prior to spray-chilling was 7.10±0.07 (Table IV.1). In this experiment, trends observed in pH shifts were similar to those observed in phase I with nontreated samples and those treated with W, ASC and CPC remaining near neutral throughout the spray-chilling process (Table IV.1). Samples treated with basic solutions, like SC and AH experienced an increase in pH, which leveled off by 24 h and remained unchanged throughout refrigerated storage (Table IV.1). Samples treated with LA experienced a decrease in initial pH after spraying and increased slowly throughout storage (Table IV.1).

Surviving total bacteria (TSA) were not different ($P \geq 0.05$) than pathogen counts (TSA+Rif), which may be indicative of the low initial natural flora on the adipose tissue combined with the high initial level of *E. coli* O157:H7 inoculation (Tables IV.3 and

IV.4). Nontreated controls and samples treated with W and SC caused similar ($P \geq 0.05$) reductions in bacterial populations throughout the chilling process, while LA, AH, ASC and CPC were comparatively more ($P < 0.05$) effective in reducing contamination (Tables IV.3 and IV.4). The antimicrobial effect of the spray-chilling solutions on acid-habituated cells increased in the order NT=W= SC < ASC=LA= AH < CPC, while on nonacid-habituated cells the order observed was NT=W=SC < AH=ASC < LA < CPC (Tables IV.3 and IV.4). Treatment with LA, ASC or AH resulted in similar reductions of acid-habituated *E. coli* O157:H7 populations; however, on nonacid-habituated populations, LA was more ($P < 0.05$) effective (approximately 1.0-1.3 logs higher reduction) than ASC and AH which caused similar reductions (Tables IV.3 and IV.4). Under the conditions of this study, ASC was more effective than W and SC, as effective as AH, and generally less effective than LA and CPC in reducing bacterial populations during spray-chilling. Although acid-habituated *E. coli* O157:H7 cells were more resistant to a low pH intervention like LA (pH 2.24) than the nonacid-habituated counterparts, the same was not observed for ASC which is also of low pH (2.9); however, ASC was less effective than LA against nonacid-habituated cells. In our work, the use of ASC (pH 2.9) as a spray application did not cause reduction of the tissue pH after the initial 10-h treatment, and thus, although ASC is an acidic treatment, it has a fast dissipation rate as opposed to that of LA and this may explain the fact that acid-habituated *E. coli* O157:H7 did not have the opportunity to form a resistance to its effects when compared to the nonacid-habituated cells as in the case of LA (Castillo et al., 1999).

Table IV.3: Mean (standard deviation) surviving total bacterial (tryptic soy agar; TSA) populations on beef carcass adipose tissue samples inoculated with acid- and nonacid-habituated *Escherichia coli* O157:H7 and sprayed at 30 min intervals with various chemical solutions during chilling at -3°C for 10 h and then stored at 1°C for 38 h

Type of Inoculum	Spray treatment	Spray application every 30 min for 10 h at -3°C		Duration (h) of refrigerated storage at 1°C		
		0 (Before spraying)	10	24	36	48
Acid-habituated	NT	5.0az (0.3)	4.9az (0.3)	4.8az (0.3)	3.9bz (0.3)	3.9bz (0.3)
	W	4.7az (0.3)	4.6az (0.2)	4.9az (0.2)	3.5bz (0.3)	3.8bz (0.2)
	SC	4.7az (0.3)	5.0az (0.3)	4.2by (0.2)	3.9bz (0.3)	3.7bz (0.4)
	AH	4.2ay (0.3)	3.4by (0.2)	2.8cx (0.2)	2.5cdy (0.3)	2.3dy (0.3)
	ASC	4.7az (0.4)	2.6bx (0.4)	2.9bx (0.2)	2.0cx (0.5)	1.7cx (0.3)
	LA	4.8az (0.3)	2.7bx (0.3)	2.8bx (0.2)	2.8by (0.2)	2.2cyx (0.3)
	0.5% CPC	5.3az (0.2)	0.3bw (0.2)	<0*cx	<0bw	<0cw
Nonacid-habituated	NT	4.8az (0.1)	4.8az (0.2)	4.6az (0.3)	4.0bz (0.2)	3.8bz (0.3)
	W	5.0az (0.3)	4.7az (0.2)	4.5az (0.2)	3.7bz (0.3)	3.1cy (0.3)
	SC	5.3az (0.3)	4.7bz (0.1)	4.0cy (0.2)	3.7cdz (0.4)	3.2dy (0.2)
	AH	4.1ay (0.3)	3.4by (0.3)	2.5cx (0.2)	2.3cdy (0.2)	2.0dw (0.2)
	ASC	5.0az (0.2)	2.6bx (0.4)	2.4bx (0.2)	1.9bcy (0.6)	1.5cw (0.3)
	LA	4.7az (0.3)	1.5bw (0.2)	1.4bw (0.2)	1.1bcx (0.2)	0.8cx (0.3)
	0.5% CPC	5.1az (0.3)	<0bv	<0bv	<0bw	<0bv

*: below 0 log CFU/cm^2, the analysis detection limit for cells attached to the adipose tissue
abcd - means in the same row with different letters are different ($P<0.05$)
zyxwv - means in the same column for each inoculum type with different letters are different ($P<0.05$)
NT- nontreated; W - water; SC – 0.005% sodium hypochlorite; AH – 0.05% ammonium hydroxide; ASC – 0.12% acidified sodium chlorite; LA - 2% lactic acid; CPC - 0.5% cetylpyridinium chloride

Table IV.4: Mean (standard deviation) surviving acid- and nonacid-habituated *Escherichia coli* O157:H7 (TSA+Rif) populations inoculated on beef carcass adipose tissue samples and sprayed at 30 min intervals with different chemical solutions during chilling at -3°C for 10 h and then stored at 1°C for 38 h

		Spray application every 30 min for 10 h at -3°C		Duration (h) of refrigerated storage at 1°C		
Type of Inoculum	Spray treatment	0 (Before spraying)	10	24	36	48
Acid-habituated	NT	5.0az (0.3)	4.8az (0.2)	4.7az (0.3)	4.0bz (0.2)	4.0bz (0.2)
	W	4.7az (0.3)	5.0az (0.2)	4.9az (0.3)	3.7bz (0.3)	4.0bz (0.2)
	SC	5.0az (0.3)	4.9az (0.3)	4.2by (0.2)	4.0bz (0.3)	3.8bz (0.3)
	AH	4.2ay (0.4)	3.3by (0.2)	2.7cx (0.3)	2.3cdx (0.2)	2.1dx (0.3)
	ASC	4.9az (0.2)	2.7bx (0.2)	3.0bx (0.3)	1.9cx (0.4)	1.8cx (0.3)
	LA	4.8az (0.4)	2.8bx (0.4)	2.8bx (0.2)	2.8by (0.2)	2.5byx (0.1)
	0.5% CPC	5.3az (0.3)	0.3bw (0.2)	<0*cw	<0cw	<0cw
Nonacid-habituated	NT	4.5ay (0.3)	4.7az (0.2)	4.5az (0.2)	3.9bz (0.1)	4.0bz (0.2)
	W	5.2az (0.3)	4.6bz (0.3)	4.7bz (0.3)	3.6cz (0.3)	3.1dy (0.2)
	SC	5.3az (0.3)	4.7bz (0.1)	4.0cy (0.4)	3.6dz (0.2)	3.5dy (0.2)
	AH	4.2ay (0.3)	3.1by (0.3)	2.2cx (0.2)	2.1cy (0.1)	1.8cx (0.2)
	ASC	4.3ay (0.6)	2.6by (0.5)	2.5bx (0.3)	2.2by (0.7)	1.5cx (0.5)
	LA	4.8az (0.3)	1.4bx (0.1)	1.5bw (0.2)	1.2bcx (0.2)	0.8cw (0.4)
	0.5% CPC	5.1az (0.6)	<0bw	<0bv	<0bw	<0bv

TSA+Rif: TSA supplemented with 100μg/ml rifampicin
*: below 0 log CFU/cm^2, the analysis detection limit for cells attached to the adipose tissue
abcd- means in the same row with different letters are different (P<0.05)
zyxwv - means in the same column for each inoculum type with different letters are different (P<0.05)
NT- nontreated; W - water; SC – 0.005% sodium hypochlorite; AH – 0.05% ammonium hydroxide; ASC – 0.12% acidified sodium chlorite; LA - 2% lactic acid; CPC - 0.5% cetylpyridinium chloride

In this study, the most effective spraying treatment was CPC, rendering nonacid-habituated cells (initial population of 5.1 log CFU/cm^2) undetectable (<0 log CFU/cm^2) within the initial 10-h of spraying and acid-habituated cells undetectable by 24-h of the chilling process (Tables IV.3 and IV.4). It has been suggested that the antimicrobial activity of CPC may be both time and concentration dependent (Mattick et al., 2002); both concentrations tested (0.1% in phase I and at 0.5% in phase II) proved to be the most effective spraying treatments evaluated. Additional investigation of CPC applied at a concentration of 0.1% and 0.5% in the spray-chilling process resulted in reductions of

nonacid-habituated *E. coli* O157:H7 populations from 4.2 log CFU/cm^2 to 1.0 and 0 log CFU/cm^2 after the initial 10-h spray treatments and 1.5 and 0 log CFU/cm^2, respectively, after the completed spray chilling process. In the case where 0.1% CPC was used after the 10-h application, there was an additional 0.5 log reduction while the use of CPC had a residual injuring effect for the remaining 38 h (data not shown in tabular form). Based on the conditions of this study, it is thus reasonable to assume that the activity of CPC may be concentration dependent and that it has a residual injuring effect on *E. coli* O157:H7 populations attached to beef carcass tissue.

The results indicated that *E. coli* O157:H7 from sublethal acid beef washings (LA/W; pH 4.12) survived better (1.0-1.3 logs) on beef adipose tissue than pathogen populations originating from water washings when exposed to LA treatments at pH 2.24, indicating that LA was less effective on acid-habituated *E. coli* O157:H7 cells as compared with nonacid-habituated cells. Bacteria may respond to stressful food conditions by developing certain tolerances like acid tolerance in the case of acid treatments or acid used for preservation in the food industry. The expression of induced acid tolerance may result in more permanent resistances depending on the intensity of the acid stress with regard to level and time of exposure. Stationary phase cells, exposed to conditions inducing acid-adaptation (i.e., media with additional glucose), may result in cells displaying higher ATR as compared to non-adapted cells (Buchanan and Edelson, 1996). In this study, a variation of acid-adaptation, termed acid-habituation, was applied and involved the inoculation (5 logs) of stationary phase cells into sublethal (pH 4.12) acidic washings which was too stressful to promote pathogen growth but did not result in death and as a result the pathogen became habituated to the effects of the low pH as

opposed to the nonacid-habituated cells in nonacidic W washings (pH 6.05). Previous research in our laboratory (Samelis et al., 2002a) indicated that *E. coli* O157:H7 cells in suspension of lethal (pH 3.1) and sublethal (pH 4.7) mixtures of lactic acid washings stored at 10°C and exposed to broth acidified with LA to pH 3.5 were more resistant than cells from water washings. Acid-habituation of *E. coli* O157:H7 cells in beef washings resulted in cells that were more (by 0.7 logs) acid tolerant than their nonacid-habituated counterparts when exposed to LA at pH 3.5 (unpublished data). However, when bacterial populations were inoculated onto a natural system of beef carcass tissue and exposed to LA at pH 2.24, the acid tolerance of acid-habituated cells was higher (by up to 1.4-1.7 logs) than that observed in the washings (Tables IV.3 and IV.4). Increased resistance observed in *E. coli* O157:H7 cells on the surface of beef carcass tissue as compared with the cells in suspension of beef decontamination washings may be the result of at least three potential factors. Firstly, it is well known that attached cells or cells adherent to a surface are more resistant to antimicrobials than those in suspension (Frank and Koffi, 1990), and thus, cells attached to the carcass tissue may be more resistant than the corresponding cells in the washings due to the protection offered by the tissue and the protection afforded by the very nature of attached bacteria that possess a stronger, more resistant exopolysaccharide layer surrounding the cells (Stopforth et al., 2002). Secondly, the use of carcass tissue provides a unique environment, which poses stressful conditions in the form of bacterial competition, nutrient limitation, oxidation and attachment and may subsequently cause cross-protection to other stresses including acid conditions. Thirdly, the physiological state of the cells after inoculation of carcass tissue may have been different than that of the inocula.

CONCLUSIONS

This study evaluated application of various pre-chilling carcass chemical decontamination interventions (Castillo et al., 1998,1999,2001b; Cutter et al., 2000; Ransom et al., 2002; Uyttendaele et al., 2001) as spraying treatments under conditions simulating the spray-chilling process of beef carcasses. In general, spray-chilling with water was similar to nonspray (dry)-chilling in reducing *E. coli* O157:H7 populations on beef carcass tissue although spray-chilling using chemicals was more effective than either dry or water spray-chilling. As applied in this study, water, sodium hydroxide and sodium hypochlorite were less effective spray-chilling treatments for reducing attached pathogen populations than peroxyacetic acid, acidified sodium chlorite, ammonium hydroxide, lactic acid or cetylpyridinium chloride. The ineffectiveness of water, sodium hydroxide and sodium hypochlorite was most likely the result of injury of pathogen cells caused by evaporative cooling. The most effective spraying treatment – that using cetylpyridinium chloride - reduced pathogen populations by almost 5 log CFU/cm^2 after 24 h of chilling. As indicated, cetylpyridinium chloride is currently not approved for decontamination of beef, and thus, of the approved solutions, lactic acid was the most effective followed closely by acidified sodium chlorite. Previous acid habituation of *E. coli* O157:H7 inocula in meat decontamination runoff fluids may have rendered the cells more resistant to the effects of spray-chilling, especially with acid, but spray-chilling with chemical solutions was still more effective than dry-chilling or spray chilling using water. Pathogen cells originating in acidic beef washings and contaminating beef carcass tissue

may experience increased resistance to subsequent acid interventions. Spray-chilling with approved chemicals may be considered as an "in-cooler" intervention and thereby constitutes another hurdle in the multiple-step decontamination system employed in U.S. packing plants.

CHAPTER V

ACID TOLERANCE RESPONSE OF NONADAPTED AND ACID-ADAPTED *ESCHERICHIA COLI* O157:H7 STRAINS GROWN AS A MIXTURE, OR GROWN INDIVIDUALLY AND MIXED, PRIOR TO INOCULATION IN BEEF DECONTAMINATION RUNOFF FLUIDS OR ON BEEF TISSUE

ABSTRACT

Escherichia coli O157:H7 contamination in fresh beef plants may consist of single or mixed strains and strain interactions may result in variable responses of cells to subsequent stressful conditions. This study assessed the acid tolerance response of stationary phase, acid-adapted (grown in TSB+1% glucose) or nonadapted (grown in glucose-free TSB) *E. coli* O157:H7 strains (ATCC43889, ATCC43895, ATCC51658 and EO139) grown individually or in a mixed culture during acid-adaptation and inoculated onto beef or into meat decontamination runoff (washings) fluids (acidic [pH 4.95] or nonacidic washings [pH 7.01]). The beef was untreated or treated by dipping for 30 s in hot water (75°C) followed by 2% lactic acid (55°C). Inoculated beef samples and washings were stored aerobically at 4 or 15°C for 6 d, and at set intervals (0, 2, and 6 d) were exposed (for 0, 60, 120, and 180 min) to pH 3.5 (adjusted with lactic acid) TSB with yeast extract. Overall, there were no significant ($P \geq 0.05$) differences in growth or survival after acid exposure of strains grown as a mixture and those grown individually

and mixed prior to inoculation. At day-0 in washings, acid-adapted populations were more resistant to the effects of acid than nonacid-adapted counterparts and remained as such at 4°C but not at 15°C throughout storage. Populations on treated meat were consistently lower than those on untreated meat during storage and subsequent exposure to acid. At day-0, there were no significant ($P \geq 0.05$) differences in acid resistance between acid-adapted and nonacid-adapted populations on meat. However, acid-adapted cells displayed consistently higher resistance than non-adapted cells through day-6, especially on dipped meat and regardless of temperature of storage. This suggests that meat contaminated with previously acid-adapted *E. coli* O157:H7 and exposed to refrigeration (4°C) temperatures such as those in a chilling cooler may result in populations resistant to subsequent lactic acid exposure.

INTRODUCTION

Escherichia coli O157:H7 has emerged with increasing frequency as a foodborne pathogen over the last 20 years and is responsible for causing serious illness and severe sequelae in susceptible humans, including hemorrhagic diarrhea, hemolytic uremic syndrome (HUS) and disseminated intravascular coagulopathy (DIC) (Bacon and Sofos, 2003). According to the Economic Research Service, the estimated cost of foodborne *E. coli* O157 illness stemming from medical costs, productivity losses and premature deaths annually in the U.S. totals $0.7 billion, while that incurred by shiga toxin-producing *E. coli* non-O157 is $0.3 billion (available at http://www.ers.usda.gov/Briefing/FoodborneDisease/ecoli/). Between 1993 and 1998,

most (72%) of the *E. coli* O157:H7 outbreaks were foodborne and of the foods implicated in the outbreaks, beef was responsible for most (45%) of the cases; 90% of the time the beef product was ground (CDC, 2000b).

In order to comply with U.S. meat and poultry inspection regulations requiring plant operation under the HACCP system and adherence to current regulatory criteria for pathogen reduction in foods (USDA-FSIS, 1996), beef processing plants employ various technologies for improving the microbiological quality of carcasses. Such technologies usually include the application of organic acid solutions and/or hot or cold water as sequential spray-washing interventions (Sofos and Smith, 1998). The decontamination strategies used for fresh beef can significantly reduce microbial contamination (Hardin et al., 1995; Sofos and Smith, 1998); however, they may create potentially stressful sublethal environments that may induce tolerances or resistances over time to further processing or consumer-related interventions and subsequently allow presence of resistant survivors (Samelis and Sofos, 2003).

The runoff from spray-washing applications may settle and collect on the floor or on meat contact surfaces resulting in meat decontamination washings that may harbor and allow stress-adaptation of pathogenic microorganisms (Samelis et al., 2002a; Stopforth et al., 2002). Furthermore, pathogens on beef carcasses that have survived decontamination interventions may potentially become stress-adapted to the residual effects of the decontamination interventions. It is hypothesized that the inherent acid resistance of *E. coli* O157:H7 may enhance its ability to resist acidic conditions faced in food processing environments or in food itself (Berry and Cutter, 2000; Goodson and Rowbury, 1989; Leyer et al., 1995). It is, thus, important to consider the acid tolerance of stress-adapted

E. coli O157:H7 originating from environments representative of the processing plant such as meat washings or meat.

Previous research (Avery and Buncic, 1997; Dykes and Moorhead, 2000; Vialette et al., 2003) has shown that foodborne pathogen strains isolated from clinical, food and environmental samples, have different pathogenicity potential and may respond differently to changing environmental conditions. Differences among strains of foodborne pathogens may be related to their varied ability to survive and grow under stressful conditions like acid and osmolarity (Vialette et al. 2003), presence of antimicrobials (Gill et al., 2002), heat (Wang and Doyle, 1998), and unfavorable storage temperatures (Barkocy-Gallagher et al., 2002; Janes et al., 2002). Although the ability of *E. coli* O157:H7 isolates to cause human illness is suggested to be correlated with genomic differences, it may not be the virulence of the strain that differs but rather their ability to arrive at the site of infection in the human gastrointestinal system (Kim et al., 1999). With the knowledge that pathogen strains have different responses to environmental conditions, it is likely that research investigating stress responses of only one strain may not always provide the most accurate evaluation of the worst-case scenario. Since testing multiple strains individually is highly impratical, it is important to mix strains and create a mixed inoculum of different pathogenic strains, particularly those from different environments like food, clinical samples or processing environments, with which to study the stress responses in a food. Considering this, the hypothesis was formed that *E. coli* O157:H7 strains may have different behavior in meat or meat environments when prepared as a mixed culture of different strains or prepared as single strains and then mixed prior to inoculation. The objective of this study was to assess the

ATR of a composite of four stationary phase acid-adapted or nonacid-adapted *E. coli* O157:H7 strains previously grown individually or in a mixed culture and inoculated into meat decontamination runoff (washings) fluids (acidic [pH 4.95 with 2% lactic acid] or nonacidic washings [pH 7.01]) or onto beef left untreated, or after treatment by dipping (30 s) in hot water (75°C) followed by lactic acid (2%).

MATERIALS AND METHODS

Strains and acid-adaptation of *E. coli* O157:H7

The strains of *E. coli* O157:H7 used in this study were ATCC43889, ATCC43895, ATCC51658 and EO139 (venison jerky isolate provided by Dr. M.P. Doyle, University of Georgia, Griffin, GA). Stock cultures of each strain were maintained at -70°C in tryptic soy broth (Difco, Becton Dickinson Co., Sparks, MD) containing 20% glycerol (Mallinckrodt Baker, Inc., Paris, KY). Working cultures were stored on TSA (Difco) slants at 4°C and were activated by transferring a loopful of each individual strain from TSA slants to TSB without dextrose (Difco, TSB-G) and incubating at 35°C for 24 h. The activated strains were subcultured once in TSB-G and streaked on Sorbitol MacConkey agar (Difco) supplemented with cefixime-tellurite (Dynal Inc., Lake Success, NY) (SMAC-CT) plates which were incubated at 35°C for 24 h. Colonies from the SMAC-CT plates were tested using a Dry Spot Latex Agglutination test (Oxoid, Ogdensburg, NY) to confirm that cultures possessed the O157 antigen. The four activated strains from broth were subsequently acid-adapted individually, or in a mixture (in equal proportions) of strains. Nonacid- or acid-adapted inocula were prepared by transferring 0.1 ml portion of

the activated cultures (individual or mixed) into 10-ml of glucose-free TSB (TSB-G) or glucose-free TSB with 1% added glucose (Sigma) (TSB+G), respectively, as originally described by Buchanan and Edelson (1996). After incubation at 35°C for 24 h, the resulting pH of the acid-adapted broth cultures for the individual strains and the mixed culture of *E. coli* O157:H7 was 5.10 and 5.14, respectively, while the pH of the nonacid adapted cultures was 7.07 and 7.13, respectively. These pH values are in agreement with those previously obtained by Stopforth et al. (2003b) for acid-adaptation of *E. coli* O157:H7 in tryptic soy broth.

Preparation and inoculation of fresh beef decontamination runoff fluids (washings)

Nonacidic washings (pH 7.01) were obtained by collecting spray-washing runoff fluids from decontamination of beef carcasses at a commercial slaughtering plant using water (84°C at spraying). Lactic acid washings were collected from the spray-washing (55°C at spraying) runoff fluids from beef carcasses decontaminated using lactic acid (2.5% in runoff; pH 2.47). Acidic washings were obtained by mixing the lactic acid washings with water washings to a final pH of 4.95. Washings were stored in 10-l sterile bottles (Nalgene, Nalge Co., Rochester, NY) for ≤30 d at -30°C and thawed (at 4°C overnight) prior to inoculation with the nonacid- or acid-adapted *E. coli* O157:H7 strains to yield inocula of approximately 5-6 log CFU/ml of washings. The inoculated washings were subsequently incubated for 6 d at 4 or 15°C to represent conditions likely to occur in meat processing plants such as a chilling or fabrication room, respectively. The pH of the washings was measured before storage and on each day of analysis with a digital pH

meter (*Ultra*Basic, UB-10; Denver Instrument Co., Denver, CO) and a glass pH electrode (pH/ATC Electrode #300729.1; Denver Instrument).

Preparation and inoculation of fresh beef tissue

Fresh decontaminated (approximately 82°C water) lean beef tissue (*biceps femoris*) was obtained from a local commercial slaughtering plant, stored at 4°C and used within 72 h postmortem. For use in the studies, the beef was cut into 5 x 2.5 x 1 cm (total surface area of 40 cm^2) pieces. Beef pieces were inoculated by spreading 100 µl of either the nonacid- or acid-adapted broth cultures on one side and allowed to attach for 15 min at 4°C before the same procedure was repeated on the reverse side to achieve a level of approximately 6 log CFU/cm^2. Following inoculation, the meat pieces were either left untreated or treated by dipping for 30 s in hot water (distilled, 75°C) followed by an additional 30 s in 2% lactic acid (55°C, pH 2.47) (Purac® FCC 88; Purac America, Lincolnshire, IL); 20 beef pieces were dipped in 1 L of each solution. After the application of decontamination treatments, individual beef pieces were either analyzed immediately or placed on retail foam trays (7.5 x 12.5 cm, Pactiv, Lake Forest, IL), covered with air-permeable film (Omnifilm; Pliant Corporation, Uniontown, OH), and stored at 4 or 15°C for 6 d. After preparation, uninoculated washings and meat were tested microbiologically, as described below, to determine the numbers of natural flora associated with each substrate.

Assessment of acid tolerance and physical analyses

To assess the acid tolerance of nonacid- and acid-adapted *E. coli* O157:H7 present in beef washings or on beef tissue, samples were exposed to TSBYE acidified to pH 3.5 with

lactic acid (Purac® FCC 88). Lactic acid was selected as an acidulant due to its commercial application for meat decontamination and because the pathogen may be transferred to such an acidic environment in the waste fluids following meat decontamination. More specifically, 1 ml of the inoculated washings was mixed with 9 ml of TSBYE acidified (with lactic acid; Purac) to pH 3.5 and kept at 25°C for the acid challenge. To test the acid tolerance of *E. coli* O157:H7 present on beef tissue, meat samples (40 cm^2) were placed into 18-oz Whirl-Pak® filter sterile plastic bags (Nasco, Fort Atkinson, Wisconsin) containing 40 ml of acidified TSBYE (adjusted to pH 3.5 with lactic acid) and pummeled (Masticator, IUL Instruments, Barcelona, Spain) for 2 min. Samples were exposed to acidified TSBYE for a duration of 180 min and at 60 min intervals, 1-ml aliquots were removed from the bags for determination of survivors. Initial (time-0 of exposure) population levels were determined by inoculated samples not exposed to acidified TSBYE. Bacterial populations in the washings before exposure to acidified TSBYE were determined by mixing 1 ml aliquots of washings with 9 ml of sterile 0.1% BPW (Difco) and serially diluted for subsequent microbiological analysis.

For meat samples, 40 ml of sterilized MRD was added to Whirl-Pak® (Nasco) bags to determine initial populations on the meat prior to acid exposure. Appropriate dilutions of all samples were plated on TSA for determination of total bacterial populations and on SMAC-CT for the selective enumeration of inoculated *E. coli* O157:H7 populations. Colonies were counted after incubation at 35°C for 48 h. The detection limit was estimated as 0 log CFU/cm^2 or ml based on the assumption that plating 1 ml of the initial sample homogenate could yield at least 1 CFU on the agar

plate. However, if only a minimum of 25 CFU per plate was acceptable, the detection limit would be 1.4 log CFU/cm^2 or ml.

The pH of homogenized samples was measured after microbiological analysis using a digital pH meter (*Ultra*Basic, UB-10; Denver Instrument, Denver, CO) with a glass pH electrode (pH/ATC Electrode #300729.1; Denver Instrument). Biochemical characterization of colonies grown on countable TSA plates was performed by observing colony morphology, testing for gram-, catalase- and oxidase-reactions and streaking onto SMAC-CT plates followed by the Dry Spot Latex Agglutination test (Oxoid) to confirm that cultures were positive for the O157 antigen.

Statistical analysis

Two replicate experiments were conducted with two samples per treatment in each replicate. Cell counts recovered from washings were converted to log CFU/ml while those attached to beef tissue were converted to log CFU/cm^2 before being analyzed. Values for the mean log and standard deviation of each set of bacterial counts were calculated on the assumption of a log-normal distribution of microorganisms. Data for cells from washings and from beef tissue were analyzed separately. Preliminary analysis of fixed effects for data from washings and from beef tissue using the GLM procedure of SAS® v 8.2 (SAS, 2002) indicated that surviving populations were dependent on the recovery media (TSA or SMAC-CT), temperature of incubation (4 or 15°C), acid-adaptation (nonacid- or acid-adapted), treatment (untreated/treated for washing or nonacidic/acidic for meat), day of analysis (0, 2, 4 or 6) and time of analysis (0, 60, 120 or 180 min) but not on the type of inoculum (prepared in a mixture or as individual

strains). Data for washings and beef tissue were thus separated by type of inoculum and recovery medium and within such subsets, by temperature of incubation and evaluated using a 2 x 2 x 3 x 4 (adaptation x treatment x day of analysis x time of analysis, respectively) factorial design. For each inoculum/media/temperature subset, individual fixed effects and up to four-way interactions were evaluated with ANOVA using the model $y = a + x_1 + x_2 + x_3 + x_4 + x_1x_2x_3x_4$ in the GLM procedures of SAS® v 8.2 (SAS, 2002); where x_1 represents adaptation, x_2 represents treatment, x_3 represents day of analysis and x_4 represents time of analysis. Least-squares means were separated using a protected pairwise t-test of SAS® v 8.2 (SAS, 2002). All differences were reported at a significance level of alpha = 0.05.

RESULTS AND DISCUSSION

Effect of recovery media

In general, bacterial populations recovered with TSA from meat or washings not exposed to acidified (pH 3.5) TSBYE (time-0 in Tables V.1-V.4) did not differ ($P \geq 0.05$) from those recovered with selective media (SMAC-CT) (time-0 in Tables V.5-V.8); however, the recovery of populations after exposure to acidified TSBYE was significantly ($P < 0.05$) higher on TSA (Tables V.1-V.4) than on SMAC-CT (Tables V.5-V.8) indicating the extensive injury caused by the acidic conditions used in this study. Due to that extent of injury, populations recovered on SMAC-CT were often undetectable (< 0 log CFU/ml [cm^2]) or too low to observe treatment effects. For this reason, discussion of changes in microbial populations on meat and in washings during storage and survival following

acid exposure is based on TSA counts. It should be noted that counts on TSA consisted almost exclusively (> 95%) of *E. coli* O157:H7 as determined by biochemical and immunological methods and may have been a result of the high (> 4 logs) inoculation level of *E. coli* O157:H7 in or on the washings and meat and the low initial levels of natural flora which may have allowed the pathogen to predominate. The level of natural contamination in uninoculated washings was 0.5 and <0 log CFU/ml for nonacidic and acidic washings, respectively, while that on beef decontaminated with hot (82°C) water obtained from the plant was $\leq$0.7 log CFU/cm^2.

Changes in pH of washings and meat during storage and acid exposure

Overall, acid-adaptation and mixing of strains prior to inoculation did not affect the pH values of inoculated meat and washings. In general, acidic washings and treated meat had lower pH values throughout storage as compared with nonacidic washings and untreated meat. The pH of acidic and nonacidic washings ranged from 4.89 to 5.22 and 6.97 to 7.41, respectively (Table V.9). The pH of treated and untreated meat ranged from 4.72 to 5.28 and 5.42 to 5.95, respectively (Table V.9). Generally, the pH values of both substrates (meat and washings) remained relatively consistent during storage at 4°C, while it increased slightly at 15°C (Table V.9). Regardless of acid-adaptation, strain-mixing, temperature of incubation or acid treatment, the pH of acidified (pH 3.5) TSBYE ranged from 3.67 to 4.07 following addition of meat, pummeling and exposure for 180 min. The pH of acidified TSBYE remained relatively unchanged at a maximum of 3.62 following addition of washings and during the 180 min of exposure.

Table V.1: Mean (standard deviation) populations (log CFU/ml; tryptic soy agar) of acid-adapted and nonacid-adapted *Escherichia coli* O157:H7 strains grown individually and mixed prior to inoculation into nonacidic (water; pH 7.01) and acidic (water washings adjusted to pH 4.95 with lactic acid [LA] washings) washings and exposed periodically to tryptic soy broth with 0.6% yeast extract (adjusted to pH 3.5 with LA) during storage for 6 d at 4 or 15°C

Storage Temperature (°C)	Day	Inoculum	Washings	Exposure to Acidified (pH 3.5) TSBYE (min) 0	60	120	180
4	0	Acid-adapted	Nonacidic	4.9abcz (0.1)	4.9az (0.1)	4.3abz (0.2)	3.3by (0.7)
			Acidic	5.1abcz (0.1)	4.9az (0.1)	4.6az (0)	4.3az (0.1)
		Nonadapted	Nonacidic	4.9abcz (0.2)	3.1bcy (0.4)	1.1defx (0.2)	1.0defx (0.0)
			Acidic	4.9bcz (0.2)	3.3bcy (0.1)	1.6dex (0.5)	0.2fgw (0.4)
	2	Acid-adapted	Nonacidic	4.9bcz (0.1)	2.1dy (0.2)	1.8dyx (0.2)	1.1defx (0.3)
			Acidic	5.0abcz (0.1)	3.3bcy (0.8)	3.4bcy (0.1)	1.7cdx (1.3)
		Nonadapted	Nonacidic	4.4cz (0.1)	0ey (0)	0gy (0)	0gy (0.0)
			Acidic	4.6cz (0.1)	2.6cdy (0.2)	0.7efgx (0.8)	0gx (0)
	4	Acid-adapted	Nonacidic	4.7c (0)	nd	nd	nd
			Acidic	5.1abc (0.6)	nd	nd	nd
		Nonadapted	Nonacidic	5.1abc (0.2)	nd	nd	nd
			Acidic	4.7c (0)	nd	nd	nd
	6	Acid-adapted	Nonacidic	4.5cz (0.2)	2.5dy (0.1)	1.7dyx (0.3)	0.8efgx (0.3)
			Acidic	4.8bcz (0.3)	3.9bzy (0.3)	3.2cyx (0.4)	2.6bcx (0.6)
		Nonadapted	Nonacidic	4.4cz (0.1)	2.1dy (0.2)	0.3fgx (0.5)	0.3fgx (0.5)
			Acidic	4.7cz (0.1)	3.2bcy (0.4)	1.3dex (0.3)	1.4dex (0.4)
15	0	Acid-adapted	Nonacidic	4.9fz (0.1)	4.9cz (0.1)	4.3cz (0.2)	3.3dy (0.7)
			Acidic	5.1fz (0.1)	4.9cz (0.1)	4.6bcz (0)	4.3bcz (0.1)
		Nonadapted	Nonacidic	4.9fz (0.2)	3.1dy (0.4)	1.1gx (0.2)	1.0fx (0)
			Acidic	4.9fz (0.2)	3.3dy (0.1)	1.6fgx (0.5)	0.2fw (0.4)
	2	Acid-adapted	Nonacidic	7.0cdez (0.1)	5.9by (0.2)	4.5bcx (0.3)	3.4cdw (0.2)
			Acidic	6.6ez (0.1)	3.3dy (0.3)	2.5efy (0.4)	2.7dey (0.2)
		Nonadapted	Nonacidic	6.8dez (0.1)	5.7bcy (0.2)	3.9cdx (0.2)	3.3dx (0.3)
			Acidic	6.5ez (0)	3.5dy (0.3)	3.0deyx (0.2)	2.3ex (0.6)
	4	Acid-adapted	Nonacidic	7.5ab (0.6)	nd	nd	nd
			Acidic	7.3abc (0.7)	nd	nd	nd
		Nonadapted	Nonacidic	7.7a (0.6)	nd	nd	nd
			Acidic	7.4abc (0.7)	nd	nd	nd
	6	Acid-adapted	Nonacidic	7.2bcdez(0.1)	6.9azy (0.2)	6.6azy (0.3)	6.2ay (0.2)
			Acidic	7.2bcdez (0.3)	5.9by (0.1)	5.4by (0.2)	5.0by (0.1)
		Nonadapted	Nonacidic	7.8abcz (0.2)	7.0azy (0.1)	6.6ay (0.2)	6.6ay (0.1)
			Acidic	7.7abcdz (0.1)	6.5aby (0.2)	6.5ay (0.3)	6.0ay (0.4)

abcd – means in the same column for each temperature of incubation with different superscript letters are different ($P < 0.05$)
zyxw – means in the same row with different superscript letters are different ($P < 0.05$)
nd- not determined; according to study design acid tolerance was assessed at days 0, 2 and 6.

Table V.2: Mean (standard deviation) populations (log CFU/ml; tryptic soy agar) of acid-adapted and nonacid-adapted *Escherichia coli* O157:H7 strains grown in a mixture prior to inoculation into nonacidic (water; pH 7.01) and acidic (water washings adjusted to pH 4.95 with lactic acid [LA] washings) washings and exposed periodically to tryptic soy broth with 0.6% yeast extract (adjusted to pH 3.5 with LA) during storage for 6 d at 4 or 15°C

Storage Temperature (°C)	Day	Inoculum	Washings	Exposure to Acidified (pH 3.5) TSBYE (min) 0	60	120	180
4	0	Acid-adapted	Nonacidic	5.3abcdez (0.1)	4.6azy (0.1)	3.6byx (0.3)	3.2bx (0.2)
			Acidic	5.3abcdez (0.2)	4.7azy (0.1)	4.7azy (0.1)	4.3ay (0.1)
		Nonadapted	Nonacidic	5.2bcdefz (0.1)	2.5efy (0.4)	1.0cdx (0)	1.0dex (0)
			Acidic	5.0cdefz (0)	3.5bcdy (0.3)	1.6cx (0.7)	1.0dex (0)
	2	Acid-adapted	Nonacidic	5.1cdefz (0)	3.3cdey (0.9)	1.9cx (0.7)	1.6cdx (0.4)
			Acidic	5.2bcdefz (0.1)	4.6abzy (0.1)	3.6byx (0.3)	2.7bx (0.5)
		Nonadapted	Nonacidic	4.8defz (0.1)	0.9ghy (0.6)	0.2dy (0.4)	0ey (0)
			Acidic	5.0cdefz (0.3)	2.7defy (0.7)	1.1cdx (1.1)	0ex (0)
	4	Acid-adapted	Nonacidic	4.9def (0.3)	nd	nd	nd
			Acidic	5.2bcdef (0)	nd	nd	nd
		Nonadapted	Nonacidic	5.3abcde (0.5)	nd	nd	nd
			Acidic	5.1cdef (0.1)	nd	nd	nd
	6	Acid-adapted	Nonacidic	4.2fz (0.2)	1.1gy (0.2)	0dx (0)	0ex (0)
			Acidic	5.2bcdefz (0.2)	4.1abcy (0.3)	3.4byx (0.2)	2.4bcx (0.6)
		Nonadapted	Nonacidic	4.4efz (0.1)	0hy (0)	0dy (0)	0ey (0)
			Acidic	5.1cdefz (0.1)	1.9fgy (0.7)	0.1dx (0.2)	0.1ex (0.2)
15	0	Acid-adapted	Nonacidic	5.3ez (0.1)	4.6cdzy (0.1)	3.6dyx (0.3)	3.2dex (0.2)
			Acidic	5.3ez (0.2)	4.7cdzy (0.1)	4.7czy (0.1)	4.3cy (0.1)
		Nonadapted	Nonacidic	5.2ez (0.1)	2.5gy (0.4)	1.0gx (0)	1.0gx (0)
			Acidic	5.0ez (0)	3.5efy (0.3)	1.6fgx (0.7)	1.0gx (0)
	2	Acid-adapted	Nonacidic	7.0cdz (0.2)	6.1abz (0.3)	4.7cy (0.5)	3.6cdx (0.5)
			Acidic	6.6dz (0.1)	3.8dey (0.4)	2.8deyx (0.7)	2.7dex (0.6)
		Nonadapted	Nonacidic	6.8dz (0.1)	5.0bcy (0.6)	3.2dx (0.2)	2.2efw (0.9)
			Acidic	6.6dz (0.2)	2.8fgy (0.5)	2.1efyx (0.8)	1.4fgx (0.8)
	4	Acid-adapted	Nonacidic	7.8a (0.4)	nd	nd	nd
			Acidic	7.4abcd (0.6)	nd	nd	nd
		Nonadapted	Nonacidic	7.8a (0.5)	nd	nd	nd
			Acidic	7.7ab (0.6)	nd	nd	nd
	6	Acid-adapted	Nonacidic	7.2bcdz (0.2)	6.7azy (0.1)	6.3abzy (0.1)	6.0aby (0.1)
			Acidic	7.1cdz (0.1)	5.8aby (0.1)	5.5bcy (0.3)	5.3by (0.1)
		Nonadapted	Nonacidic	7.6abcdz (0.3)	6.7az (0.1)	6.7az (0.2)	6.7az (0.1)
			Acidic	7.4abcdz (0.1)	6.4azy (0.3)	6.0aby (0.4)	5.6aby (0.2)

abcd – means in the same column for each temperature of incubation with different superscript letters are different ($P < 0.05$)
zyxw – means in the same row with different superscript letters are different ($P < 0.05$)
nd- not determined; according to study design acid tolerance was assessed at days 0, 2 and 6.

Table V.3: Mean (standard deviation) populations (log CFU/cm^2; tryptic soy agar) of acid-adapted and nonacid-adapted *Escherichia coli* O157:H7 strains grown individually and mixed prior to inoculation on lean beef tissue left untreated or treated (by dipping in hot water [75°] followed by lactic acid [LA; 2%, 55°C]) and exposed periodically to tryptic soy broth with 0.6% yeast extract (adjusted to pH 3.5 with LA) during storage for 6 d at 4 or 15°C

Storage Temperature (°C)	Day	Inoculum	Beef	Exposure to Acidified (pH 3.5) TSBYE (min) 0	60	120	180
4	0	Acid-adapted	Untreated	6.2az (0.2)	5.9az (0.2)	5.2abz (0.3)	5.5az (0)
			Treated	4.5bcz (0.5)	4.9abz (0.2)	4.3abcz (0.5)	4.2abcdz (0.4)
		Nonadapted	Untreated	6.2az (0.2)	5.5az (0.2)	5.3abz (0.0)	4.8abcz (0.5)
			Treated	4.7abzc (0.5)	3.3cdzy (0.9)	3.1cdy (0.5)	2.7defy (0.4)
	2	Acid-adapted	Untreated	6.2az (0.3)	5.6az (0.2)	5.4az (0.1)	5.0abz (0.1)
			Treated	4.6bcz (0.4)	3.6bcdz (0.7)	3.5cz (0.5)	3.2dez (0.7)
		Nonadapted	Untreated	5.9azb (0.4)	4.7abczy (0.6)	3.7bcyx (0.4)	3.0defx (0.6)
			Treated	4.1cz (0.5)	3.0dzy (0.8)	1.9dy (0.7)	1.8efy (0.7)
	4	Acid-adapted	Untreated	6.2a (0.3)	nd	nd	nd
			Treated	4.8abc (0.2)	nd	nd	nd
		Nonadapted	Untreated	6.0ab (0.4)	nd	nd	nd
			Treated	4.6bc (0.6)	nd	nd	nd
	6	Acid-adapted	Untreated	6.0abz (0.4)	5.2az (0.5)	5.2abz (0.6)	5.1az (0.6)
			Treated	4.3cz (0.3)	3.7bcdz (0.2)	3.6cz (0.3)	3.5bcdz (0.4)
		Nonadapted	Untreated	6.0azb (0.2)	5.0abzy (0.2)	4.4abcyx (0.3)	3.4cdx (0.5)
			Treated	3.7cz (0.6)	2.2dzy (0.8)	1.9dy (0.5)	1.6fy (0.7)
15	0	Acid-adapted	Untreated	6.2bcz (0.2)	5.9abcz (0.2)	5.2abcz (0.3)	5.5abz (0.0)
			Treated	4.5dz (0.5)	4.9cdz (0.2)	4.3bcdz (0.5)	4.2bcdz (0.4)
		Nonadapted	Untreated	6.2bcz (0.2)	5.5bcdz (0.2)	5.3abcz (0)	4.8abcz (0.5)
			Treated	4.7cdz (0.5)	3.3efzy (0.9)	3.1dey (0.5)	2.7dy (0.4)
	2	Acid-adapted	Untreated	7.2abz (0.4)	6.0abczy (0.3)	5.7aby (0.4)	5.4aby (0.2)
			Treated	4.5dz (0.6)	4.1dez (0.4)	3.9cdz (0.4)	3.7cdz (0.4)
		Nonadapted	Untreated	6.9abz (0.3)	5.4bcdzy (0.5)	4.1cdyx (0.3)	3.6dx (0.3)
			Treated	4.2dz (0.4)	2.4fy (0.5)	1.6efyx (0.7)	0.9ex (0.9)
	4	Acid-adapted	Untreated	7.7ab (0.2)	nd	nd	nd
			Treated	4.4d (0.8)	nd	nd	nd
		Nonadapted	Untreated	7.6ab (0.2)	nd	nd	nd
			Treated	4.3d (1.1)	nd	nd	nd
	6	Acid-adapted	Untreated	7.8az (0.6)	7.0azy (0.2)	6.6azy (0.4)	6.2ay (0.7)
			Treated	4.7cdz (0.7)	4.2dez (0.5)	3.6dz (0.5)	3.7cdz (0.5)
		Nonadapted	Untreated	7.7abz (0.4)	6.6abzy (0)	6.4azy (0.2)	5.6aby (0.1)
			Treated	3.6dz (1.0)	2.0fy (1.2)	0.9fy (0.7)	0.7ey (0.8)

abcd – means in the same column for each temperature of incubation with different superscript letters are different ($P < 0.05$)
zyxw – means in the same row with different superscript letters are different ($P < 0.05$)
nd- not determined; according to study design acid tolerance was assessed at days 0, 2 and 6.

Table V.4: Mean (standard deviation) populations (log CFU/cm^2; tryptic soy agar) of acid-adapted and nonacid-adapted *Escherichia coli* O157:H7 strains grown in a mixture prior to inoculation on lean beef tissue left untreated or treated (by dipping in hot water [75°] followed by lactic acid [LA; 2%, 55°C]) and exposed periodically to tryptic soy broth with 0.6% yeast extract (adjusted to pH 3.5 with LA) during storage for 6 d at 4 or 15°C

Storage Temperature (°C)	Day	Inoculum	Beef	Exposure to Acidified (pH 3.5) TSBYE (min) 0	60	120	180
4	0	Acid-adapted	Untreated	6.0abz (0.4)	5.0azy (0.3)	4.3abyx (0)	3.6cx (0)
			Treated	4.2cdz (0.2)	4.6az (0.2)	3.8bz (0.5)	3.7cz (0.6)
		Nonadapted	Untreated	6.2abz (0.3)	5.2azy (0.4)	4.6abyx (0.2)	3.8cx (0.2)
			Treated	4.2cdz (0.4)	4.4az (0.3)	3.9bz (0.5)	3.6cz (0.4)
	2	Acid-adapted	Untreated	6.1abz (0.3)	5.5az (0.2)	5.1az (0.3)	5.1abz (0.2)
			Treated	5.1bzc (0.8)	4.6az (0.1)	4.4abz (0.1)	4.3abcz (0.3)
		Nonadapted	Untreated	6.1abz (0.4)	5.0azy (0.4)	4.7aby (0.4)	4.3abcy (0.5)
			Treated	4.5cz (0.7)	2.8by (0.4)	1.7cy (0.4)	1.6dy (0.3)
	4	Acid-adapted	Untreated	6.0ab (0.2)	nd	nd	nd
			Treated	4.5cd (0.1)	nd	nd	nd
		Nonadapted	Untreated	6.1ab (0.3)	nd	nd	nd
			Treated	4.2cd (0.5)	nd	nd	nd
	6	Acid-adapted	Untreated	6.4az (0.1)	5.5az (0.3)	5.5az (0.3)	5.2az (0.4)
			Treated	4.5cdz (0.4)	4.3az (0.2)	3.9bz (0.4)	3.8cz (0.4)
		Nonadapted	Untreated	6.3abz (0.1)	4.6ay (0.3)	4.3aby (0.3)	3.9bcy (0.2)
			Treated	3.7dz (0.6)	2.3by (0.5)	1.7cyx (0.5)	0.8dx (0.4)
15	0	Acid-adapted	Untreated	6.0cz (0.4)	5.0cdzy (0.3)	4.3bcyx (0.0)	3.6dx (0.0)
			Treated	4.2dz (0.2)	4.6cdez (0.2)	3.8cz (0.5)	3.7cdz (0.6)
		Nonadapted	Untreated	6.2cz (0.3)	5.2bcdzy (0.4)	4.6bcyx (0.2)	3.8bcdx (0.2)
			Treated	4.2dz (0.4)	4.4dez (0.3)	3.9cz (0.5)	3.6dz (0.4)
	2	Acid-adapted	Untreated	6.9cz (0.3)	5.7abczy (0.2)	5.2aby (0.2)	4.9abcy (0.6)
			Treated	3.9fz (0.6)	3.7efz (0.2)	3.4cz (0.5)	3.2dz (0.4)
		Nonadapted	Untreated	6.5cz (0.5)	4.3dey (0.4)	3.6cyx (0.7)	2.7dx (0.9)
			Treated	4.3dz (0.4)	2.6fgy (0.3)	1.8dyx (0.6)	1.3ex (0.3)
	4	Acid-adapted	Untreated	7.8a (0.4)	nd	nd	nd
			Treated	4.3d (0.3)	nd	nd	nd
		Nonadapted	Untreated	4.2e (0.6)	nd	nd	nd
			Treated	7.6b (0.5)	nd	nd	nd
	6	Acid-adapted	Untreated	8.0az (0.4)	6.3aby (0.5)	6.0ayx (0.8)	5.0abx (0.2)
			Treated	3.7fz (0.4)	3.8efz (0.3)	3.4cz (0.3)	3.2dz (0.4)
		Nonadapted	Untreated	8.1az (0.2)	6.7ay (0.2)	6.3ay (0.2)	5.6ay (0.7)
			Treated	4.0fz (0.4)	2.4gy (0.8)	1.6dy (0.5)	1.2ey (0.4)

abcd – means in the same column for each temperature of incubation with different superscript letters are different ($P < 0.05$)
zyxw – means in the same row with different superscript letters are different ($P < 0.05$)
nd- not determined; according to study design acid tolerance was assessed at days 0, 2 and 6.

Table V.5: Mean (standard deviation) populations (log CFU/ml; Sorbitol MacConkey Agar with cefixime and tellurite supplement) of acid-adapted and nonacid-adapted *Escherichia coli* O157:H7 strains grown individually and mixed prior to inoculation into nonacidic (water; pH 7.01) and acidic (water washings adjusted to pH 4.95 with lactic acid [LA] washings) washings and exposed periodically to tryptic soy broth with 0.6% yeast extract (adjusted to pH 3.5 with LA) during storage for 6 d at 4 or 15°C

Storage Temperature (°C)	Day	Inoculum	Washings	Exposure to Acidified (pH 3.5) TSBYE (min) 0	60	120	180
4	0	Acid-adapted	Nonacidic	5.0az (0.1)	3.4az (0.6)	1.1az (0.2)	1.0az (0)
			Acidic	4.9az (0.2)	2.4az (0.5)	1.8az (1.1)	0az (0)
		Nonadapted	Nonacidic	4.8az (0.1)	1.0az (0.0)	1.0az (0)	1.0az (0)
			Acidic	4.7az (0.2)	0.0az (0.0)	0az (0)	0az (0)
	2	Acid-adapted	Nonacidic	4.4az (0.5)	0az (0)	0az (0)	0az (0)
			Acidic	4.7az (0.2)	0.1az (0.2)	0az (0)	0az (0)
		Nonadapted	Nonacidic	4.4az (0.5)	0az (0)	0az (0)	0az (0)
			Acidic	4.7az (0.2)	0.1az (0.2)	0az (0)	0az (0)
	4	Acid-adapted	Nonacidic	4.6a (0.1)	nd	nd	nd
			Acidic	4.4a (0.2)	nd	nd	nd
		Nonadapted	Nonacidic	4.8a (0.1)	nd	nd	nd
			Acidic	4.1a (0.2)	nd	nd	nd
	6	Acid-adapted	Nonacidic	3.8az (0.2)	1.1az (0.3)	0az (0)	0az (0)
			Acidic	4.2az (0.2)	0.8az (0.9)	0az (0)	0az (0)
		Nonadapted	Nonacidic	3.6az (0.3)	0az (0)	0az (0)	0az (0)
			Acidic	3.8az (0.3)	0az (0)	0az (0)	0az (0)
15	0	Acid-adapted	Nonacidic	5.0az (0.1)	3.4az (0.6)	1.1bz (0.2)	1.0az (0)
			Acidic	4.9az (0.2)	2.4az (0.5)	1.8bz (1.1)	0az (0)
		Nonadapted	Nonacidic	4.8az (0.1)	1.0az (0)	1.0bz (0)	1.0az (0)
			Acidic	4.7az (0.2)	0az (0)	0bz (0)	0az (0)
	2	Acid-adapted	Nonacidic	6.9az (0.1)	4.6az (0.3)	0.5bz (0.6)	0az (0)
			Acidic	6.2az (0.1)	0az (0)	0bz (0)	0az (0)
		Nonadapted	Nonacidic	6.7az (0)	2.5az (0.2)	0bz (0)	0az (0)
			Acidic	6.3az (0.1)	0az (0)	0bz (0)	0az (0)
	4	Acid-adapted	Nonacidic	7.2a (0.7)	nd	nd	nd
			Acidic	6.6a (0.3)	nd	nd	nd
		Nonadapted	Nonacidic	7.4a (0.7)	nd	nd	nd
			Acidic	7.5a (0.2)	nd	nd	nd
	6	Acid-adapted	Nonacidic	6.7az (0.3)	6.5az (0.1)	5.3bz (0.2)	4.1az (0.1)
			Acidic	6.0az (0.2)	3.1az (0.6)	1.3bz (0.6)	0.3az (0.4)
		Nonadapted	Nonacidic	7.3az (0.3)	6.5az (0.4)	5.4ay (0.1)	4.8ax (0.1)
			Acidic	7.1az (0.3)	4.5az (0.3)	4.1bz (0.4)	2.7az (0.3)

abcd – means in the same column for each temperature of incubation with different superscript letters are different ($P < 0.05$)
zyxw – means in the same row with different superscript letters are different ($P < 0.05$)
nd- not determined; according to study design acid tolerance was assessed at days 0, 2 and 6.

Table V.6: Mean (standard deviation) populations (log CFU/ml; Sorbitol MacConkey Agar with cefixime and tellurite supplement) of acid-adapted and nonacid-adapted *Escherichia coli* O157:H7 strains grown in a mixture prior to inoculation into nonacidic (water; pH 7.01) and acidic (water washings adjusted to pH 4.95 with lactic acid [LA] washings) washings and exposed periodically to tryptic soy broth with 0.6% yeast extract (adjusted to pH 3.5 with LA) during storage for 6 d at 4 or 15°C

Storage				Exposure to Acidified (pH 3.5) TSBYE (min)			
Temperature (°C)	Day	Inoculum	Washings	0	60	120	180
4	0	Acid-adapted	Nonacidic	5.1abz (0.2)	1.9aby (0.6)	1.0ax (0)	1.0ax (0)
			Acidic	5.1abz (0)	2.5ay (0.4)	0bx (0)	0bx (0)
		Nonadapted	Nonacidic	5.0abcz (0.1)	1.0bcdy (0)	1.0aby (0)	1.0aby (0)
			Acidic	5.0abcz (0.2)	0dy (0)	0by (0)	0by (0)
	2	Acid-adapted	Nonacidic	4.4abcdz (0.2)	0dy (0)	0by (0)	0by (0)
			Acidic	4.8abcz (0.3)	1.4bcy (1.0)	0bx (0)	0bx (0)
		Nonadapted	Nonacidic	4.0cdz (0.2)	0dy (0)	0by (0)	0by (0)
			Acidic	4.7abcz (0.1)	0.4cdy (0.8)	0by (0)	0by (0)
	4	Acid-adapted	Nonacidic	4.7abc (0.5)	nd	nd	nd
			Acidic	4.0cd (0)	nd	nd	nd
		Nonadapted	Nonacidic	5.0a (0.5)	nd	nd	nd
			Acidic	4.6abcd (0.4)	nd	nd	nd
	6	Acid-adapted	Nonacidic	4.2bcdz (0.2)	1.1bcdy (0.2)	0by (0)	0by (0)
			Acidic	3.5dz (2.1)	1.2bcy (0.3)	0bx (0)	0bx (0)
		Nonadapted	Nonacidic	3.5dz (1.0)	0dy (0)	0by (0)	0by (0)
			Acidic	4.5abcdz (0.1)	0dy (0)	0by (0)	0by (0)
15	0	Acid-adapted	Nonacidic	5.1dz (0.2)	1.9dey (0.6)	1.0cx (0)	1.0bcx (0)
			Acidic	5.1dz (0)	2.5dy (0.4)	0dx (0)	0dx (0)
		Nonadapted	Nonacidic	5.0dz (0.1)	1.0efgy (0)	1.0cdy (0)	1.0bcdy (0)
			Acidic	5.0dz (0.2)	0gy (0)	0dy (0)	0dy (0)
	2	Acid-adapted	Nonacidic	7.0abcz (0.1)	3.6bcy (1.4)	0.4cdx (0.7)	0dx (0)
			Acidic	5.9cdz (0.1)	1.5defy (1.0)	0dx (0)	0dx (0)
		Nonadapted	Nonacidic	6.7abcz (0.1)	0.5fgy (1.0)	0dy (0)	0dy (0)
			Acidic	6.3bcz (0.4)	0gy (0)	0dy (0)	0dy (0)
	4	Acid-adapted	Nonacidic	7.6a (0.4)	nd	nd	nd
			Acidic	6.6bc (0.3)	nd	nd	nd
		Nonadapted	Nonacidic	7.5a (0.6)	nd	nd	nd
			Acidic	7.3a (0.7)	nd	nd	nd
	6	Acid-adapted	Nonacidic	7.0abcz (0.2)	6.3azy (0.2)	5.5ay (0.1)	4.0ax (0.1)
			Acidic	6.5bcz (0.1)	2.6cdy (0.5)	1.3cx (0.4)	0.5cdx (0.3)
		Nonadapted	Nonacidic	7.1abz (0.4)	6.3azy (0.1)	5.2ayx (0.3)	4.6ax (0.2)
			Acidic	6.8abcz (0.2)	4.5by (0.1)	3.8by (0.5)	1.6bx (0.7)

abcd – means in the same column for each temperature of incubation with different superscript letters are different ($P < 0.05$)
zyxw – means in the same row with different superscript letters are different ($P < 0.05$)
nd- not determined; according to study design acid tolerance was assessed at days 0, 2 and 6.

Table V.7: Mean (standard deviation) populations (log CFU/cm^2; Sorbitol MacConkey Agar with cefixime and tellurite supplement) of acid-adapted and nonacid-adapted *Escherichia coli* O157:H7 strains grown individually and mixed prior to inoculation on lean beef tissue left untreated or treated (by dipping in hot water [75°] followed by lactic acid [LA; 2%, 55°C]) and exposed periodically to tryptic soy broth with 0.6% yeast extract (adjusted to pH 3.5 with LA) during storage for 6 d at 4 or 15°C

Storage Temperature (°C)	Day	Inoculum	Beef	Exposure to Acidified (pH 3.5) TSBYE (min) 0	60	120	180
4	0	Acid-adapted	Untreated	6.1az (0.3)	4.1az (0.1)	3.1az (0.2)	3.5az (0)
			Treated	4.1az (0.6)	2.6az (0.4)	3.0az (0.4)	3.7az (0.7)
		Nonadapted	Untreated	6.0az (0.4)	3.7az (0.2)	2.0az (1.1)	2.1az (0.6)
			Treated	4.0az (0.2)	2.0az (0.6)	0.8az (0.9)	0.4az (0.5)
	2	Acid-adapted	Untreated	5.7az (0.8)	3.6az (0.5)	2.6az (0.5)	2.0az (0.8)
			Treated	4.0az (0.5)	2.0az (0.8)	1.1az (1.3)	1.0az (1.2)
		Nonadapted	Untreated	5.6az (0.5)	2.8az (0.7)	1.5az (0.7)	1.6az (0.7)
			Treated	2.9az (0.6)	1.6az (0.7)	0.7az (0.9)	0.7az (0.8)
	4	Acid-adapted	Untreated	5.8a (0.5)	nd	nd	nd
			Treated	4.2a (0.2)	nd	nd	nd
		Nonadapted	Untreated	5.5a (0.3)	nd	nd	nd
			Treated	4.0a (0.6)	nd	nd	nd
	6	Acid-adapted	Untreated	6.0az (0.5)	3.4az (0.7)	2.5az (0.6)	2.8az (0.2)
			Treated	3.6az (0.2)	2.0az (0.3)	1.1az (0.9)	0.6az (0.7)
		Nonadapted	Untreated	5.9az (0.2)	2.9az (0.6)	2.0az (0.5)	1.7az (0.6)
			Treated	2.9az (0.8)	1.1az (1.1)	0.6az (0.7)	0.4az (0.5)
15	0	Acid-adapted	Untreated	6.1abz (0.3)	4.1abz (0.1)	3.1az (0.2)	3.5az (0)
			Treated	4.1abz (0.6)	2.6abz (0.4)	3.0az (0.4)	3.7az (0.7)
		Nonadapted	Untreated	6.0abz (0.4)	3.7abz (0.2)	2.0az (1.1)	2.1az (0.6)
			Treated	4.0abz (0.2)	2.0abz (0.6)	0.8az (0.9)	0.4az (0.5)
	2	Acid-adapted	Untreated	7.1abz (0.2)	3.6abzy (0.4)	3.2azy (0.5)	2.3ay (0.4)
			Treated	3.2bz (0.5)	2.5abz (0.5)	1.4az (1.2)	1.1az (1.2)
		Nonadapted	Untreated	6.3abz (0.9)	2.7abz (0.2)	2.2az (0.8)	2.0az (0.7)
			Treated	3.5abz (0.4)	1.3bz (0.6)	0.7az (0.9)	0.4az (0.5)
	4	Acid-adapted	Untreated	7.7a (0.2)	nd	nd	nd
			Treated	3.7ab (1.1)	nd	nd	nd
		Nonadapted	Untreated	7.5ab (0.2)	nd	nd	nd
			Treated	3.7ab (0.8)	nd	nd	nd
	6	Acid-adapted	Untreated	7.8az (0.4)	5.8azy (1.1)	3.9azy (1.2)	2.7ay (0.8)
			Treated	4.4abz (0.8)	2.2abz (0.5)	1.0az (1.2)	0.6az (0.8)
		Nonadapted	Untreated	7.7az (0.4)	4.4abzy (0.7)	3.7azy (0.7)	2.4ay (0.4)
			Treated	3.2bz (0.7)	1.1bz (1.4)	0.7az (0.8)	0.5az (0.6)

abcd – means in the same column for each temperature of incubation with different superscript letters are different ($P < 0.05$)
zyxw – means in the same row with different superscript letters are different ($P < 0.05$)
nd- not determined; according to study design acid tolerance was assessed at days 0, 2 and 6.

Table V.8: Mean (standard deviation) populations (log CFU/cm^2; Sorbitol MacConkey Agar with cefixime and tellurite supplement) of acid-adapted and nonacid-adapted *Escherichia coli* O157:H7 strains grown in a mixture prior to inoculation on lean beef tissue left untreated or treated (by dipping in hot water [75°] followed by lactic acid [LA; 2%, 55°C]) and exposed periodically to tryptic soy broth with 0.6% yeast extract (adjusted to pH 3.5 with LA) during storage for 6 d at 4 or 15°C

Storage Temperature (°C)	Day	Inoculum	Beef	Exposure to Acidified (pH 3.5) TSBYE (min) 0	60	120	180
4	0	Acid-adapted	Untreated	5.6abcz (0.7)	2.6aby (0.7)	2.0abcy (0.1)	1.4abcy (0.1)
			Treated	3.7dz (0.3)	3.5azy (0.6)	2.4abzy (0.4)	1.7abcy (0.5)
		Nonadapted	Untreated	5.9az (0.5)	2.7aby (0.8)	2.1abcy (0.1)	1.6abcy (0.7)
			Treated	3.7dz (0.5)	2.9abzy (0.7)	2.3abzy (0)	1.8aby (0.5)
	2	Acid-adapted	Untreated	5.5abcz (0.9)	3.0aby (0.7)	2.5abyx (0.4)	0.9abcx (0.8)
			Treated	4.0cdz (0.6)	2.5abzy (0.1)	1.0bcdy (0.7)	0.8abcy (1.0)
		Nonadapted	Untreated	5.9az (0.6)	2.6aby (0.2)	1.9abcy (0.5)	1.7abcy (0.5)
			Treated	3.4dz (0.6)	1.4bcy (0.5)	0.5cdy (0.6)	0cy (0)
	4	Acid-adapted	Untreated	5.8ab (0.4)	nd	nd	nd
			Treated	3.6d (0.4)	nd	nd	nd
		Nonadapted	Untreated	6.0a (0.3)	nd	nd	nd
			Treated	3.3d (0.5)	nd	nd	nd
	6	Acid-adapted	Untreated	6.0az (0.5)	2.3aby (0.4)	3.0ay (0.7)	2.1ay (0.8)
			Treated	4.1bcdz (0.5)	2.2abcy (0.5)	0.9bcdyx (0.7)	0.2bcx (0.4)
		Nonadapted	Untreated	5.9az (0.4)	2.6aby (0.4)	2.3aby (0.3)	1.5abcy (0.6)
			Treated	3.2dz (0.7)	0.6cy (0.7)	0.2dy (0.4)	0cy (0)
15	0	Acid-adapted	Untreated	5.6bz (0.7)	2.6bcdy (0.7)	2.0bcdey (0.1)	1.4abcdy (0.1)
			Treated	3.7cz (0.3)	3.5abcz (0.6)	2.4abczy (0.4)	1.7abcdy (0.5)
		Nonadapted	Untreated	5.9bz (0.5)	2.7bcy (0.8)	2.1bcdy (0.1)	1.6abcdy (0.7)
			Treated	3.7cz (0.5)	2.9bczy (0.7)	2.3abczy (0)	1.8abcy (0.5)
	2	Acid-adapted	Untreated	6.7abz (0.4)	2.9bcy (0.3)	2.5abcy (0.7)	1.6abcdy (0.7)
			Treated	3.5cz (0.3)	2.2cdzy (0.6)	0.8cdey (1.0)	0.7cdy (0.8)
		Nonadapted	Untreated	6.0bz (0.8)	1.8cdy (0.2)	1.3cdey (0.6)	1.0bcdy (0.1)
			Treated	3.2cz (0.5)	0.9dy (0.9)	0.3ey (0.5)	0dy (0)
	4	Acid-adapted	Untreated	7.8a (0.3)	nd	nd	nd
			Treated	3.6c (0.5)	nd	nd	nd
		Nonadapted	Untreated	7.3ab (0.7)	nd	nd	nd
			Treated	3.6c (0.3)	nd	nd	nd
	6	Acid-adapted	Untreated	7.9az (0.3)	5.0ay (0.6)	3.5abyx (0.6)	2.5abx (0.7)
			Treated	3.2cz (0.4)	2.4bcdz (0.5)	0.5dey (0.6)	0.1cdy (0.2)
		Nonadapted	Untreated	8.0az (0.4)	3.9aby (0.8)	3.9ay (0.9)	2.8ay (0.8)
			Treated	3.4cz (0.5)	0.9dy (1.1)	0.4dey (0.5)	0.3cdy (0.4)

abcd – means in the same column for each temperature of incubation with different superscript letters are different ($P < 0.05$)
zyxw – means in the same row with different superscript letters are different ($P < 0.05$)
nd- not determined; according to study design acid tolerance was assessed at days 0, 2 and 6.

Changes in bacterial populations in washings and on meat inoculated with *E. coli* O157:H7

Acid-adaptation of cultures and strain-mixing did not influence ($P \geq 0.05$) pathogen populations in washings incubated at 4 or 15°C (Time-0 in Tables V.1 and V.2). In addition, there were no differences ($P \geq 0.05$) in bacterial populations during incubation in nonacidic or acidic washings (Tables V.1 and V.2). Populations of bacteria in washings stored at 4°C remained unchanged following inoculation at approximately 5 log CFU/ml; however, washings stored at 15°C experienced increases in populations to approximately 7.5 log CFU/ml (Tables V.1 and V.2).

As with washings, pathogen populations on meat were not influenced ($P \geq 0.05$) by acid-adaptation of cultures or strain-mixing (Tables V.1-V.4). Pathogen populations attached to meat at approximately 6 log CFU/cm^2 after inoculation and remained as such on untreated meat while immediately decreasing by up to 2.0 log CFU/cm^2 on meat treated by dipping in hot (75°C) water and 2% lactic acid (55°C) (Tables V.3 and V.4). On treated meat stored at 4 and 15°C, there were no changes in pathogen populations during storage; however, on untreated meat stored at 15°C, pathogen populations increased to up to 8 log CFU/cm^2 (Tables V.3 and V.4).

Table V.9: Mean (standard deviation) pH values of acid-adapted or nonacid-adapted *Escherichia coli* O157:H7 strains grown individually (IN) and mixed or grown in a mixture (CK) prior to inoculation on lean beef tissue left untreated or treated (by dipping in hot water [75°] followed by lactic acid [LA; 2%, 55°C]) or into nonacidic (water) and acidic (water washings adjusted to pH 4.95 with lactic acid washings) washings

				Days of Storage			
Substrate	Inoculum	Mix	Substrate	0	2	4	6
Meat (4°C)	Acid-adapted	IN	Untreated	5.58az (0.20)	5.61az (0.07)	5.64az (0.13)	5.69az (0.16)
			Treated	4.73bz (0.30)	4.72bz (0.17)	4.77bz (0.14)	4.77bz (0.23)
		CK	Untreated	5.47az (0.16)	5.50az (0.05)	5.54az (0.09)	5.55az (0.18)
			Treated	4.86bz (0.21)	4.86bz (0.13)	4.90bz (0.10)	4.92bz (0.24)
	Nonadapted	IN	Untreated	5.42az (0.30)	5.47az (0.23)	5.53az (0.39)	5.49az (0.40)
			Treated	4.78bz (0.17)	4.77bz (0.08)	4.86bz (0.14)	4.91bz (0.22)
		CK	Untreated	5.61az (0.09)	5.57az (0.12)	5.67az (0.14)	5.71az (0.16)
			Treated	4.85bz (0.11)	4.91bz (0.06)	4.94bz (0.07)	4.97bz (0.18)
Meat (15°C)	Acid-adapted	IN	Untreated	5.58az (0.20)	5.65az (0.32)	5.71az (0.27)	5.86az (0.16)
			Treated	4.73bz (0.30)	4.78bz (0.19)	4.81bz (0.17)	4.89bz (0.15)
		CK	Untreated	5.47az (0.16)	5.52az (0.13)	5.43az (0.13)	5.95az (0.17)
			Treated	4.86bz (0.21)	4.94bz (0.24)	4.96bz (0.18)	5.28bz (0.14)
	Nonadapted	IN	Untreated	5.42az (0.30)	5.48az (0.18)	5.57az (0.10)	5.61az (0.20)
			Treated	4.78bz (0.17)	4.87bz (0.13)	4.91bz (0.11)	4.97bz (0.14)
		CK	Untreated	5.61bz (0.09)	5.81az (0.12)	5.87az (0.21)	5.88az (0.18)
			Treated	4.85az (0.11)	4.89bz (0.13)	4.97bz (0.43)	4.98bz (0.33)
Washings (4°C)	Acid-adapted	IN	Nonacidic	7.01az (0.02)	7.17az (0.28)	7.20az (0.08)	7.22az (0.12)
			Acidic	4.95bz (0.05)	5.02bz (0.10)	5.04bz (0.05)	5.07bz (0.10)
		CK	Nonacidic	7.07az (0.13)	7.11az (0.21)	7.17az (0.13)	7.17az (0.13)
			Acidic	5.01bz (0.12)	5.11bz (0.13)	5.16bz (0.08)	5.14bz (0.10)
	Nonadapted	IN	Nonacidic	7.11az (0.09)	7.20az (0.20)	7.21az (0.17)	7.18az (0.09)
			Acidic	4.97bz (0.04)	5.03bz (0.07)	5.05bz (0.12)	5.07bz (0.13)
		CK	Nonacidic	6.97az (0.15)	7.01az (0.10)	7.08az (0.11)	7.07az (0.14)
			Acidic	4.89bz (0.20)	4.96bz (0.17)	4.97bz (0.19)	4.98bz (0.16)
Washings (15°C)	Acid-adapted	IN	Nonacidic	7.01az (0.02)	7.21az (0.08)	7.28az (0.12)	7.41az (0.15)
			Acidic	4.95bz (0.07)	5.07bz (0.10)	5.17bz (0.08)	5.21bz (0.11)
		CK	Nonacidic	7.07az (0.13)	7.16az (0.15)	7.26az (0.18)	7.36az (0.21)
			Acidic	5.01bz (0.12)	5.09bz (0.20)	5.19bz (0.11)	5.22bz (0.22)
	Nonadapted	IN	Nonacidic	7.11az (0.04)	7.19az (0.10)	7.26az (0.12)	7.31az (0.31)
			Acidic	4.97bz (0.04)	5.04bz (0.07)	5.14bz (0.09)	5.20bz (0.14)
		CK	Nonacidic	6.97az (0.15)	7.04az (0.08)	7.11az (0.2)	7.22az (0.23)
			Acidic	4.89bz (0.20)	4.95bz (0.40)	4.97bz (0.26)	5.04bz (0.07)

ab – means in the same column for each temperature of incubation with different superscript letters are different ($P < 0.05$)

z – means in the same row with different superscript letters are different ($P < 0.05$)

ATR of *E. coli* O157:H7 in washings

In general, there were no differences ($P \geq 0.05$) in the ATR of cultures grown as a mixture of strains compared with those grown as individual strains and mixed prior to inoculation (Tables V.1 and V.2). Although there was no indication of differences in the growth rates of strains grown individually or as a mixture prior to inoculation of washings (Tables V.1 and V.2) or meat (Tables V.3 and V.4), it was hypothesized that strains grown as a mixed culture might enhance survival mechanisms through strain interaction and as such be more apt to deal with acidic conditions than strains prepared individually and mixed immediately prior to inoculation. The rationale for such a hypothesis is supported by the ability of strains in a mixed culture to display higher resistance to stress conditions than those in pure culture (Smoot and Pierson, 1998; Sutherland, 2001), and furthermore, on the potential of strain interaction to enhance infection (Ferguson et al., 1999). Under the conditions of this study, it was apparent that the preparation and mixing of strains at different stages prior to inoculation of washings or meat had no effect ($P \geq 0.05$) on the ATR of the pathogen.

The ATR of acid-adapted *E. coli* O157:H7 in washings immediately following inoculation (day-0) was higher ($P < 0.05$) than that of the nonacid-adapted cultures (Tables V.1 and V.2). This was most likely a direct result of the previous application of acid stress brought about by fermentation of sugars in broth (Buchanan and Edelson, 1996). Although the pathogen's ATR decreased as storage progressed, acid-adapted populations remained more resistant than nonacid-adapted populations throughout 6 d of storage at 4°C. Considering that population levels remained stable and consistent during storage at 4°C, it is likely that the higher acid tolerance of acid-adapted populations

compared with nonacid-adapted populations was associated with maintenance of previous acid-adaptation under incubation temperatures not supporting growth and consequential generation of new, nonacid-adapted *E. coli* O157:H7 populations (Stopforth et al., 2002). Although there were no differences ($P \geq 0.05$) in population concentrations in acidic and nonacidic washings exposed to acidified TSBYE immediately following inoculation, the level of survival following exposure to acid was slightly higher in populations from acidic washings (Tables V.1 and V.2). Regardless of previous acid-adaptation, survival of pathogen populations from acidic washings throughout storage at 4°C was consistently and significantly ($P < 0.05$) higher than that of those from nonacidic washings following exposure to acidified TSBYE (Tables V.1 and V.2). This indicates that cells incubated in washings at 4°C become habituated to acidic (pH 4.89-5.22) conditions, and as a result become more tolerant to more extreme acid exposure (pH 3.5). This may pose a problem in a beef processing establishment where there is potential for development of acid-adaptation due to exposure to residual acidic runoff fluids (Samelis et al., 2002; Stopforth et al., 2002, 2003b). The results suggest that regardless of previous acid-adaptation in broth, *E. coli* O157:H7 may become habituated to the effects of sublethal acid in the form of acidic washings that may collect in a beef processing environment. The potential for such washings to collect and remain in a processing environment exists, and if left undisturbed by inadequate sanitation programs, may harbor *E. coli* O157:H7, resulting in acid-habituation of the cells. Upon cross-contamination of carcasses or meat, these acid-habituated populations may be more tolerant to subsequent acid interventions.

When *E. coli* O157:H7 was incubated in washings at 15°C, the higher ATR of acid-adapted populations, as compared with nonacid-adapted populations, observed immediately after inoculation was not maintained throughout storage (Tables V.1 and V.2). Generally, ATR was similar between acid-adapted and nonacid-adapted populations throughout storage at 15°C. The seemingly higher ATR of nonacid-adapted cells may be mainly due to a higher initial population level exposed to acidified TSBYE as compared with populations from acid-adapted inocula (Tables V.1 and V.2). A logical explanation for this effect, which is the opposite of what was observed at 4°C, may be a potential exhaustion of cells in acidic washings due to the energy required to overcome the stressful conditions of the acidic environment while simultaneously expending energy on generating new cells at a temperature permitting growth. In general, the ATR of *E. coli* O157:H7 appeared to increase as storage at 15°C progressed and was in part due to the higher initial level of populations exposed to acidified TSBYE. Pathogen populations stored in washings at 15°C became more resistant to the effects of the acid as storage progressed; however, growth only occurred between day-0 and day-2; thereafter, no significant ($P \geq 0.05$) increase in population levels was observed (Tables V.1 and V.2). This implies that the increased ATR of the pathogen during progression of storage at 15°C was not solely due to increased levels of exposure but also in part to general stress-hardening in the nutrient-limited washings. It is well known that environments with limited nutrients pose a stress to bacteria that results in adaptive mechanisms conferring increased resistance to subsequent stress application (Arnold and Kaspar, 1995; Jenkins et al., 1988; Rowe and Kirk, 1999). The general stress response associated with the stationary phase of bacterial growth may also account for the increased resistance to

acidic conditions in washings incubated at 15°C (Arnold and Kaspar, 1995; Buchanan and Edelson, 1996; Cheville et al., 1996).

ATR of *E. coli* O157:H7 on meat

There was no difference ($P \geq 0.05$) in ATR of acid-adapted *E. coli* O157:H7 as compared with nonacid-adapted populations on meat immediately following inoculation (day-0; Tables V.3 and V.4). The absence of acid-tolerance differences between acid- and nonacid-adapted cultures may have been a result of the protection offered by the transition of cells from a liquid to the solid meat matrix. The attachment to a surface may have masked the true difference in acid tolerance of the cultures considering that cells attached to surfaces are more resistant to adverse conditions than planktonic cells (Costerton et al., 1995). Interestingly, the ATR of acid-adapted *E. coli* O157:H7 populations on meat stored at 4°C was higher than that of the nonacid-adapted populations during progression of storage (Tables V.1 and V.2). In all cases, the survival and not necessarily the ATR of populations on untreated meat was higher than that on treated meat; this was most likely due to the higher initial level of exposure to acidified TSBYE considering that the difference in survival remained similar to the difference in initial levels.

The ATR of acid-adapted populations present on meat stored at 4°C did not change during storage but remained relatively consistent with that observed at day-0, while the ATR of nonacid-adapted populations decreased relative to the progression of storage. Furthermore, the reduction in acid-adapted *E. coli* O157:H7 on meat stored at 4°C due to exposure in acid was minimal throughout storage and presented a tail of

survivors following 60 min of exposure, highlighting the extreme acid resistance of acid-adapted cells from meat stored at 4°C. This may have implications on the microbiology of fresh beef that may be contaminated with acid-adapted *E. coli* O157:H7 and stored at 4°C. Acid-resistant cells incubated at 4°C may develop a secondary resistance to subsequent acid interventions or even cross-protection to processing stresses such as cooking (Ryu et al., 1998).

Similar to the ATR of populations on meat stored at 4°C, the ATR of acid-adapted *E. coli* O157:H7 on meat stored at 15°C was higher ($P < 0.05$) than that of nonacid-adapted populations throughout storage (Tables V.3 and V.4). The ATR of acid-adapted *E. coli* O157:H7 on treated meat stored at 15°C was similar to that observed on treated meat stored at 4°C; however, the ATR of acid-adapted populations on untreated meat stored at 15°C was lower than that on meat stored at 4°C (Tables V.3 and V.4). Although, survival after acid exposure, at both temperatures, was similar for acid-adapted populations present on meat, the initial level of cells exposed was higher on meat stored at 15°C (Tables V.3 and V.4). A similar trend was observed for the nonacid-adapted populations, indicating that acid resistance of *E. coli* O157:H7 on meat was enhanced during storage at 4°C as compared with 15°C. In all cases, the ATR of populations on untreated meat stored at 15°C was lower ($P < 0.05$) than that on treated meat and was in part due to lower initial levels of exposure to acid (Tables V.3 and V.4). The presence of a resistant acid-adapted sub-population on meat stored at 4°C following exposure to acid was also observed on meat stored at 15°C (Tables V.3 and V.4).

These results may imply that under the conditions of this study, acid-adapted *E. coli* O157:H7 on meat became resistant to subsequent acid conditions and that resistance

may be further enhanced by storage at refrigeration temperatures (e.g., 4°C). A logical explanation for this may be that less energy is expended by the pathogen on metabolic and enzymatic processes at 4°C and the primary energy expenditure is on maintenance of stress resistance, whereas, at 15°C, the pathogen has the ability to grow and most likely uses energy to synthesize compounds for growth and replication, thus detracting from energy dedicated to maintaining acid resistance.

CONCLUSIONS

Under the conditions of this study, mixing and subsequent co-habitation of *E. coli* O157:H7 strains prior to inoculation of meat or meat decontamination runoff fluids (washings) did not appear to affect the growth rate or ATR of the pathogen. Decontamination of meat did not affect the subsequent ATR of *E. coli* O157:H7 other than resulting in lower initial pathogen levels exposed to acid conditions. In this study, *E. coli* O157:H7 became acid-habituated and more tolerant to acid conditions after incubation in acidic washings of sublethal pH (4.89-5.22) at 4°C rather than in corresponding nonacidic washings (pH 6.97-7.41) or washings incubated at 15°C. The ATR of the pathogen originating in washings was enhanced when populations were acid-adapted and incubated at 4°C. Similarly, the ATR of *E. coli* O157:H7 on meat was increased by previous acid-adaptation in broth and enhanced by storage at 4°C. *Escherichia coli* O157:H7 in sublethal acidic washings may become acid-habituated and, as such, more resistance to subsequent acid exposure if incubated under low temperatures representative of refrigeration conditions in a processing facility. This is important for the

beef industry because meat or washings contaminated with previously acid-adapted *E. coli* O157:H7 and incubated at low temperatures may contribute to development of populations resistant to subsequent processing interventions using acid and particularly lactic acid such as a post-chill lactic acid spray.

CHAPTER VI

REDUCTION OF *ESCHERICHIA COLI* O157:H7 POPULATIONS ON LEAN BEEF TISSUE INOCULATED WITH CULTURES PREPARED UNDER DIFFERENT CONDITIONS AND EXPOSED TO INCOMPLETE HEATING AND SYNTHETIC GASTRIC FLUID

ABSTRACT

This study evaluated stress responses of different *Escherichia coli* O157:H7 inocula on untreated or decontaminated (sequential hot water and warm acid dipping) beef stored under vacuum at different temperatures and periodically transferred to aerobic storage conditions prior to exposure to consumer-induced stresses simulating incomplete cooking and consumption. Lean beef tissue samples inoculated with *E. coli* O157:H7 cultures prepared in TSB, meat decontamination runoff fluids (WASH), or dettached from moist biofilms (WETB) or dried biofilms (DRYB) were left untreated or sequentially decontaminated by dipping for 30 s in hot (75°C) water and lactic acid (2%) (decontaminated). Samples were vacuum-packaged, stored at 4 (7, 14, and 28 d) or 12°C (4 and 8 d), and periodically transferred to aerobic storage (7°C for 5 d). During storage, samples were exposed to sequential heat (55°C for up to 20 min) and synthetic gastric fluid (adjusted to pH 1.0 with HCl for up to 90 min) simulating consumption of undercooked beef. Under the conditions of this study, planktonic pathogen cells prepared in liquid media were, in general, more resistant to heat and acid stresses than those grown

as biofilms and detached prior to inoculation. Decontamination of fresh beef resulted in injury that prevented subsequent growth of the surviving populations at 12°C and decreased the pathogen's resistance to subsequent heat and acid conditions. The shift of pathogen cells, on beef stored under vacuum at 4°C, to aerobic storage did not affect cell populations nor subsequent survival after sequential exposure to heat and simulated gastric fluid. The transfer of meat stored under vacuum at 12°C, to aerobic storage, however, resulted in reduced pathogen numbers during storage and sensitization to the effects of sequential heat and acid exposure and because this sensitization did not occur at 4°C it appeared to be storage temperature-dependent.

INTRODUCTION

The hides of cattle are one of the principal sources of bacterial contamination on carcasses during slaughter; however, the carcasses may also be cross-contaminated from workers' clothes, hands or equipment, other carcasses, plant equipment, plant environment (i.e., water or air) and accidental spillage of body fluids during skinning and evisceration (Gill et al., 1998; Lundeen, 2000). Prevalence estimates of *E. coli* O157:H7 on the hides of cattle presented for slaughter have been as high as 60.6% (Barkocy-Gallagher et al., 2003) and there is evidence that positive hide samples may affect the number of positive carcass samples after harvesting (Elder et al., 2000; Ransom et al., 2003a). Contaminated raw beef products entering the food supply may result in severe economic losses and, more importantly, in foodborne illness and even death among consumers. To avoid or minimize such risk, most U.S. meat processing facilities have

implemented multiple-hurdle carcass decontamination interventions. Examples of such interventions and chemical solutions that have been determined to be effective in reducing bacterial contamination include: (i) spraying using hot or cold water; (ii) hot water pasteurization; (iii) steam pasteurization; (iv) hot water/steam vacuuming; and, (v) sanitizing solutions like organic acids, acidified sodium chlorite, peroxyacetic acid, and cetylpyridinium chloride (Ransom et al., 2003b). Multiple-hurdle systems in U.S. beef processing plants most commonly include sequential treatments of hot water (up to 85°C) followed by lactic acid (up to 5%) spraying/misting of the carcasses prior to chilling (Sofos and Smith, 1998).

Although beef decontamination interventions have demonstrated effectiveness in reducing bacterial populations on carcass surfaces, limited research has been conducted to evaluate the fate of pathogens surviving in residual decontamination runoff fluids (washings) and on subsequently recontaminated carcasses or meat. Research has demonstrated that *E. coli* O157:H7 can survive in 2% lactic acid and water meat decontamination runoff fluids for 2 and 14 d (Samelis et al., 2001b, 2002a), respectively, and for up to 14 d in a mixture (1:1) of lactic acid and water washings (Samelis et al., 2002a; Stopforth et al., 2003a,b). In addition, Stopforth et al. (2003a,b) indicated that *E. coli* O157:H7 populations were able to form biofilms on stainless steel submerged in beef decontamination runoff fluids stored at 15°C, which were more resistant to sanitizers than were populations suspended in washings. The results also demonstrated that acid washings may alter the microbial ecology of meat plant environments and select for the growth of the natural meat flora and in so doing potentially enhance the survival of attached pathogens following long-term stressing (Stopforth et al., 2003a,b). Exposure of

pathogen biofilms to stressful environments such as adverse temperature and pH, desiccation (low a_w), and sublethal concentrations of sanitizers associated with food processing may result in stress-hardened cells with the ability to survive subsequent antimicrobial treatments or processing stresses. Furthermore, detachment of even one cluster of such biofilms may recontaminate previously unadulterated or decontaminated product with sufficient organisms to comprise an infectious dose and as such enter the food supply (Stoodley et al., 2002; Stopforth et al., 2002, 2003a,b). More importantly, previous exposure to adverse environmental and processing conditions may alter pathogen tolerance to subsequent consumer-related stresses including heat from cooking and acid upon consumption.

The ability of pathogens to survive in adverse conditions plays a crucial role in foodborne disease sequellae and indeed the emergence of *E. coli* O157:H7 as a foodborne pathogen may be partly due to its increased acid tolerance (Benjamin and Datta, 1995; Leyer et al., 1995). A major requirement for foodborne pathogenicity is the ability of cells to survive the acidic gastrointestinal environment (Datta and Benjamin, 1999), and as such it is expected that pathogens with a high acid tolerance will cause disease with a lower infective dose and vice versa. An important concern for consumers is the potential for incomplete cooking of raw beef products, especially ground beef, where the pathogen may be introduced below the surface on an intact product and, as such, potentially survive inadequate cooking and increase the risk of foodborne illness. An additional concern is that incomplete cooking of contaminated product may result in a selection of hardy pathogen cells which may have the ability to survive the acidic conditions

encountered during consumption and digestion, especially among at-risk populations such as the elderly, the young and immunocompromised.

Therefore, the objective of this study was to determine the fate of *E. coli* O157:H7 cultures originating from different histories (i.e., tryptic soy broth, meat decontamination runoff fluids, moist biofilms and dried biofilms) inoculated on fresh lean beef, exposed to sequential heat (75°C) and lactic acid (2%; 55°C) decontamination or left untreated prior to exposure to sequential heat (55°C) and synthetic gastric fluid (adjusted to pH 1.0 with HCl). In addition, this study evaluated the effect of such potential consumer-generated stresses on *E. coli* O157:H7 on beef stored in vacuum packages at 4 (for 14 d) or 12°C (for 8 d), and subsequently transferred to aerobic storage (7°C for 5 d). Beef was stored in vacuum at 4°C for 14 d to simulated a commercial distribution time of two weeks while that stored at 12°C was minimized to 8 d based on the potential for beef to spoil at regfrigeration abuse temperature. Aerobic storage time of 5 d at 7°C was selected based on common retail practice for displaying fresh beef.

MATERIALS AND METHODS

Bacterial strains

Four rifampicin (Rif) resistant (100 μg/ml) derivatives of *E. coli* O157:H7 (ATCC 43895, ATCC 43889, ATCC 51658 and EO139 [venison jerky isolate provided by Dr. M.P. Doyle, University of Georgia, Griffin, GA) developed in our laboratory (using methods described by Hardin et al., 1995) were used in this study. Stock cultures of each strain were maintained at -70°C in tryptic soy broth (Difco, Becton Dickinson Co., Sparks,

MD) containing 200 μg/ml of rifampicin (Sigma Chemical Company, St. Louis, MO) and 20% glycerol (Mallinckrodt Baker, Inc., Paris, KY). Working cultures were stored on TSA (Difco) slants at 4°C and were activated by transferring a loopful of each individual strain from TSA slants to TSB without dextrose (TSB-G; Difco) and incubating at 35°C for 24 h. The activated strains were subcultured once in TSB-G supplemented with 200 μg/ml of rifampicin (to ensure that cultures retained their antibiotic resistance) as well as streaked on Sorbitol MacConkey agar (Difco) supplemented with cefixime-tellurite (Dynal Inc., Lake Success, NY) (SMAC-CT) and incubated at 35°C for 24 h. Colonies from the SMAC-CT plates were tested using a Dry Spot Latex Agglutination test (Oxoid, Ogdensburg, NY) to confirm presence of the O157 antigen. The four strains obtained from subculturing were mixed and centrifuged at 4,628 x g (Eppendorf, model 5810 R; Brinkmann Instruments. Inc., Westbury, NY) for 15 min at 4°C. The resulting pellets were washed in 10 ml of sterile phosphate-buffered saline (PBS, Sigma), centrifuged for a second time, and the final pellet was resuspended in 10 ml PBS, and serially diluted in 0.1% BPW (Difco) to yield approximately 5.0 log CFU/ml.

Inocula preparation

The four-strain composite culture was used to inoculate various substrates in order to prepare the inocula under representative environmental conditions (inoculum origin). The control inoculum (TSB) was obtained by inoculating (10^5 CFU/ml) TSB (Difco) with the composite *E. coli* O157:H7 culture and, after overnight incubation at 35°C, diluting (100,000 fold) the resulting culture in sterilized MRD.

To simulate pathogen populations surviving in meat decontamination runoff fluids, spray-washing runoff fluids (washings) from decontamination of beef carcasses at a commercial slaughtering plant using water (84°C at spraying) were collected and stored in 10-L sterile bottles (Nalgene, Nalge Co., Rochester, NY) for ≤30 d at -30°C. The washings were thawed (at 4°C overnight) prior to use and were passed through four layers of cheesecloth, three times, to remove large beef tissue particles. Following initial filtering, the washings were passed through Fisherbrand® filter paper (Qualitative P8; Fisher Scientific, Houston, TX) four times, using a Buchner funnel under vacuum to remove remaining small beef particles. The resulting fluid was filter-sterilized through 500-ml Millipore™ Stericups (0.22 μm GV Durapore Membrane; Millipore Corporation, Bedford, MA) under vacuum. After preparation, the meat washings were tested microbiologically to ensure sterility by plating onto TSA. To generate suspended populations originating in washings (WASH), the *E. coli* O157:H7 composite was inoculated (10^5 CFU/ml) in 40 ml of the filtered meat washings, and after incubation at 35°C for 24 h it was diluted (100,000 fold) in sterilized MRD.

To simulate pathogen populations originating from meat decontamination runoff fluids and attached to meat processing surfaces, stainless steel (type 304, #2b finish, 0.08 mm thick) coupons (2 x 5 cm) were submersed in washings to serve as a platform for biofilm formation. The stainless steel coupons were cleaned and prepared as described by Stopforth et al. (2002, 2003a,b). After cleaning, the stainless steel coupons were individually placed in an upright position into sterilized centrifugation tubes (50 ml, 28.5 x 104 mm [O.D. x L]; Nalgene) containing 50-ml aliquots of the sterilized washings. The washings were then inoculated (10 CFU/ml) with the composite *E. coli* O157:H7 culture

and incubated at 35°C for 24 h. Two types of biofilm cells were prepared based on conditions prior to harvesting of cells, namely, dry biofilms (DRYB) and moist biofilms (WETB). Dry biofilms (DRYB) were obtained by subjecting the stainless steel coupons to air drying for 12 h after being removed from the overnight incubation in washings and prior to harvesting, while WETB were harvested immediately after incubation without a drying step. Cells from WETB were harvested from the stainless steel by first rinsing the coupon with sterile distilled water to remove loosely attached cells and organic material, whereas coupons for DRYB were not rinsed. The coupons were placed in a centrifuge tube containing 45 ml of sterile MRD and ten glass beads (4 mm diameter, Fisher) to aid in removal of attached cells. The tube was then vortexed (3200 rpm; Vortex-Genie 2, Scientific Industries Inc., Bohemia, NY) for 2 min to remove cells attached to coupons after which the coupons were rinsed with 1 ml sterile distilled water to collect cells loosely associated with the coupon before the process was repeated with another four coupons in the same tube in an effort to concentrate cells.

Inoculation and decontamination of fresh lean beef

Fresh untreated beef top rounds were obtained from a local commercial slaughtering plant, stored at 4°C, and used within 72 h postmortem. The beef was cut into 5 x 2.5 x 1 cm (total surface area of 40 cm^2) pieces. Separate beef pieces were inoculated with 200 µl of each of the TSB, WASH, WETB or DRYB inocula on one side and allowed to attach for 15 min at 4°C before the same procedure was repeated on the reverse side to achieve a level of approximately 3 log CFU/cm^2. The following levels were achieved 3.0, 3.9, 2.8, and 1.6 log CFU/cm^2 for TSB, WASH, WETB, and DRYB, respectively. The level of the

pathogen from TSB and WETB inocula was close to the target, whereas, WASH resulted in approximately 1 log CFU/cm^2 higher than the target and DRYB was limited due to the extreme injury caused by the drying process (unpublished data). Following inoculation, the meat was either left untreated or exposed to sequential decontamination treatments (decontaminated) comprised of dipping beef pieces in hot water (distilled, 75°C) for 30 s followed by dipping in 2% lactic acid (55°C, pH 2.14) (Mallinckrodt Baker, Inc.) for 30 s (20 beef pieces per 1 L fluid). After the application of the decontamination treatment, individual beef pieces were placed into 18-oz Whirl-Pak® filter sterile plastic bags (Nasco, Fort Atkinson, Wisconsin) for immediate analysis or into vacuum bags (15 x 20 cm, 3 ml std barrier, Nylon/PE vacuum pouch; Koch, Kansas City, MO), sealed (Hollymatic, Corp., Countryside, IL) and stored at 4 (for up to 28 d) or 12°C (for 8 d). Triplicate beef pieces from each inoculum (TSB, WASH, WETB or DRYB) per treatment (untreated or decontaminated) were removed on days 7, 14, and 28 of storage at 4°C and on days 4, 8, and 16 of storage at 12°C for microbiological analyses.

Transfer of vacuum-packaged refrigerated beef to aerobic storage

Triplicate samples were removed from 4°C vacuum storage at days 7 and 14 or from 12°C vacuum storage at days 4 and 8 and placed on retail foam trays (7.5 x 12.5 cm, Pactiv, Lake Forest, IL), covered with air-permeable film (Omnifilm; Pliant Corporation, Uniontown, OH), and stored at 7°C for 5 days. When samples were removed from aerobic storage they were placed into a sterile 18-oz Whirl-Pak® filter sterile plastic bag (Nasco) for microbiological analyses, or for further stress exposure.

Exposure of fresh beef to heat

In order to assess the effect of sublethal heat exposure or incomplete heating, samples from aerobic storage were removed and sealed in vacuum bags (Koch). The vacuum bags were submersed for 10 or 20 min in a water bath (Isotemp 228, Fisher) held at 55°C. Three sets of samples representing each inoculum by treatment combination were submersed. One set of samples was submersed for 10 min and analyzed microbiologically immediately after heating to determine the survival of bacterial populations on the beef. The other two sets of samples were submersed for 20 min and one set was analyzed microbiologically immediately following heating while the remaining set was retained for use in the subsequent exposure of samples to a synthetic gastric fluid.

Exposure of fresh beef to synthetic gastric fluid

To assess the susceptibility of *E. coli* O157:H7 to inactivation at a low pH similar to the stomach environment, use was made of a synthetic gastric fluid (Beumer et al., 1992; Czuprynski et al., 2002) consisting of 8.3 g proteose peptone (Sigma), 3.5 g D-glucose (dextrose, ACS, anhydrous; Fisher), 2.05 g sodium chloride (ACS, crystallized; Fisher), 0.6 g potassium phosphate (monobasic, anhydrous; Sigma), 0.11 g calcium chloride (dehydrate; Sigma), 0.37g potassium chloride (ACS, crystallized; Fisher), 0.1 g lysozyme (from chicken egg white, dialyzed, crystallized; Sigma), 50 mg bile (bovine bile [ox gall powder], dried, unfractionated; Sigma) and 13.3 mg pepsin (Pepsin A, from porcine stomach mucosa, dessicated; Sigma) per liter of distilled water. The synthetic gastric fluid in this experiment was adjusted to pH 1.0 using hydrochloric acid (AR®,

approximately 37%, Mallinckrodt). To test the acid tolerance of *E. coli* O157:H7 populations surviving on beef pieces previously submersed in water (55°C), 50 ml of synthetic gastric fluid (GF; 25°C) was added to the bags containing the samples followed by pummeling (Masticator, IUL Instruments, Barcelona, Spain) for 2 min. Samples were exposed to GF for a duration of 90 min and at 30 min intervals, aliquots of the samples were removed from the bags for determination of survivors. Determination of populations on inoculated but untreated samples prior to heating served as controls or as time-0 of exposure to determine initial populations. Control samples received 40 ml of sterilized MRD as it allowed for a more sensitive detection level as compared to the 50 ml of GF, which was selected based on preliminary investigations to achieve a pH simulating the stomach after addition of beef.

Microbiological and physical analyses of samples

For microbiological analysis of time-0 (initial level) and time-10 and -20 (heat treated) samples, 40 ml of sterilized MRD was added to the Whirl-Pak® bag (Nasco) containing the beef pieces and homogenized (IUL Instruments) for 2 min. The GF-treated samples were analyzed directly from the bag containing GF. A portion (1 ml) of the homogenized samples was serially diluted in 9 ml of sterile 0.1% buffered peptone water (Difco) and appropriate dilutions were plated onto TSA for determination of total bacterial counts and onto TSA+Rif (TSA supplemented with 200 μg/ml of rifampicin) for the selective enumeration of inoculated rifampicin-resistant *E. coli* O157:H7 populations. Colonies were counted after incubation at 35°C for 48 h. The detection limit was estimated as 0 log CFU/cm^2 for time-0, -10, and -20 samples, based on the assumption that plating 1 ml of

the initial sample homogenate could yield at least 1 CFU on the agar plate. The detection limit for the GF-treated samples was estimated as 0.1 log CFU/cm^2 based on the same procedure as described above except for the dilution created by homogenizing beef with a surface area of 40 cm^2 in 50 ml of GF. However, if a minimum of 25 CFU per plate is required to be acceptable, then the detection limit would be 1.4 log CFU/cm^2.

The pH of homogenized control samples was measured after each microbiological analysis while that of samples exposed to gastric fluid was randomly measured after the 90-min exposure time due to time constraints. The pH was measured using a digital pH meter (*Ultra*Basic, UB-10; Denver Instrument, Denver, CO) with a glass pH electrode (pH/ATC Electrode #300729.1; Denver Instrument). Partial biochemical characterization of randomly selected colonies on countable TSA plates was performed by observing colony morphology, testing for gram-, catalase- and oxidase-reactions (Stopforth et al., 2003b).

Statistical analysis

Two replicate experiments were conducted with three samples tested per treatment at each sampling time in each replicate. Microbiological data were converted to log CFU/cm^2 before being analyzed. Values for the mean log and standard deviation of each set of bacterial counts were calculated on the assumption of a log-normal distribution of microorganisms. Data were separated by temperature of incubation as the days of analyses were different based on the expected growth rates at different temperatures and thus they were not compared statistically. For the 12°C data set, analysis of fixed effects using the GLM procedure of SAS® v 8.2 (SAS, 2002) indicated that log CFU/cm^2

populations were dependent on type of medium (TSA or TSA+Rif) (F-statistic=149.20; P<0.0001), inoculum type (TSB, WASH, WETB, or DRYB) (F-statistic=62.63; P<0.0001), treatment (decontaminated or untreated) (F-statistic=4107.57; P<0.0001), day of analysis (0, 4, 8, 9, 13, or 16) (F-statistic=382.60; P<0.001), and time of analysis (time-0, -10 [heat], -20 [heat], -30 [GF], -60 [GF], or -90 [GF]) (F-statistic=623.68; P<0.0001). For the 4°C data set, analysis of fixed effects using the GLM procedure of SAS® v 8.2 (SAS, 2002) indicated that log CFU/cm^2 populations were dependent on type of medium (TSA or TSA+Rif) (F-statistic=368.13; P<0.0001), inoculum type (TSB, WASH, WETB, or DRYB) (F-statistic=43.02; P<0.0001), treatment (untreated or decontaminated) (F-statistic=1039.65; P<0.0001), day of analysis (0, 7, 12, 14, 19, or 28) (F-statistic=13.60; P<0.001), and time of analysis (time-0, -10 [heat], -20 [heat], -30 [GF], -60 [GF], or -90 [GF]) (F-statistic=709.53; P<0.0001). Data were too numerous to present in a single table and, thus, for each temperature of incubation data were further separated by each medium-treatment combination using a 4 x 6 x 6 (inoculum type x day of analysis x time of analysis, respectively) factorial design. Among each medium by temperature combination, individual fixed effects and up to three-way interactions were evaluated with ANOVA using the model $y = x_1 + x_2 + x_3 + x_1x_2 + x_1x_3 + x_2x_3 + x_1x_2x_3$ in the GLM procedures of SAS® v 8.2 (SAS, 2002); where x_1 represents inoculum type, x_2 represents day of analysis and x_3 represents time of analysis. Least-squares means were separated using a protected pairwise t-test of SAS® v 8.2 (SAS, 2002). All differences were reported at a significance level of alpha = 0.05.

RESULTS AND DISCUSSION

In the present study, fresh beef was contaminated with *E. coli* O157:H7 and stored at 4 or 12°C under vacuum before being transferred to aerobic storage or exposed to heat and subsequent acid. The application of heat in this study was selected to simulate incomplete cooking of beef that would subsequently be exposed to acidic conditions representing ingestion and consumption by an individual. Exposure of food to heat is one of the oldest methods of preservation or preparation (Cassens, 1994). There are several methods to cook meat, although the main objective is to achieve a specific internal temperature; the type of product, the rate and type of heat, the type of equipment used and several other factors affect the cooking process (Aberle et al., 2001). Furthermore, various factors may influence the heat resistance of microorganisms including its metabolic phase, growth temperature and nutrient availability (Pflug and Holcomb, 1991). Numerous foodborne outbreaks involving infection with *E. coli* O157:H7 are thought to originate with the consumption of hamburger meat and other beef products that were cooked inadequately (CDC, 2001; Kassenborg et al., 1998). The potential for *E. coli* O157:H7 to survive cooking and mount an ATR to the acidic conditions faced in the human digestive tract is important as it may have severe public health consequences.

The initial pH of the untreated meat inoculated with *E. coli* O157:H7 from different origins ranged from 4.99 to 5.60 and that of decontaminated meat ranged from 4.74 to 5.47 (Table VI.1). The pH of the meat samples remained relatively unchanged during storage (Table VI.1). The pH of meat homogenized and exposed in gastric fluid for 90 min ranged from 1.76 to 2.23 (Table VI.1).

In general, the recovery of bacterial populations from fresh beef tissue during storage and after exposure to stresses was higher ($P < 0.05$) on general growth media (TSA) (initial level in Tables VI.2-VI.5) than that recovered on selective media (TSA+Rif) (initial level in Tables VI.6-VI.9). The increased levels in total bacterial populations (TSA) during storage, especially on untreated beef, compared with the pathogen populations (TSA+Rif) specifically, was most likely due to increases in psychrophilic/psychrotrophic natural flora associated with fresh beef. Furthermore, the difference in total bacterial populations (TSA) compared to pathogen populations (TSA+Rif) following exposure to stresses may have been a result of extensive injury caused by heat and acidic conditions and/or higher initial levels of total bacteria exposed to the stresses. Due to the predominance of the gram-negative, catalase-negative, oxidase-positive *Pseudomonas*-like natural flora on TSA plates, data regarding the changes in pathogen populations on beef during storage and the survival following stress exposure are discussed with reference to TSA+Rif counts. Injury sustained by these cells is not discounted and as such it should be noted that cell counts on TSA+Rif do not represent the absolute level of pathogen survivors.

Table VI.1: Mean (standard deviation) pH values of fresh beef inoculated with *Escherichia coli* O157:H7 of different origins (TSB, WASH, WETB or DRYB), left untreated or decontaminated with sequential treatments of hot water (75°C) and 2% lactic acid (55°C) for 30 s prior to storage under vacuum at 4 or 12°C for 28 and 16 d, respectively

Temperature	Treatment	Day of Storage	Inoculum origin TSB	WASH	WETB	DRYB
4	Untreated	0	5.59cz (0.17)	4.99bcy (0.09)	5.60abz (0.15)	5.02cdy (0.05)
		7	5.60bcz (0.24)	5.05yb (0.14)	5.57bz (0.08)	5.14bcy (0.16)
		7*	5.52cz (0.10)	5.18aby (0.16)	5.67abz (0.30)	5.25by (0.25)
		14	5.55cz (0.11)	5.08by (0.16)	5.57bz (0.18)	5.00cdy (0.10)
		14*	5.70bcz (0.20)	5.18aby (0.07)	5.57bz (0.12)	5.24by (0.21)
		28	5.53cz (0.15)	5.13aby (0.23)	5.53bz (0.40)	5.04cdy (0.20)
	Decontaminated	0	5.46c (0.04)	4.74c (0.06)	5.47b (0.04)	4.87d (0.38)
		7	5.52c (0.04)	4.97bc (0.12)	5.70a (0.12)	5.02cd (0.16)
		7*	5.82ab (0.42)	5.37a (0.13)	5.18c (0.16)	5.33ab (0.14)
		14	5.89ab (0.49)	5.17ab (0.07)	5.77a (0.18)	5.29ab (0.06)
		14*	6.15a (0.51)	5.36a (0.28)	5.78a (0.30)	5.49a (0.25)
		28	5.71bc (0.34)	5.38a (0.35)	5.62ab (0.25)	5.07c (0.39)
12	Untreated	0	5.59az (0.17)	4.99dey (0.09)	5.60az (0.15)	5.02dey (0.05)
		4	5.43abz (0.02)	5.49abz (0.35)	5.44abz (0.07)	5.51abz (0.07)
		4*	5.61az (0.06)	5.56az (0.14)	5.53az (0.08)	5.51abz (0.11)
		8	5.45abz (0.12)	5.42abz (0.14)	5.58az (0.11)	5.53az (0.14)
		8*	5.68az (0.43)	5.53az (0.17)	5.60az (0.19)	5.70az (0.15)
		16	5.43abz (0.10)	5.55az (0.15)	5.60az (0.13)	5.53az (0.12)
	Decontaminated	0	5.46aby (0.04)	4.74ezy (0.06)	5.47aby (0.04)	4.87ez (0.38)
		4	5.08cz (0.20)	4.99dez (0.15)	4.99dz (0.24)	5.13cdz (0.22)
		4*	5.26bcz (0.27)	5.15cdz (0.14)	5.27bcz (0.24)	5.30bcz (0.25)
		8	4.99cz (0.08)	5.02dz (0.13)	5.04dz (0.09)	5.10cdz (0.05)
		8*	5.24bcz (0.30)	5.29bcz (0.15)	5.30bcz (0.28)	5.33bz (0.43)
		16	5.17cz (0.13)	5.09cdz (0.10)	5.19cdz (0.11)	5.16cdz (0.17)

TSB – suspension cells originating in tryptic soy broth; WASH – suspension cultures originating in meat decontamination runoff fluids (washings); WETB – biofilm cells formed in washings and detached from stainless steel; DRYB - dessicated biofilms detached from stainless steel
abcd – means in the same column with different superscript letters are different ($P<0.05$)
zy – means in the same row with different superscript letters are different ($P<0.05$)
pH of meat homogenized in simulated gastric fluid ranged from 1.76 (0.13) to 2.23 (0.22) after 90-min exposure time.
* pH obtained after transfer from vacuum to aerobic storage at 7°C for 5 d

Table VI.2: Mean (standard deviation) total bacterial (TSA) populations (log CFU/cm^2) on fresh beef inoculated with *Escherichia coli* O157:H7 of different origins (TSB, WASH, WETB or DRYB), and decontaminated with sequential treatments of hot water (75°C) and 2% lactic acid (55°C) for 30 s prior to storage under vacuum at 4°C for 28 d and periodic exposure to heat (55°C) for 20 min followed by simulated gastric fluid (pH 1.0) for 90 min

			Time of Heat and Subsequent Simulated Gastric Fluid Exposure (min)				
			Heat (55°C)		Simulated Gastric Fluid (pH 1.0)		
Day	Inoculum	Initial Level	10	20	30	60	90
0	TSB	1.8dez (0.4)	0.9cdy (0.7)	0.7bcy (0.4)	0cx (0)	0bx (0)	0cx (0)
	WASH	1.4efz (0.4)	1.2cz (1.0)	0.5cdy (0.6)	0cx (0)	0bx (0)	0cx (0)
	WETB	0.6gz (0.2)	0.1ey (0.2)	0ex (0)	0cx (0)	0bx (0)	0cx (0)
	DRYB	1.0fgz (0.7)	0.3ey (0.7)	0.5cdy (0.6)	0cx (0)	0bx (0)	0cx (0)
7	TSB	1.7ez (0.4)	0.4dey (0.6)	0.3cdy (0.6)	0cx (0)	0bx (0)	0cx (0)
	WASH	1.9dez (0.3)	0.9cdy (0.6)	0.6bcy (0.8)	0cx (0)	0bx (0)	0cx (0)
	WETB	1.5efz (0.4)	0.3ey (0.7)	0ex (0)	0cx (0)	0bx (0)	0cx (0)
	DRYB	0.4gzy (0.6)	0.9cdz (0.6)	0.2dy (0.3)	0cx (0)	0bx (0)	0cx (0)
7*	TSB	1.9dez (0.2)	1.1cy (0.6)	0.4cdx (0.5)	0cw (0)	0bw (0)	0cw (0)
	WASH	1.7ez (0.4)	1.0cy (0.2)	0.1dx (0.2)	0cw (0)	0bw (0)	0cw (0)
	WETB	1.6ez (0.6)	0.8cdy (0.4)	0.4cdy (0.6)	0cx (0)	0bx (0)	0cx (0)
	DRYB	2.4cdz (1.2)	0.9cdy (0.7)	0ex (0)	0cx (0)	0bx (0)	0cx (0)
14	TSB	1.7ez (0.6)	1.2czy (0.4)	0.7bcy (0.7)	0.8ay (0.9)	0.6ay (0.7)	0.7ay (0.7)
	WASH	1.9dez (0.8)	0.5cdey (0.5)	0.2dy (0.5)	0cx (0)	0.1ay (0.3)	0cx (0)
	WETB	2.4cdz (0.8)	0.4dey (0.7)	0.4cdy (0.7)	0.3aby (0.6)	0.2ay (0.6)	0cx (0)
	DRYB	1.7ez (1.0)	0.4dey (0.7)	0.5cdy (0.8)	0.2by (0.6)	0.3ay (0.6)	0cx (0)
14*	TSB	2.5cz (1.1)	0.8cdy (0.4)	0.4cdx (0.4)	0.3abx (0.5)	0.2ax (0.5)	0.1bx (0.3)
	WASH	2.7bcz (1.2)	1.0cy (0.6)	0.5cdx (0.8)	0.2bx (0.4)	0bw (0)	0cw (0)
	WETB	3.3abz (0.8)	2.9az (0.2)	0.1dy (0.2)	0cx (0)	0bx (0)	0cx (0)
	DRYB	4.1az (2.0)	1.4bcy (0.7)	0.1dx (0.2)	0cw (0)	0bw (0)	0cw (0)
28	TSB	3.7az (1.4)	2.4by (0.6)	1.6ax (0.6)	0cw (0)	0bw (0)	0cw (0)
	WASH	1.6ez (0.4)	0.9cdz (0.7)	0ey (0)	0cy (0)	0by (0)	0cy (0)
	WETB	2.2cdz (0.7)	1.7bczy (1.1)	1.5ay (0.6)	0cx (0)	0bx (0)	0cx (0)
	DRYB	3.3abz (1.9)	2.2by (1.1)	1.3abx (0.7)	0cw (0)	0bw (0)	0cw (0)

TSB – suspension cells originating in tryptic soy broth; WASH – suspension cultures originating in meat decontamination runoff fluids (washings); WETB – biofilm cells formed in washings and detached from stainless steel; DRYB - dessicated biofilms detached from stainless steel

abcdefg – means in the same column with different superscript letters are different ($P<0.05$)

zyxw – means in the same row with different superscript letters are different ($P<0.05$)

* Counts obtained after transfer from vacuum to aerobic storage at 7°C for 5 d

Table VI.3: Mean (standard deviation) total bacterial (TSA) populations (log CFU/cm^2) on fresh beef inoculated with *Escherichia coli* O157:H7 of different origins (TSB, WASH, WETB or DRYB), stored under vacuum at 4°C for 28 d and periodically exposed to heat (55°C) for 20 min followed by simulated gastric fluid (pH 1.0) for 90 min

			Time of Heat and Subsequent Simulated Gastric Fluid Exposure (min)				
			Heat (55°C)		Simulated Gastric Fluid (pH 1.0)		
Day	Inoculum	Initial Level	10	20	30	60	90
0	TSB	3.0hz (0.6)	3.1bcz (0.4)	2.9abz (0.6)	0dy (0)	0cy (0)	0cy (0)
	WASH	4.0gz (0.3)	3.5by (0.2)	3.1ay (0.3)	0dx (0)	0cx (0)	0cx (0)
	WETB	3.0hz (0.3)	2.3dy (0.2)	2.1cdy (0.2)	0dx (0)	0cx (0)	0cx (0)
	DRYB	1.9iz (0.3)	1.5efzy (0.2)	1.0fy (0.1)	0dx (0)	0cx (0)	0cx (0)
7	TSB	4.1gz (0.4)	2.8cy (0.9)	2.2cy (1.2)	1.1ax (1.1)	1.0ax (1.1)	1.2ax (0.9)
	WASH	4.7fgz (0.7)	2.9bcy (0.6)	2.9aby (0.2)	0.2cx (0.2)	0.1bx (0.3)	0cw (0)
	WETB	4.1gz (1.2)	1.8dey (0.2)	1.4efy (0.4)	0.3cx (0.4)	0cw (0)	0cw (0)
	DRYB	3.1hz (1.5)	1.9dey (0.6)	0.6gx (0.4)	0.1cx (0.1)	0cw (0)	0cw (0)
7*	TSB	4.8fz (1.3)	2.8cy (0.9)	2.4bcy (0.8)	1.0abx (1.1)	0.8abxw (1.1)	0.4bw (0.8)
	WASH	5.9dez (1.4)	3.1bcy (0.5)	2.0cdx (0.5)	0.9abw (0.4)	0.2bv (0.2)	0.1bv (0.1)
	WETB	4.4gz (0.8)	1.8dey (0.3)	1.0fx (0.3)	0dw (0)	0cw (0)	0cw (0)
	DRYB	5.7ez (0.9)	1.1fy (0.4)	0.3gx (0.3)	0dw (0)	0cw (0)	0cw (0)
14	TSB	5.7ez (0.7)	3.1bcy (1.4)	2.2cx (0.4)	0.6abcw (0.7)	0.3bw (0.5)	0.2bw (0.4)
	WASH	5.8ez (1.1)	4.2ay (1.9)	1.7dex (0.6)	0.1cw (0.3)	0.1bw (0.3)	0cv (0)
	WETB	6.2dez (0.9)	3.0bcy (0.9)	2.8aby (0.4)	0.5bcx (0.8)	0.4bx (0.6)	0.3bx (0.5)
	DRYB	5.5efz (1.8)	2.9bcy (1.3)	2.5bcy (1.0)	0.7abx (0.8)	0.5abx (0.7)	0cw (0)
14*	TSB	8.2az (0.5)	3.5by (0.3)	2.8abx (0.5)	0.4cw (0.5)	0.5abw (0.6)	0.1bw (0.2)
	WASH	7.2bcz (0.9)	3.4by (0.5)	2.4bcx (0.4)	0.2cw (0.4)	0.1bw (0.2)	0cv (0)
	WETB	8.1az (0.4)	4.6ay (0.6)	2.3bcx (1.1)	0.6abcw (1.1)	0.4bw (1.0)	0.4bw (0.9)
	DRYB	7.7abz (1.1)	4.5ay (0.7)	3.3ax (0.6)	0dw (0)	0cw (0)	0cw (0)
28	TSB	6.9bcz (0.3)	4.1ay (0.8)	2.8abx (0.7)	0.8abw (0.9)	0.3bw (0.5)	0.3bw (0.5)
	WASH	6.6cdz (0.9)	4.3ay (2.1)	3.6ay (2.1)	0.8abx (0.9)	0.3bx (0.6)	0cw (0)
	WETB	6.5dz (1.0)	3.0bcy (1.7)	2.5bcy (1.6)	0dx (0)	0cx (0)	0cx (0)
	DRYB	6.7cdz (1.1)	2.9bcy (1.3)	2.4bcy (1.8)	0dx (0)	0cx (0)	0cx (0)

TSB – suspension cells originating in tryptic soy broth; WASH – suspension cultures originating in meat decontamination runoff fluids (washings); WETB – biofilm cells formed in washings and detached from stainless steel; DRYB - dessicated biofilms detached from stainless steel

abcdefghi – means in the same column with different superscript letters are different ($P<0.05$)

zyxw – means in the same row with different superscript letters are different ($P<0.05$)

*Counts obtained after transfer from vacuum to aerobic storage at 7°C for 5 d

Table VI.4: Mean (standard deviation) total bacterial (TSA) populations (log CFU/cm^2 ± SD) on fresh beef inoculated with *Escherichia coli* O157:H7 of different origins (TSB, WASH, WETB or DRYB), and decontaminated with sequential treatments of hot water (75°C) and 2% lactic acid (55°C) for 30 s prior to storage under vacuum at 12°C for 16 d and periodic exposure to heat (55°C) for 20 min followed by simulated gastric fluid (pH 1.0) for 90 min

			Time of Heat and Subsequent Simulated Gastric Fluid Exposure (min)				
			Heat (55°C)		Simulated Gastric Fluid (pH 1.0)		
Day	Inoculum	Initial Level	10	20	30	60	90
0	TSB	1.8hiz (0.4)	0.9efy (0.7)	0.7cy (0.4)	0dx (0)	0dx (0)	0bx (0)
	WASH	1.4ijz (0.4)	1.2efz (1.0)	0.5cdy (0.6)	0dx (0)	0dx (0)	0bx (0)
	WETB	0.6kz (0.2)	0.1gz (0.2)	0ey (0)	0dy (0)	0dy (0)	0by (0)
	DRYB	1.0jkz (0.7)	0.3gy (0.7)	0.5cdy (0.6)	0dx (0)	0dx (0)	0bx (0)
4	TSB	3.4cdz (1.9)	2.0bcy (1.2)	0.5cdx (0.7)	0dw (0)	0dw (0)	0bw (0)
	WASH	2.4fgz (0.8)	1.4dey (1.2)	1.4aby (1.1)	0.4bcx (0.8)	0dw (0)	0bw (0)
	WETB	2.1ghz (1.3)	1.3efy (1.0)	0.8bcy (0.9)	0 dx (0)	0dx (0)	0bx (0)
	DRYB	2.2ghz (1.3)	0.8fy (1.2)	0.6cdy (1.2)	0.3cx (0.5)	0dx (0)	0bx (0)
4*	TSB	4.1abz (2.0)	0.9efy (0.2)	0.7cy (0.4)	0.3cx (0.4)	0.2bcx (0.3)	0bw (0)
	WASH	2.4fgz (0.9)	1.0efy (1.3)	0.4cdx (0.5)	0.2cx (0.3)	0dw (0)	0bw (0)
	WETB	2.4fgz (1.5)	0.9efy (0.5)	0.3dx (0.4)	0dw (0)	0dw (0)	0bw (0)
	DRYB	1.3ijz (0.8)	0.9efz (0.9)	0ey (0)	0dy (0)	0dy (0)	0by (0)
8	TSB	3.0defz (0.8)	1.9cdy (0.8)	1.7ay (0.4)	0.8abx (0.5)	0.4abx (0.6)	0.2ax (0.6)
	WASH	3.1dez (1.2)	2.1bcy (0.9)	1.9ay (0.4)	1.1ax (1.0)	0.7ax (0.8)	0.6ax (0.7)
	WETB	3.2dez (1.4)	3.2az (1.2)	1.9ay (0.6)	0.5abcx (0.5)	0.1cx (0.1)	0.1ax (0.2)
	DRYB	2.0ghz (1.0)	1.2efy (0.5)	0.3dx (0.4)	0.2cx (0.3)	0.1cx (0.2)	0bw (0)
8*	TSB	2.8efgz (0.8)	1.0efy (0.3)	0ex (0)	0dx (0)	0dx (0)	0bx (0)
	WASH	4.0abcz (1.5)	1.0efy (0.6)	0.4cdx (0.6)	0.1cx (0.2)	0.1cx (0.1)	0bw (0)
	WETB	2.7efgz (1.3)	2.8az (1.3)	0.8bcy (1.1)	0dx (0)	0dx (0)	0bx (0)
	DRYB	2.4fgz (1.4)	0.8fy (1.0)	0.1dx (0.2)	0dw (0)	0dw (0)	0bw (0)
16	TSB	4.8az (1.9)	1.9cdy (1.5)	0.8bcx (0.9)	0dw (0)	0dw (0)	0bw (0)
	WASH	3.6bcdz (1.2)	2.6aby (0.5)	1.7ax (0.9)	1.1ax (1.2)	0.6abw (0.9)	0.5aw (0.8)
	WETB	4.8az (1.1)	2.5aby (1.8)	1.5ax (1.5)	0dw (0)	0dw (0)	0bw (0)
	DRYB	4.2abz (1.8)	2.7ay (0.9)	0.9bcx (1.0)	0dw (0)	0dw (0)	0bw (0)

TSB – suspension cells originating in tryptic soy broth; WASH – suspension cultures originating in meat decontamination runoff fluids (washings); WETB – biofilm cells formed in washings and detached from stainless steel; DRYB - dessicated biofilms detached from stainless steel

abcdefghijk – means in the same column with different superscript letters are different (P<0.05)

zyxw – means in the same row with different superscript letters are different (P<0.05)

*Counts obtained after transfer from vacuum to aerobic storage at 7°C for 5 d

Table VI.5: Mean (standard deviation) total bacterial (TSA) populations (log CFU/cm^2) on fresh beef inoculated with *Escherichia coli* O157:H7 of different origins (TSB, WASH, WETB or DRYB), stored under vacuum at 12°C for 16 d and periodically exposed to heat (55°C) for 20 min followed by simulated gastric fluid (pH 1.0) for 90 min

			Time of Heat and Subsequent Simulated Gastric Fluid Exposure (min)				
			Heat (55°C)		Simulated Gastric Fluid (pH 1.0)		
Day	Inoculum	Initial Level	10	20	30	60	90
0	TSB	3.0jz (0.6)	3.1gz (0.4)	2.9fgz (0.6)	0iy (0)	0hy (0)	0gy (0)
	WASH	4.0iz (0.3)	3.5gzy (0.2)	3.1fy (0.3)	0ix (0)	0hx (0)	0gx (0)
	WETB	3.0jz (0.3)	2.3hiy (0.2)	2.1hiy (0.2)	0ix (0)	0hx (0)	0gx (0)
	DRYB	1.9kz (0.3)	1.5jzy (0.2)	1.0ky (0.1)	0ix (0)	0hx (0)	0gx (0)
4	TSB	6.7dez (0.3)	5.9cdy (0.9)	4.0dex (0.7)	1.5gw (0.3)	1.1fw (0.6)	0.9ew (0.6)
	WASH	6.7dez (0.7)	6.2bcz (0.7)	5.1bcy (1.0)	2.7fx (1.0)	2.8cdx (0.6)	2.8cdx (0.7)
	WETB	6.2fgz (0.7)	5.4dey (0.7)	5.3by (0.8)	3.0efx (0.5)	2.6dxw (0.7)	2.3dw (0.8)
	DRYB	5.8gz (1.2)	4.5fy (1.6)	3.2fx (0.9)	1.6gw (1.0)	1.5efw (1.0)	1.3ew (1.0)
4*	TSB	5.4ghz (1.1)	3.5gy (2.0)	2.3hix (1.1)	0.9hw (0.7)	0.6gw (0.5)	0.3fw (0.4)
	WASH	6.4efz (1.7)	3.5gy (1.3)	3.4efy (1.4)	0.8hx (0.5)	0.2gw (0.4)	0.1fw (0.2)
	WETB	4.8hz (0.8)	2.3hiy (1.1)	1.8ijy (0.7)	0ix (0)	0hx (0)	0gx (0)
	DRYB	4.8hz (0.3)	1.7ijy (1.1)	1.3jky (1.1)	0ix (0)	0hx (0)	0gx (0)
8	TSB	7.6bcz (0.5)	7.3az (0.4)	6.5ay (0.4)	4.7ax (0.8)	4.2axw (1.1)	4.0aw (1.0)
	WASH	7.7bcz (0.4)	7.3az (0.6)	6.3ay (0.6)	4.5abx (1.6)	4.5ax (0.9)	4.2ax (0.7)
	WETB	7.7bcz (0.4)	7.1az (0.4)	6.4ay (0.6)	4.0abcdx (0.7)	3.2bcw (0.9)	3.3bw (0.9)
	DRYB	7.8bcz (0.3)	7.1az (0.3)	5.2bcy (1.5)	3.6dex (1.0)	3.2bcx (0.8)	3.2bcx (0.8)
8*	TSB	8.1abz (0.5)	5.5dey (0.7)	5.5by (1.7)	4.0abcdx (1.4)	2.8cdw (1.2)	2.5dw (1.7)
	WASH	7.3cdz (1.1)	5.4dey (0.4)	4.7cx (0.5)	2.0gw (0.9)	1.8ew (0.9)	1.3ev (1.1)
	WETB	7.0cdez (1.0)	5.9cdy (0.2)	4.2dx (0.6)	2.9fw (0.4)	1.7ev (0.7)	1.3ev (1.0)
	DRYB	6.6efz (1.3)	5.3ey (0.6)	2.4ghx (0.9)	1.9gw (0.9)	1.3efv (0.7)	1.1ev (0.9)
16	TSB	8.6az (0.7)	6.9aby (1.3)	6.7ay (1.1)	3.9bcdx (1.0)	3.4bx (1.3)	3.2bcx (1.7)
	WASH	8.0abz (0.6)	7.2ay (0.6)	6.9ay (0.7)	4.5abx (0.9)	4.2ax (1.0)	4.1ax (0.9)
	WETB	8.1abz (0.5)	7.0ay (1.3)	6.5ay (0.9)	4.3abcx (0.7)	4.4ax (0.4)	4.2ax (0.9)
	DRYB	7.7bcz (1.1)	7.1az (0.7)	6.3ay (1.0)	3.7cdx (0.9)	3.5bx (0.8)	3.4bx (0.8)

TSB – suspension cells originating in tryptic soy broth; WASH – suspension cultures originating in meat decontamination runoff fluids (washings); WETB – biofilm cells formed in washings and detached from stainless steel; DRYB - dessicated biofilms detached from stainless steel

abcdefghijk – means in the same column with different superscript letters are different ($P<0.05$)

zyxwv – means in the same row with different superscript letters are different ($P<0.05$)

*Counts obtained after transfer from vacuum to aerobic storage at 7°C for 5 d

Table VI.6: Mean (standard deviation) *Escherichia coli* O157:H7 (TSA+Rif) populations (log CFU/cm^2) of different origins (TSB, WASH, WETB or DRYB), inoculated onto fresh beef and decontaminated with sequential treatments of hot water (75°C) and 2% lactic acid (55°C) for 30 s prior to storage under vacuum at 4°C for 28 d and periodic exposure to heat (55°C) for 20 min followed by simulated gastric fluid (pH 1.0) for 90 min

			Time of Heat and Subsequent Simulated Gastric Fluid Exposure (min)				
			Heat (55°C)		Simulated Gastric Fluid (pH 1.0)		
Day	Inoculum	Initial Level	10	20	30	60	90
0	TSB	1.8az (0.4)	0.8aby (0.5)	0.5ay (0.5)	0ax (0)	0ax (0)	0ax (0)
	WASH	1.4abz (0.4)	1.2az (1.0)	0.5ay (0.6)	0ax (0)	0ax (0)	0ax (0)
	WETB	0.3fgz (0.5)	0.1cz (0.3)	0by (0)	0ay (0)	0ay (0)	0ay (0)
	DRYB	0.8cdez (0.8)	0.3bcy (0.6)	0bx (0)	0ax (0)	0ax (0)	0ax (0)
7	TSB	1.1bcz (0.4)	0.3bcy (0.5)	0.2ay (0.6)	0ax (0)	0ax (0)	0ax (0)
	WASH	1.5abz (0.3)	0.5bcy (0.5)	0.3ay (0.4)	0ax (0)	0ax (0)	0ax (0)
	WETB	0.4efgz (0.6)	0dy (0)	0by (0)	0ay (0)	0ay (0)	0ay (0)
	DRYB	0lz (0)	0dz (0)	0.1az (0.2)	0az (0)	0az (0)	0az (0)
7*	TSB	1.0bcdz (0.5)	1.0az (0.6)	0.2ay (0.3)	0ax (0)	0ax (0)	0ax (0)
	WASH	1.2bcz (0.4)	0.9az (0.4)	0by (0)	0ay (0)	0ay (0)	0ay (0)
	WETB	0.3fgz (0.5)	0.4bcz (0.4)	0.2az (0.3)	0ay (0)	0ay (0)	0ay (0)
	DRYB	0.1gz (0.2)	0.2cz (0.4)	0by (0)	0ay (0)	0ay (0)	0ay (0)
14	TSB	1.1bcz (0.5)	0.7abz (0.6)	0.2ay (0.3)	0ax (0)	0ax (0)	0ax (0)
	WASH	0.9cdz (0.5)	0.3bcy (0.3)	0bx (0)	0ax (0)	0ax (0)	0ax (0)
	WETB	0.2gz (0.2)	0dy (0)	0by (0)	0ay (0)	0ay (0)	0ay (0)
	DRYB	0hz (0)	0dz (0)	0bz (0)	0az (0)	0az (0)	0az (0)
14*	TSB	1.2bcz (0.4)	0.5bcy (0.5)	0.2ay (0.3)	0ax (0)	0ax (0)	0ax (0)
	WASH	0.7defz (0.6)	0.6abz (0.6)	0by (0)	0ay (0)	0ay (0)	0ay (0)
	WETB	0.6defz (0.7)	0.3bcz (0.4)	0by (0)	0ay (0)	0ay (0)	0ay (0)
	DRYB	0.6defz (0.6)	0.5bcz (0.6)	0by (0)	0ay (0)	0ay (0)	0ay (0)
28	TSB	1.2bcz (0.4)	0dy (0)	0by (0)	0ay (0)	0ay (0)	0ay (0)
	WASH	0.7defz (0.6)	0.1cy (0.3)	0bx (0)	0ax (0)	0ax (0)	0ax (0)
	WETB	0.6defz (0.7)	0dy (0)	0by (0)	0ay (0)	0ay (0)	0ay (0)
	DRYB	0.6defz (0.6)	0dy (0)	0y (0)	0ay (0)	0ay (0)	0ay (0)

TSB – suspension cells originating in tryptic soy broth; WASH – suspension cultures originating in meat decontamination runoff fluids (washings); WETB – biofilm cells formed in washings and detached from stainless steel; DRYB - dessicated biofilms detached from stainless steel

abcdefg – means in the same column with different superscript letters are different ($P<0.05$)

zyx – means in the same row with different superscript letters are different ($P<0.05$)

*Counts obtained after transfer from vacuum to aerobic storage at 7°C for 5 d

Table VI.7: Mean (standard deviation) *Escherichia coli* O157:H7 (TSA+Rif) populations (log CFU/cm^2) of different origins (TSB, WASH, WETB or DRYB), inoculated onto fresh beef stored under vacuum at 4°C for 28 d and periodically exposed to heat (55°C) for 20 min followed by simulated gastric fluid (pH 1.0) for 90 min

			Time of Heat and Subsequent Simulated Gastric Fluid Exposure (min)				
			Heat (55°C)		Simulated Gastric Fluid (pH 1.0)		
Day	Inoculum	Initial Level	10	20	30	60	90
0	TSB	3.0bcdz (0.5)	3.0abcz (0.4)	2.8az (0.6)	0cy (0)	0cy (0)	0cy (0)
	WASH	3.9az (0.3)	3.4azy (0.2)	3.0ay (0.3)	0cx (0)	0cx (0)	0cx (0)
	WETB	2.8cdz (0.2)	2.2dy (0.2)	2.0cdy (0.2)	0cx (0)	0cx (0)	0cx (0)
	DRYB	1.6fgz (0.5)	1.3ghz (0.2)	0.5fgy (0.4)	0cx (0)	0cx (0)	0cx (0)
7	TSB	3.3abz (1.0)	2.5cdzy (1.2)	2.1bcy (1.2)	1.0ax (1.2)	0.8ax (1.1)	0.9ax (1.1)
	WASH	3.4abz (0.7)	2.6bcdy (0.3)	2.7aby (0.3)	0.1bx (0.2)	0.1bx (0.1)	0cw (0)
	WETB	2.2ez (0.4)	1.5fgy (0.1)	1.0ey (0.5)	0cx (0)	0cx (0)	0cx (0)
	DRYB	1.6fgz (0.1)	1.0hy (0.1)	0hx (0)	0cx (0)	0cx (0)	0cx (0)
7*	TSB	2.7cdz (0.8)	2.6bcdz (1.2)	2.1bcz (1.0)	0.9ay (1.0)	0.7ay (0.9)	0.4by (0.7)
	WASH	3.2bcz (0.1)	3.0abcz (0.4)	1.8cdy (0.6)	0.5abx (0.5)	0.1bx (0.2)	0cw (0)
	WETB	2.2ez (0.1)	1.8efz (0.2)	0.7efy (0.3)	0cx (0)	0cx (0)	0cx (0)
	DRYB	1.3gz (0.2)	0.9hz (0.4)	0.1gy (0.1)	0cx (0)	0cx (0)	0cx (0)
14	TSB	3.0bcdz (0.7)	2.6bcdz (1.0)	1.7dy (0.6)	0.3bx (0.5)	0.2bx (0.3)	0.1bx (0.2)
	WASH	3.4abz (0.7)	2.1dey (0.7)	1.0ex (0.7)	0.1bw (0.2)	0.1bw (0.1)	0cv (0)
	WETB	2.3dez (0.2)	1.4fgy (0.2)	0.9ex (0.4)	0cw (0)	0cw (0)	0cw (0)
	DRYB	2.1efz (0.4)	1.6efgy (0.7)	1.0ey (1.2)	0cx (0)	0cx (0)	0cx (0)
14*	TSB	3.0bcdz (0.8)	2.9abcz (0.4)	2.2bcy (0.6)	0.3bx (0.6)	0.2bx (0.4)	0cw (0)
	WASH	3.6abz (0.3)	3.1abz (0.5)	2.1bcy (0.4)	0cx (0)	0cx (0)	0cx (0)
	WETB	2.6dez (0.5)	2.2dz (0.7)	1.0ey (0.4)	0cx (0)	0cx (0)	0cx (0)
	DRYB	1.7fgz (0.8)	1.0hy (0.9)	0.3hx (0.3)	0cw (0)	0cw (0)	0cw (0)
28	TSB	3.0bcdz (0.8)	2.0dey (1.2)	1.6dy (0.8)	0cx (0)	0cx (0)	0cx (0)
	WASH	3.6abz (0.3)	2.2dy (0.6)	0.8efx (0.7)	0cw (0)	0cw (0)	0cw (0)
	WETB	2.6dez (0.5)	1.1ghy (0.3)	0.4gx (0.5)	0cw (0)	0cw (0)	0cw (0)
	DRYB	1.7fgz (0.8)	0.3iy (0.4)	0.3gy (0.5)	0cx (0)	0cx (0)	0cx (0)

TSB – suspension cells originating in tryptic soy broth; WASH – suspension cultures originating in meat decontamination runoff fluids (washings); WETB – biofilm cells formed in washings and detached from stainless steel; DRYB - dessicated biofilms detached from stainless steel

abcdefgh – means in the same column with different superscript letters are different ($P<0.05$)

zyxw – means in the same row with different superscript letters are different ($P<0.05$)

*Counts obtained after transfer from vacuum to aerobic storage at 7°C for 5 d

Table VI.8: Mean (standard deviation) *Escherichia coli* O157:H7 (TSA+Rif) populations (log CFU/cm^2) of different origins (TSB, WASH, WETB or DRYB), inoculated onto fresh beef and decontaminated with sequential treatments of hot water (75°C) and 2% lactic acid (55°C) for 30 s prior to storage under vacuum at 12°C for 16 d and periodic exposure to heat (55°C) for 20 min followed by simulated gastric fluid (pH 1.0) for 90 min

			Time of Heat and Subsequent Simulated Gastric Fluid Exposure (min)				
			Heat (55°C)		Simulated Gastric Fluid (pH 1.0)		
Day	Inoculum	Initial Level	10	20	30	60	90
0	TSB	1.8defz (0.4)	0.8efgy (0.5)	0.5cdy (0.5)	0cx (0)	0bx (0)	0bx (0)
	WASH	1.4fghz (0.4)	1.2dez (1.0)	0.5cdy (0.6)	0cx (0)	0bx (0)	0bx (0)
	WETB	0.3jz (0.5)	0.1hz (0.3)	0ey (0)	0cy (0)	0by (0)	0by (0)
	DRYB	0.8hiz (0.8)	0.3hz (0.6)	0ey (0)	0cy (0)	0by (0)	0by (0)
4	TSB	3.0az (1.6)	1.8bcy (1.2)	0.1dx (0.2)	0cw (0)	0bw (0)	0bw (0)
	WASH	1.8defz (0.9)	1.0defz (0.8)	0.6bcy (0.8)	0.3by (0.8)	0bx (0)	0bx (0)
	WETB	0.8hiz (1. 3)	0.1hy (0.2)	0.1dy (0.2)	0cx (0)	0bx (0)	0bx (0)
	DRYB	0.7iz (1.2)	0.7fgz (1.2)	0.5cdz (1.2)	0.1bz (0.1)	0by (0)	0by (0)
4*	TSB	2.1abcdz (1.0)	0.8efgy (0.4)	0.2dy (0.4)	0.2by (0.3)	0bx (0)	0bx (0)
	WASH	1.4fghz (0.5)	1.0defz (1.3)	0.3dy (0.4)	0cx (0)	0bx (0)	0bx (0)
	WETB	1.0ghiz (0.9)	0.2hy (0.3)	0ex (0)	0cx (0)	0bx (0)	0bx (0)
	DRYB	0.1jz (0.2)	0iy (0)	0ey (0)	0cy (0)	0by (0)	0by (0)
8	TSB	2.4abcz (0.7)	1.7bcy (0.8)	1.5ay (0.5)	0.1bx (0.1)	0.1ax (0.1)	0bw (0)
	WASH	2.0bcdez (1.0)	1.2dey (0.8)	1.1aby (0.7)	0.6abx (0.6)	0.2ax (0.5)	0.2ax (0.4)
	WETB	1.9cdefz (1.3)	2.2abz (1.0)	0.3dy (0.5)	0.1by (0.2)	0bx (0)	0bx (0)
	DRYB	0.3jz (0.3)	0.2hz (0.4)	0ey (0)	0cy (0)	0by (0)	0by (0)
8*	TSB	2.1abcdz (1.1)	1.0defy (0.3)	0ex (0)	0cx (0)	0bx (0)	0bx (0)
	WASH	2.1abcdz (0.8)	0.4ghy (0.4)	0.2dy (0.4)	0cx (0)	0bx (0)	0bx (0)
	WETB	2.6abz (1.6)	2.6az (1.6)	0.7bcy (1.0)	0cx (0)	0bx (0)	0bx (0)
	DRYB	0.1jz (0.3)	0.2hz (0.4)	0ey (0)	0cy (0)	0by (0)	0by (0)
16	TSB	1.9cdefz (1.3)	1.1defy (1.3)	0.4cdx (0.9)	0cw (0)	0 bw (0)	0 bw (0)
	WASH	1.7efgz (0.8)	2.1abz (0.6)	1.2ay (1.0)	1.0ay (1.1)	0.6ax (0.9)	0.4ax(0.7)
	WETB	1.8defz (1.8)	1.9abz (1.9)	1.0abcy (1.6)	0cx (0)	0bx (0)	0bx (0)
	DRYB	2.2abcdz (1.8)	1.3cdy (1.3)	0ex (0)	0cx (0)	0bx (0)	0bx (0)

TSB – suspension cells originating in tryptic soy broth; WASH – suspension cultures originating in meat decontamination runoff fluids (washings); WETB – biofilm cells formed in washings and detached from stainless steel; DRYB - dessicated biofilms detached from stainless steel

abcdefghij – means in the same column with different superscript letters are different ($P<0.05$)

zyxw – means in the same row with different superscript letters are different ($P<0.05$)

*Counts obtained after transfer from vacuum to aerobic storage at 7°C for 5 d

Table VI.9: Mean (standard deviation) *Escherichia coli* O157:H7 (TSA+Rif) populations (log CFU/cm^2) of different origins (TSB, WASH, WETB or DRYB), inoculated onto fresh beef stored under vacuum at 12°C for 16 d and periodically exposed to heat (55°C) for 20 min followed by simulated gastric fluid (pH 1.0) for 90 min

			Time of Heat and Subsequent Simulated Gastric Fluid Exposure (min)				
			Heat (55°C)		Simulated Gastric Fluid (pH 1.0)		
Day	Inoculum	Initial Level	10	20	30	60	90
0	TSB	3.0gz (0.5)	3.0gz (0.4)	2.8iz (0.6)	0iy (0)	0hy (0)	0hy (0)
	WASH	3.9fz (0.3)	3.4fgzy (0.2)	3.0hiy (0.3)	0ix (0)	0hx (0)	0hx (0)
	WETB	2.8ghz (0.2)	2.2hzy (0.2)	2.0jy (0.2)	0ix (0)	0hx (0)	0hx (0)
	DRYB	1.6iz (0.5)	1.3ijz (0.2)	0.5ly (0.4)	0ix (0)	0hx (0)	0hx (0)
4	TSB	5.9bcz (1.0)	5.2cz (0.8)	3.2ghy (0.8)	1.4fx (0.4)	0.9fw (0.4)	0.7fw (0.6)
	WASH	6.4abz (0.7)	6.1bz (0.6)	5.0dy (1.0)	2.5ex (1.0)	2.4dx (0.7)	2.5cdx (1.0)
	WETB	4.9ez (0.7)	4.6dez (0.3)	4.4ez (0.6)	2.6ey (0.6)	2.4dy (0.7)	2.1dy (0.7)
	DRYB	4.8ez (0.9)	4.0efz (1.5)	2.7iy (0.5)	1.1fgx (1.2)	1.0fx (1.2)	0.9fx (1.1)
4*	TSB	3.8fz (1.7)	3.3gz (2.2)	2.0jy (1.1)	0.8ghx (0.7)	0.3gw (0.5)	0.1gw (0.2)
	WASH	3.8fz (0.9)	3.0gz (0.9)	3.1hiz (0.8)	0.4hy (0.4)	0.1gy (0.1)	0hx (0)
	WETB	2.5hz (0.5)	1.7iy (0.3)	1.0ky (0.7)	0ix (0)	0hx (0)	0hx (0)
	DRYB	2.4hz (1.3)	0.9jy (0.3)	0.5ly (0.4)	0ix (0)	0hx (0)	0hx (0)
8	TSB	5.6cdz (1.8)	6.3bz (1.3)	6.0bcz (0.9)	3.6abcy (1.2)	3.3by (1.3)	3.1by (1.1)
	WASH	7.1az (0.4)	7.1az (0.4)	5.9bcy (0.8)	4.0abx (1.7)	4.0ax (1.3)	3.7ax (0.9)
	WETB	6.2bcz (1.0)	6.2bz (1.0)	5.7cz (0.7)	3.3cdy (0.8)	2.9bcdy (1.0)	3.0by (0.8)
	DRYB	6.1bcz (1.1)	5.4cz (1.2)	4.2efy (1.9)	2.8dex (0.7)	2.7cdx (0.9)	2.4cdx (0.9)
8*	TSB	4.9ez (1.9)	4.8cdz (1.1)	3.8efy (1.6)	3.1cdyx (0.9)	2.5dx (1.2)	1.6ew (2.0)
	WASH	5.5dz (0.6)	4.8cdy (0.7)	3.7fgx (0.8)	1.6fw (0.6)	1.3efwv (0.8)	0.9fv (0.8)
	WETB	4.7ez (1.4)	4.8cdz (1.2)	3.4ghy (1.0)	2.8dey (0.4)	1.7ex (0.8)	1.1efx (1.2)
	DRYB	4.1fz (0.8)	3.6fgy (0.9)	1.2kx (0.7)	0.3hw (0.2)	0.2gw (0.3)	0.1gw (0.1)
16	TSB	5.7cdz (1.7)	6.2bz (1.3)	6.4az (1.1)	3.4bcy (1.3)	3.0bcy (1.5)	2.8bcy (1.4)
	WASH	6.8az (1.2)	7.1az (0.7)	6.8az (0.7)	4.2ay (1.0)	4.1ay (1.0)	3.9ay (0.8)
	WETB	6.6az (1.4)	6.6abz (1.3)	6.3abz (0.9)	4.0aby (0.7)	4.1ay (0.6)	3.6ay (1.0)
	DRYB	6.8az (1.1)	6.5bz (0.8)	6.0bcz (0.9)	3.3cdy (0.8)	3.0bcyx (0.5)	2.6bcx (0.6)

TSB – suspension cells originating in tryptic soy broth; WASH – suspension cultures originating in meat decontamination runoff fluids (washings); WETB – biofilm cells formed in washings and detached from stainless steel; DRYB - dessicated biofilms detached from stainless steel
abcdefghij – means in the same column with different superscript letters are different ($P<0.05$)
zyxw – means in the same row with different superscript letters are different ($P<0.05$)
*Counts obtained after transfer from vacuum to aerobic storage at 7°C for 5 d

In this study, it appeared that pathogen populations prepared in TSB and in WASH were, in general, more resistant to heat and acid stresses than those grown as biofilms and detached prior to inoculation (Tables VI.6-VI.9). It should be noted that populations from WETB were always more resistant to stresses than those from DRYB

which may have been due to lower initial levels of DRYB populations exposed to stresses and increased sensitivity of DRYB cultures in comparison to all other cultures due to the severe desiccation and starvation stresses faced during preparation of this inoculum (Tables VI.6-VI.9). In general, and regardless of temperature, populations from DRYB decreased as storage progressed, possibly due to the injury caused by the drying process further sensitizing cells to stresses faced on beef tissue (Tables VI.6-VI.9). Although, in some cases, the lower heat and acid tolerance of cells derived from biofilms may have been due to lower initial pathogen levels than TSB or WASH cells, this result is not consistent for all conditions studied. Pathogen populations from TSB and WASH stored at 4°C were consistently more ($P < 0.05$) resistant to heat and acid stresses than WETB and DRYB (Tables VI.6 and VI.7). In this case, it appears that higher initial pathogen levels from TSB and WASH were responsible for higher resistance to stresses than those from a biofilm history. Furthermore, it was apparent that pathogen populations from TSB were more resistant ($P < 0.05$) to heat and acid than those from WASH (Tables VI.6 and VI.7). The higher tolerance of TSB cultures compared to WASH was not a result of higher initial pathogen levels exposed to the stresses but more likely an inherent tolerance associated with the TSB cultures. It has been evidenced (Buchanan and Edelson, 1996) that broth containing glucose even at levels as low as 0.25% may induce a mild acid-adaptation in *E. coli* O157:H7 and it is well known (Leyer and Johnson, 1993) that acid-adaptation during logarithmic growth increases resistance of pathogens to other environmental stresses. This may explain the observation that TSB cultures were more ($P < 0.05$) resistant to heat and acid than were WASH cultures during storage at 4°C but not

necessarily at 12°C, where new cultures were generated that may not have retained the original resistance of the inoculum.

Contrary to the trend observed with cultures originating at 4°C, pathogen populations from WASH stored at 12°C were slightly more resistant to stresses than those from TSB throughout storage; however, this trend was reversed when cultures were transferred to aerobic storage (Tables VI.8 and VI.9). Pathogen populations originating from WETB stored at 12°C were more resistant than, or as resistant as, cultures from TSB and WASH at day-4 and -0, respectively (Tables VI.8 and VI.9). This may be due to fact that WETB cultures were formed under competitive conditions associated with biofilm formation in the presence of mixed species (Costerton et al., 1994, 1995; Sutherland, 2001) and, as such, were able to generate populations with more resistance to stresses than those from TSB or WASH. Storage of beef at 4°C contributed positively to the reduction of the pathogen during heat and acid exposure and was mostly due to the no-growth profile of the pathogen at 4°C which maintained the populations at low levels (Tables VI.6 and VI.7).

Decontamination not only reduced levels of the pathogen attached to beef immediately following its application but also prevented growth of the pathogen at 12°C (Tables VI.6-VI.9). Moreover, decontamination reduced the heat and acid tolerance of the pathogen, which may have been a result of reduced population levels exposed to the stresses. The findings of this study support the decontamination of fresh beef with hot water and lactic acid. In addition to the knowledge that these decontamination treatments result in an immediate decrease in pathogen levels, the results of this study indicate that decontamination resulted in injury that prevented subsequent growth of the surviving

populations even at 12°C for up to 16 d as well as decreasing the pathogen's resistance to heat and acid conditions. Results of this study indicate that, in general, the pathogen populations on beef stored at 4°C were more susceptible to the effects of the subsequent acid exposure than to prior heating (Tables VI.6 and VI.7). This trend was not repeated when beef was stored at 12°C and that may be because the meat originating at refrigeration temperatures (4°C) and heated at 55°C for the same amount of time as that originating at refrigeration-abuse temperatures (12°C) did not reach the same internal temperature. This result has serious implications for consumers; that is, refrigerated beef contaminated with *E. coli* O157:H7 and cooked for a specific time, rather than to a specific internal temperature may only serve to exhaust pathogen cells and not necessarily to kill or inactivate them. Heat application in this study was achieved by water immersion of beef pieces contained within a bag and does not represent the actual destruction that may be achieved by direct heating such as grilling, broiling or frying. In almost all cases, regardless of inoculum history, temperature of incubation and day of storage, *E. coli* O157:H7 on decontaminated beef was reduced to below 0 log CFU/cm^2 when exposed to heat (55°C) and acid (pH 1.0) conditions (Tables VI.6-VI.9). Conversely, growth of the pathogen during storage at 12°C on untreated meat was accompanied by increased heat and acid tolerance, pointing to the development of resistance to stresses over time (Tables VI.8 and VI.9). This raises important safety concerns for the risk of *E. coli* O157:H7 contamination if temperature abuse is not prevented and if, subsequently, consumers do not treat beef, even whole muscle cuts like steaks, as adulterated product.

An important phenomenon identified in this study was the remarkable reduction in pathogen populations on decontaminated, and especially on untreated, beef upon transfer from anaerobic storage in vacuum packages at 12°C to aerobic storage in retail packages at 7°C for 5 d (Tables VI.8 and VI.9). Moreover, the transfer of beef from anaerobic conditions during storage at 12°C, to aerobic conditions, resulted in a decrease in the pathogen's heat and acid tolerance (Tables VI.8 and VI.9). This result was not repeated for beef stored at 4°C indicating that pathogen populations and subsequent resistance to heat and acid exposure remained similar ($P \geq 0.05$) upon transfer from vacuum packages to aerobic storage. Considering that changes in pathogen populations on beef stored at 4°C remained similar after transfer to aerobic storage, it is more likely that stress resistance due to a shift in atmospheric environment is associated with habituation of pathogen cells to chill conditions rather than the physiological growth phase or general stress response of stationary phase cells. Previous research using laboratory media (George and Peck, 1998; George et al., 1998) indicated that the heat resistance of *E. coli* O157:H7 was higher when grown under anaerobic conditions compared to that when grown under aerobic conditions. Results of the present study support results of the latter studies and, furthermore, imply that temperature-abuse of vacuum-packaged beef poses a higher risk to consumers than that which occurs when vacuum-packaged beef is shifted to aerobic storage prior to cooking and consumption.

CONCLUSIONS

Escherichia coli O157:H7 on beef stored at chill temperatures (such as 4°C) may become habituated to the stresses faced by transfer from vacuum storage at 4°C, to aerobic storage at 7°C. Temperature-abuse of vacuum-packaged beef as opposed to abuse that occurs when vacuum-packaged beef is transferred to aerobic storage prior to cooking and consumption may pose a higher risk of *E. coli* O157:H7 infection to consumers. In this study, growth of *E. coli* O157:H7 on whole beef muscle stored under temperature-abuse conditions such as 12°C was associated with an increase in the heat and acid tolerance of the pathogen making it an important food safety risk for consumers not treating whole muscle cuts as adulterated product. Under conditions of this study, planktonic pathogen cells from incubation in tryptic soy broth and meat decontamination runoff fluids were, in general, more resistant than those grown as biofilms and detached prior to inoculation of fresh beef exposed to heat and acid stresses. Decontamination of fresh beef resulted in injury that prevented subsequent growth of the surviving *E. coli* O157:H7 populations even at 12°C, as well as decreasing the pathogen's resistance to subsequent heat and acid conditions. In summary, heat and acid tolerance of *E. coli* O157:H7 are influenced by inoculum history and, to a greater extent, by the temperature of storage and by the transfer of beef from anaerobic conditions in vacuum packages to aerobic conditions in retail packages.

CHAPTER VII

REDUCTION OF *LISTERIA MONOCYTOGENES* POPULATIONS DURING EXPOSURE TO A SIMULATED GASTRIC FLUID FOLLOWING STORAGE OF INOCULATED FRANKFURTERS FORMULATED AND TREATED WITH PRESERVATIVES

ABSTRACT

The effect of a simulated gastric fluid (adjusted to pH 1.0 with HCl) on *Listeria monocytogenes*, inoculated postprocessing on pork frankfurters formulated with sodium lactate (SL) and sodium diacetate (SD) and not dipped or dipped in solutions of lactic acid or acetic acid, was evaluated during storage of the frankfurters at 10°C for 40 days. Pork frankfurters containing 1.8% SL, 0.25% SD, 1.8% SL+0.125% SD, or 1.8% SL+0.25% SD were inoculated with 10^2-10^3 CFU/cm^2 of a 10-strain preparation of *L. monocytogenes* and were not dipped or dipped for 2 min in solutions of 2.5% lactic or acetic acid before they were vacuum-packaged and stored. Survival of *L. monocytogenes* was determined after exposure of frankfurters for 0, 20, 40 and 60 min to the simulated gastric fluid after storage for 0, 10, 20, 30 or 40 days. Growth of *L. monocytogenes* on frankfurters formulated with antimicrobials was inhibited in the order control < SL < 0.25%SD < SL+0.125%SD < SL+0.25% SD. Survival of *L. monocytogenes* during exposure to simulated gastric fluid was higher on those frankfurters on which there had

been extensive growth of the pathogen than on frankfurters on which growth had been inhibited. Inclusion of single antimicrobials in the formulation of not dipped frankfurters appeared to increase resistance of *L. monocytogenes* to simulated gastric fluid provided the initial level of the pathogen exposed to the simulated gastric fluid was higher than 5 logs. Although, the combinations of SL and SD used in the formulation of not dipped frankfurters inhibited growth of *L. monocytogenes*, prolonged (40 d) storage of such frankfurters induced slight resistance to simulated gastric fluid. The frankfurter formulation of 0.25% SD and dipping in 2.5% lactic acid appeared to increase resistance of *L. monocytogenes* to simulated gastric fluid following growth during prolonged (day-20 to day-40) storage. Use of 2.5% acetic acid dipping combined with antimicrobials in the formulation inhibited growth of *L. monocytogenes* on frankfurters and subsequent detectable survival after exposure to simulated gastric fluid.

INTRODUCTION

Listeria monocytogenes causes an estimated 2,500 cases of foodborne illness and approximately 500 deaths (approximately 20% case fatality rate) annually in the U.S. (Mead et al., 1999). The onset of listeriosis in humans is promoted by various risk factors with the young and elderly, immunocompromised individuals and pregnant women being more susceptible than the general population (Ryser and Marth, 1999). *L. monocytogenes* is now regarded as an important meatborne pathogen, following recent outbreaks of listeriosis that involved consumption of postprocessing contaminated ready-to-eat (RTE) meat and poultry products (CDC, 1999, 2000, 2002). In response to the risks associated

with this pathogen, the United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) maintains a policy of "zero-tolerance" for *L. monocytogenes* in RTE foods.

L. monocytogenes is difficult to control in foods as it is ubiquitous, potentially resistant to many food preservation methods (Lou and Yousef, 1999), and has the ability to survive in meat plants (Wong, 1998; Samelis and Metaxopoulos, 1999). Traditional strategies to prevent postprocessing contamination of RTE products with *L. monocytogenes* may be inadequate (Samelis and Metaxopoulos, 1999; Tompkin et al., 1999; Tompkin, 2002). For this reason, pre- and post-packaging hurdle technologies are needed to prevent and control growth of or destroy *L. monocytogenes* in RTE products during storage. In an attempt to increase RTE product safety, antimicrobials generally recognized as safe (GRAS) such as sodium chloride, nitrite, trisodium phosphate, lactates, acetates, benzoates and sorbates or naturally occurring antimicrobials such as lysozyme, plant extracts and bacteriocins are being incorporated in RTE products (Buncic et al., 1995; Blom et al., 1997; Kuntz, 1999; Juncher et al., 2000). Recent studies (Bedie et al., 2001; Samelis et al., 2001, 2002) using sodium lactate, sodium acetate and sodium diacetate, either singly or in combination, have shown that they may control postprocessing *L. monocytogenes* contamination of RTE meat products. It has been hypothesized that postprocessing application of antimicrobials may be more advantageous for controlling postprocessing *L. monocytogenes* contamination than incorporation of antimicrobials in the product, as then the active compound is applied after processing on the product surface where the contaminating pathogen is attached (Farber and Peterkin, 1999; Tompkin et al., 1999). Research has shown that application

of organic acids and/or their salts to RTE pork frankfurters after peeling and before packaging was effective for controlling postprocessing *L. monocytogenes* contamination (Samelis et al., 2001).

L. monocytogenes infection via the gastrointestinal tract is confronted by many host defenses or challenges including innate immune mechanisms (Conlan, 1997; Havell et al., 1999) and cellular immunity (Okamoto et al., 1994; Miller et al., 2002). However, the primary defense against enteric pathogens is the low pH of gastric secretions (Peterson et al., 1989; Waterman and Small, 1998). The potential for a pathogen like *L. monocytogenes* to survive killing by acid during transit through the stomach increases the likelihood of its colonization of the intestines and the virulence associated with the pathogen, increasing its potential to cause infection (Waterman and Small, 1998). There are no studies to date evaluating the contribution of antimicrobials incorporated as ingredients in RTE meats on the subsequent acid tolerance of *L. monocytogenes* in a simulated gastric fluid. The objective of this study was to evaluate the effect of a simulated gastric fluid (adjusted to pH 1.0 with HCl) on *L. monocytogenes* inoculated postprocessing on pork frankfurters formulated with organic acid salts, treated by dipping in organic acid solutions, and stored in vacuum packages at 10°C.

MATERIALS AND METHODS

Frankfurter preparation

The formulation of the frankfurters included (%, wt/wt): pork trimmings (82.2), ice (10), sodium chloride (2), dextrose (2), dry mustard (0.9), corn syrup solids (2), polyphosphate

(0.4), sodium nitrite (0.0156), sodium erythorbate (0.05), paprika (0.25), onion powder (0.05), garlic powder (0.05), coriander (0.05) and white pepper (0.05). Fresh pork trimmings (approximately 30% fat) were obtained from Swift and Co. (Greeley, CO); spices and seasonings were purchased from AC Legg and Co. (Birmingham, AL) (sodium tripolyphosphate and sodium hexameta-phosphate, Heller Inc., Bedford Park, IL). Separate batches contained: no antimicrobials (control); sodium lactate (1.8% pure sodium lactate, Purac Inc., Lincolnshire, IL - SL); sodium diacetate (0.25%, Niacet, Niagara Falls, NY - SD); sodium lactate (1.8%) combined with 0.125% sodium diacetate (SL+0.125% SD); and sodium lactate (1.8%) combined with 0.25% sodium diacetate (SL+0.25% SD). The ingredients of each batch were emulsified in a bowl chopper, extruded into 24 mm diameter fibrous cellulose casings, weighed, smoked, and cooked as described by Bedie et al. (2001) and Samelis et al. (2001c, 2002c,d). After overnight cooling, the casings were removed and the frankfurters were cut into 10-cm length pieces for inoculation.

Product inoculation

The strains of *L. monocytogenes* used in this study included Scott A (serotype 4b, human isolate), NA-3 (serotype 4b), NA-19 (serotype 3b), 101M (serotype 4b) and 103M (serotype 1a): all pork sausage isolates, 558 (serotype 1/2, pork meat isolate), and PVM1, PVM2, PVM3 and PVM4 (pork variety meat isolates, serotype unknown). The inoculum was prepared as described by Bedie et al. (2001) and Samelis et al. (2001c, 2002c,d) and serially diluted to yield a population of 10^4-10^5 CFU/ml. Two frankfurters from each batch were placed in vacuum bags (15 by 20 cm, 3 mil std. barrier, Nylon/PE vacuum

pouch, Koch Supplies Inc., North Kansas City, MO) and inoculated with 0.25 ml of the washed composite culture per frankfurter (73 cm^2 surface area). The frankfurters were massaged in the bag to spread the inoculum uniformly over the surface. The organisms were allowed to attach for 15 min at 5°C before the frankfurters were treated with organic acid solutions and/or vacuum packaged.

Frankfurter dipping treatments

Frankfurters from each batch were removed from the bags in which they were inoculated. Ten frankfurters per batch were not dipped or dipped into 1 L of 2.5% acetic (Mallinckrodt and Baker, Inc., Paris, KY) or 2.5% lactic acid (Sigma) solution and stirred occasionally for two minutes before pairs of frankfurters were vacuum packaged in new bags and stored at 10°C. Thus, including antimicrobials in the formulation and as dipping solutions, the following treatments were evaluated: (1) Not dipped control; (2) Not dipped SL; (3) Not dipped SD; (4) Not dipped SL+0.125% SD; (5) Not dipped SL+0.25% SD; (6) 2.5% acetic acid-dipped control; (7) 2.5% acetic acid-dipped SL; (8) 2.5% acetic acid-dipped SD; (9) 2.5% acetic acid-dipped SL+0.125% SD; (10) 2.5% acetic acid-dipped SL+0.25% SD; (11) 2.5% lactic acid-dipped control; (12) 2.5% lactic acid-dipped SL; (13) 2.5% lactic acid-dipped SD; (14) 2.5% lactic acid-dipped SL+0.125% SD; and, (15) 2.5% lactic acid-dipped SL+0.25% SD. At day 0, bacterial populations were determined within 60 min after dipping.

Exposure of *L. monocytogenes* to simulated gastric fluid

To assess the susceptibility of *L. monocytogenes* to inactivation at a low pH similar to that of the stomach environment, use was made of a simulated gastric fluid (Beumer et al., 1992; Cotter et al., 1999; Czuprynski et al., 2002) consisting of: 8.3 g proteose peptone (Sigma), 3.5 g D-glucose (dextrose, anhydrous; Fisher), 2.05 g sodium chloride (crystallized; Fisher), 0.6 g potassium phosphate (monobasic, anhydrous; Sigma), 0.11 g calcium chloride (dehydrate; Sigma), 0.37g potassium chloride (crystallized; Fisher), 0.1 g lysozyme (from chicken egg white, dialyzed, crystallized; Sigma), 50 mg bile (bovine bile, dried, unfractionated; Sigma) and 13.3 mg pepsin (Pepsin A, from porcine stomach mucosa, dessicated; Sigma) per liter of distilled water. The simulated gastric fluid in this experiment was adjusted to pH 1.0 using hydrochloric acid (AR®, approximately 37%, Mallinckrodt). To test the effects of the simulated gastric environment on *L. monocytogenes* originating on the frankfurters, one inoculated frankfurter from each formulation/dipping combination was removed at 0, 10, 20, 30 and 40 d during storage, placed into a plastic Whirlpak® bag (Nasco, Fort Atkinson, WI) in which 100 ml of simulated gastric fluid (25°C) was added before pummeling in a masticator (IUL Instruments, Barcelona, Spain) for 2 min. Control (time-0) samples (inoculated/not treated) were placed in bags in which 50 ml of 0.1% BPW (Difco) was added prior to pummeling. During 1 h, at 20-min intervals, portions of the samples exposed to simulated gastric fluid were removed from the bags for determination of survivors.

Microbiological and physical analysis of samples

Determination of microbial populations and measurements of the pH of homogenized samples were performed at time-0 (inoculated/not treated) and at 20, 40 and 60 min

following addition of simulated gastric fluid to samples. For microbiological analysis, 1 ml of the homogenized sample was serially diluted in 9 ml of sterile 0.1% BPW and appropriate dilutions were plated onto TSA (Difco) supplemented with 0.1% pyruvate (Fisher) (TSAP) for determination of total bacterial counts and onto PALCAM (Difco) agar with PALCAM selective supplement (Dalynn Biological, Calgary, Canada) for the selective enumeration of *L. monocytogenes* populations. Colonies were counted after plates were incubated at 30°C for 48 h. The detection limits of the microbiological analyses were -0.16 and 0.13 log CFU/cm^2 for attached cells from time-0 samples and samples exposed to simulated gastric fluid, respectively.

The pH values of homogenized samples were measured after microbiological analysis using a digital pH meter (*Ultra*Basic, UB-10; Denver Instrument, Denver, CO) with a glass pH electrode (pH/ATC Electrode #300729.1; Denver Instrument). Colonies that were suspected to be *L. monocytogenes* were tested for Gram-reaction by mixing colonies with a 3% KOH solution for the presence (Gram-negative) or absence (Gram-positive) of a slimy suspension (Samelis et al., 2001c). All Gram-positive colonies on plates were subjected to the catalase test by dropping a 3% hydrogen peroxide (Target™, Dayton, Hudson Corp., Minneapolis, MN) solution directly onto them to observe effervescence. Gram-positive, catalse-positive colonies identified by this procedure were subsequently tested for oxidase using a test kit (BBL™ DrySlide™, Oxidase slides; Becton Dickinson, Sparks, MD).

Statistical analysis

Two replicate experiments were conducted with three samples tested per treatment at each sampling time in each replicate. Microbiological data obtained by plating the liquid from homogenized samples were converted to log CFU/cm^2 based on the outside surface area (73 cm^2) of the frankfurter in the appropriate volume of simulated gastric fluid (100 ml) or 0.1% BPW (50 ml) before being analyzed. Values for the mean log and standard deviation of each set of bacterial counts were calculated on the assumption of a log-normal distribution of microorganisms. Preliminary analysis of fixed effects using the general linear models procedure of SAS® version 8.2 (SAS, 2002) indicated that numbers of bacteria recovered were dependent on formulation (Control, SL, SD, SL+0.125% SD or SL+0.25% SD) (F-statistic=682.07, P<0.0001), dipping treatment (not dipped, dipped in 2.5% acetic or lactic acid) (F-statistic=258.80; P<0.0001), day of analysis (day-0, -10, -20, -30 or -40) (F-statistic=216.59, P<0.0001) and time of analysis (time-0, -20, -40 or -60) (F-statistic=769.66; P<0.0001). Data regarding viable populations for each day of analysis were evaluated using a 5 x 4 x 3 (formulation x time of analysis x treatment) factorial design. For each medium/day of analysis combination, individual fixed effects and up to three-way interactions were evaluated with ANOVA using the model $y = x_1 + x_2 + x_3 + x_1x_2 + x_1x_3 + x_2x_3 + x_1x_2x_3$ in the general linear models procedures of SAS® version 8.2 (SAS, 2002); where x_1 represents formulation (control, SL, SD, SL+0.125% SD or SL+0.25% SD), x_2 represents time of analysis (0, 20, 40 or 60 min) and x_3 represents dipping treatment (not dipped, dipped in 2.5% acetic or lactic acid). Least-squares means were separated using a protected pairwise t-test of SAS® version 8.2 (SAS, 2002). All differences were reported at a significance level of alpha = 0.05.

RESULTS AND DISCUSSION

Populations recovered on TSAP agar are not presented as the plates were almost entirely covered by colonies of gram-positive, catalase-positive, spore-forming *Bacillus*-like (Fischer et al., 1986) bacteria of the natural microbial flora; making enumeration of *L. monocytogenes* colonies on these plates impossible. Rare colonies of the *Bacillus*-like organism were also observed on PALCAM agar and should not have interfered with growth of *L. monocytogenes*. Further investigation suggested that most of the spices used in this experiment harbored the *Bacillus*-like bacteria that were encountered on the plates. Future experiments should consider the exclusive use of irradiated spices to minimize introduction of such contamination or strains of *L. monocytogenes* with selective markers such as green-fluorescent protein or antibiotic resistance to aid in recovery and enumeration of the test organism. The data presented and discussed in this paper are those obtained by plating on PALCAM agar.

The initial pH values of inoculated frankfurters that were not dipped or dipped in acid prior to simulated gastric fluid exposure ranged between 5.47 and 6.07 (Table VII.1-VII.3). Overall, the pH values of frankfurters exposed to simulated gastric fluid were similar ($P \geq 0.05$) regardless of different formulations, different dipping treatments, and the storage times. Therefore, pH values were pooled to obtain means and standard deviations of pH values representing time of exposure. In general, there was a significant ($P < 0.05$) decrease in product pH to 2.0 ± 0.3 after 20 min of simulated gastric fluid

exposure which increased significantly (P<0.05) to 2.4±0.4 during the remaining time of exposure (Table VII.1-VII.3).

Table VII.1: Mean (standard deviation) pH values of nondipped pork frankfurter samples inoculated with *Listeria monocytogenes* and exposed to simulated gastric fluid (pH 1.0; for up to 60 min) periodically during storage under vacuum at 10°C for 40 d

Day of Storage	Antimicrobial in Formulation	Gastric Acid Exposure (min)			
		0	20	40	60
0	None (Control)	6.17abcz (0.06)	1.84fgx (0.26)	2.04ghx (0.35)	2.25ghy (0.45)
	1.8% SL	6.32az (0.04)	2.11cdw (0.13)	2.34efx (0.22)	2.55dey (0.22)
	0.25% SD	6.12bcz (0.05)	2.04dex (0.26)	2.23fx (0.30)	2.44efy (0.32)
	1.8% SL + 0.125% SD	6.15bcz (0.03)	2.32bcx (0.21)	2.51cdx (0.29)	2.75cdy (0.18)
	1.8% SL + 0.25% SD	6.20abz (0.06)	2.35abw (0.35)	2.59cx (0.41)	2.83bcy (0.29)
10	None (Control)	6.17abcz (0.06)	1.84fgx (0.26)	2.04ghx (0.35)	2.25ghy (0.45)
	1.8% SL	6.32az (0.04)	2.11cdw (0.13)	2.34efx (0.22)	2.55dey (0.22)
	0.25% SD	6.12bcz (0.05)	2.04dex (0.26)	2.23fy (0.30)	2.44efy (0.32)
	1.8% SL + 0.125% SD	6.15bcz (0.03)	2.32bcx (0.21)	2.51cdx (0.29)	2.75cdy (0.18)
	1.8% SL + 0.25% SD	6.20abz (0.06)	2.35abw (0.35)	2.59cx (0.41)	2.83bcy (0.29)
20	None (Control)	5.79ez (0.16)	1.89fgx (0.12)	2.06ghxy (0.13)	2.18ghy (0.11)
	1.8% SL	6.30az (0.06)	2.41abx (0.16)	2.70bcy (0.17)	2.90aby (0.13)
	0.25% SD	6.10bcz (0.06)	1.96efx (0.24)	2.07ghxy (0.22)	2.26ghy (0.27)
	1.8% SL + 0.125% SD	6.17abcz (0.02)	2.45abx (0.46)	2.92ay (0.17)	2.93aby (0.40)
	1.8% SL + 0.25% SD	6.22abz (0.03)	2.57ax (0.28)	2.84aby (0.19)	3.03ay (0.13)
30	None (Control)	5.33fz (0.17)	1.55ix (0.09)	1.69jxy (0.12)	1.85iy (0.15)
	1.8% SL	6.23abz (0.06)	1.75ghw (0.23)	2.01hx (0.31)	2.30fgy (0.34)
	0.25% SD	6.07cdz (0.11)	1.82fghx (0.14)	1.97hixy (0.20)	2.12hy (0.23)
	1.8% SL + 0.125% SD	6.17abcz (0.03)	2.13cdw (0.54)	2.56cdx (0.40)	2.82bcy (0.35)
	1.8% SL + 0.25% SD	6.18abcz (0.12)	2.06dew (0.39)	2.41dex (0.47)	2.81bcy (0.22)
40	None (Control)	4.97gz (0.10)	1.68hiy (0.08)	1.79ijy (0.16)	1.86iy (0.12)
	1.8% SL	6.03cdz (0.14)	2.05dex (0.28)	2.36dey (0.34)	2.51ey (0.28)
	0.25% SD	5.89dez (0.31)	1.67hix (0.06)	1.80ijxy (0.08)	1.89iy (0.07)
	1.8% SL + 0.125% SD	6.11bcz (0.05)	1.97efx (0.34)	2.21fgy (0.37)	2.41efy (0.39)
	1.8% SL + 0.25% SD	6.10bcz (0.19)	2.22cx (0.44)	2.51cdy (0.43)	2.63cdy (0.32)

SL – sodium lactate; SD – sodium diacetate
abcdefghij - means in the same row with different letters are significantly different (P<0.05)
zyxw - means in the same column with different letters are significantly different (P<0.05)

Table VII.2: Mean (standard deviation) pH values of pork frankfurter samples inoculated with *Listeria monocytogenes* and dipped in 2.5% lactic acid and subsequently exposed to simulated gastric fluid (pH 1.0; for up to 60 min) periodically during storage under vacuum at 10°C for 40 d

Day of Storage	Antimicrobial in Formulation	Gastric Acid Exposure (min)			
		0	20	40	60
0	None (Control)	5.31fz (0.20)	1.59iy (0.11)	1.68ky (0.11)	1.72jy (0.12)
	1.8% SL	6.08abz (0.05)	1.98dex (0.46)	2.18efgy (0.52)	2.28fghy (0.47)
	0.25% SD	5.20fz (0.71)	1.63iy (0.14)	1.77jky (0.25)	1.79jy (0.16)
	1.8% SL + 0.125% SD	5.98abcz (0.04)	2.04dex (0.58)	2.27efy (0.58)	2.43efy (0.60)
	1.8% SL + 0.25% SD	6.05abz (0.12)	1.72ghiw (0.46)	2.01hix (0.53)	2.21ghy (0.58)
10	None (Control)	6.12az (0.06)	1.94efw (0.24)	2.16ghx (0.39)	2.36fgy (0.45)
	1.8% SL	6.12az (0.07)	2.17cx (0.26)	2.69aby (0.39)	2.84bcy (0.28)
	0.25% SD	5.87cdz (0.08)	1.95efw (0.27)	2.17fgx (0.36)	2.39fy (0.44)
	1.8% SL + 0.125% SD	5.98abcz (0.03)	2.53ax (0.50)	2.81ay (0.46)	2.97aby (0.34)
	1.8% SL + 0.25% SD	6.05abz (0.05)	2.30bw (0.30)	2.58bcdx (0.27)	2.92aby (0.19)
20	None (Control)	5.90bcz (0.18)	1.80ghx (0.17)	1.93ixy (0.15)	2.06iy (0.16)
	1.8% SL	6.11az (0.08)	2.22bcx (0.27)	2.50cdy (0.28)	2.69cdy (0.25)
	0.25% SD	5.86cdz (0.10)	1.84fgx (0.09)	2.07ghy (0.11)	2.21ghy (0.10)
	1.8% SL + 0.125% SD	5.98abcz (0.04)	2.54aw (0.39)	2.87ax (0.29)	3.05ay (0.28)
	1.8% SL + 0.25% SD	6.05abz (0.03)	2.56aw (0.24)	2.80abx (0.17)	2.99aby (0.15)
30	None (Control)	5.56ez (0.14)	1.70hix (0.21)	1.91ijy (0.20)	2.05iy (0.21)
	1.8% SL	6.13az (0.05)	2.21cx (0.34)	2.66bcy (0.23)	2.68dy (0.34)
	0.25% SD	5.76dz (0.15)	1.81ghx (0.26)	2.06ghiy (0.31)	2.20hiy (0.30)
	1.8% SL + 0.125% SD	5.91bcz (0.12)	2.24bcx (0.58)	2.46dy (0.61)	2.55dey (0.57)
	1.8% SL + 0.25% SD	6.05abz (0.08)	2.11cdx (0.16)	2.50cdy (0.27)	2.61dy (0.29)
40	None (Control)	5.31fz (0.20)	1.59iy (0.11)	1.68ky (0.11)	1.72jy (0.12)
	1.8% SL	6.08abz (0.05)	1.98dex (0.46)	2.18efgxy (0.52)	2.28fghy (0.47)
	0.25% SD	5.20fz (0.71)	1.63iy (0.14)	1.77jky (0.25)	1.79jy (0.16)
	1.8% SL + 0.125% SD	5.98abcz (0.04)	2.04dex (0.58)	2.27efxy (0.58)	2.43efy (0.60)
	1.8% SL + 0.25% SD	6.05abz (0.12)	1.72ghiw (0.46)	2.01hix (0.53)	2.21ghy (0.58)

SL – sodium lactate; SD – sodium diacetate
abcdefghij - means in the same row with different letters are significantly different ($P<0.05$)
zyxw - means in the same column with different letters are significantly different ($P<0.05$)

Table VII.3: Mean (standard deviation) pH values of pork frankfurter samples inoculated with *Listeria monocytogenes* and dipped in 2.5% acetic acid and subsequently exposed to simulated gastric fluid (pH 1.0; for up to 60 min) periodically during storage under vacuum at 10°C for 40 d

Day of Storage	Antimicrobial in Formulation	Gastric Acid Exposure (min)			
		0	20	40	60
0	None (Control)	6.01abz (0.07)	1.75gx (0.22)	1.93ixy (0.25)	2.05ijy (0.22)
	1.8% SL	6.04az (0.04)	2.32bcx (0.24)	2.62cdy (0.18)	2.68cdy (0.28)
	0.25% SD	5.86cdez (0.05)	1.86fgx (0.24)	2.06hiy (0.28)	2.20hiy (0.28)
	1.8% SL + 0.125% SD	5.91bcz (0.05)	2.19cdw (0.25)	2.57dex (0.24)	2.80cy (0.15)
	1.8% SL + 0.25% SD	5.96abcz (0.17)	2.18cdw (0.22)	2.44efx (0.28)	2.77cy (0.08)
10	None (Control)	6.02az (0.10)	2.00dex (0.11)	2.17ghxy (0.14)	2.28ghy (0.13)
	1.8% SL	5.99abz (0.08)	2.30bcw (0.37)	2.61cdx (0.32)	2.86bcy (0.20)
	0.25% SD	5.77dez (0.04)	2.09dex (0.13)	2.34fgy (0.14)	2.49dey (0.12)
	1.8% SL + 0.125% SD	5.88bcdz (0.04)	2.63ax (0.23)	2.94ay (0.17)	3.12ay (0.13)
	1.8% SL + 0.25% SD	5.96abcz (0.06)	2.65aw (0.38)	2.86abx (0.25)	3.05ay (0.20)
20	None (Control)	6.08az (0.04)	1.76gx (0.11)	1.96iy (0.11)	2.12hijy (0.09)
	1.8% SL	6.04az (0.07)	2.30bcw (0.28)	2.59dex (0.20)	2.80cy (0.17)
	0.25% SD	5.87cdez (0.04)	1.89efx (0.19)	2.09hixy (0.22)	2.29fghy (0.18)
	1.8% SL + 0.125% SD	5.91bcz (0.03)	2.44abw (0.27)	2.78bcx (0.25)	2.98aby (0.19)
	1.8% SL + 0.25% SD	5.97abcz (0.04)	2.40bw (0.13)	2.73bcx (0.08)	2.95aby (0.09)
30	None (Control)	6.01abz (0.07)	1.75gx (0.22)	1.93ixy (0.25)	2.05ijy (0.22)
	1.8% SL	6.04az (0.04)	2.32bcx (0.24)	2.62cdy (0.18)	2.68cdy (0.28)
	0.25% SD	5.86cdez (0.05)	1.86fgx (0.24)	2.06hixy (0.28)	2.20hiy (0.28)
	1.8% SL + 0.125% SD	5.91bcz (0.05)	2.19cdw (0.25)	2.57dex (0.24)	2.80cy (0.15)
	1.8% SL + 0.25% SD	5.96abcz (0.17)	2.18cdw (0.22)	2.44efx (0.28)	2.77cy (0.08)
40	None (Control)	5.52fz (0.19)	1.44hy (0.29)	1.52ky (0.34)	1.62ky (0.40)
	1.8% SL	6.01abz (0.05)	1.49hx (0.22)	1.75jxy (0.28)	1.97jy (0.40)
	0.25% SD	5.70efz (0.08)	1.48hx (0.29)	1.61jkxy (0.35)	1.78ky (0.44)
	1.8% SL + 0.125% SD	5.72ez (0.33)	1.85fgx (0.58)	2.16ghy (0.67)	2.35efgy (0.72)
	1.8% SL + 0.25% SD	5.92bcz (0.11)	1.99efy (0.51)	2.19ghy (0.58)	2.39efgy (0.67)

SL – sodium lactate; SD – sodium diacetate
abcdefghij - means in the same row with different letters are significantly different (P<0.05)
zyxw - means in the same column with different letters are significantly different (P<0.05)

Irrespective of antimicrobial formulation, dipping frankfurters in 2.5% acetic or lactic acid resulted in immediate reductions in microbial counts as compared with not dipped samples (Table VII.4). Growth of the pathogen during storage of not dipped frankfurters increased in the following order: control > SL > SD > SL + 0.125% SD > SL

+ 0.25% SD with SL + 0.25% SD totally inhibiting growth throughout storage (time-0 of Tables VII.4-VII.7). Growth of the pathogen during storage in control (no antimicrobials in the formulation) frankfurters was faster and more extensive when dipped in 2.5% lactic as compared to acetic acid (time-0 of Tables 1-4). Dipping in 2.5% acetic acid totally inhibited the pathogen's growth in frankfurters when an antimicrobial was included in the product formulation (Tables VII.4-VII.7). Although, 2.5% lactic acid dipping inhibited growth of the pathogen up to day 10, thereafter the pathogen on frankfurters including SL and SD singly but not in combination were able to grow (time-0 of Tables VII.4-VII.7).

At day-0, there was no recovery of *L. monocytogenes* after 20 min of exposure to simulated gastric fluid (Table VII.5). At day-10, there was still no survival of *L monocytogenes* in gastric fluid, despite the fact that populations of the pathogen on the controls with no dipping or dipped in 2.5% lactic acid had increased above 5 logs (Table VII.5). From day-20 to -40, however, the growth of *L. monocytogenes* in frankfurters (control, SL and SD) was followed by an increased survival after exposure to simulated gastric fluid (Tables VII.5-VII.7). Reductions of pathogen populations in not dipped frankfurters after 20 min of exposure to simulated gastric fluid at day-30 (Table VII.6) were comparable (4-5 logs) between control frankfurter samples and those with SL and SD in the formulation. On day-30, pathogen survivors from not dipped frankfurters formulated with SL and SD were evident after 60 min of exposure to simulated gastric fluid (Table VII.6). At day-40 (Table VII.7), reduction in pathogen cells in not dipped frankfurters with SL or SD in the formulation was greater than in control samples. There was a major tailing effect in survival between 20 and 60 min of exposure to the simulated gastric fluid. The combination of SL and 0.125% SD in the formulation allowed only

limited growth during prolonged storage as well as some (≤0.7 logs) survival during the first 40 min of exposure to simulated gastric fluid. Although, the combination of SL and 0.25% SD did not permit growth of *L. monocytogenes* during storage, it allowed some survivors (≤0.3 logs) upon exposure to simulated gastric fluid (Table VII.7).

The effect of dipping in acid solutions on the acid tolerance of *L. monocytogenes* is most clear when comparing the changes in populations of control frankfurters. As indicated, acid tolerance of the pathogen in control, not dipped frankfurters increased during storage. A similar trend was apparent with control frankfurters dipped in 2.5% lactic acid. Although acid tolerance trends of *L. monocytogenes* on control frankfurters dipped or not dipped in acid were similar, it should be noted that the numbers of pathogen cells exposed to simulated gastric fluid were consistently greater on the not dipped than on the dipped samples. Frankfurter formulation with 0.25% SD and dipping in 2.5% lactic acid appeared to increase resistance of *L. monocytogenes* to simulated gastric fluid following growth during storage after 20 d. Use of acetic acid dipping combined with antimicrobials in the formulation inhibited growth of *L. monocytogenes* on frankfurters and detection of survivors following exposure to simulated gastric fluid. However, dipping of control frankfurters in acetic acid allowed growth and survival after exposure to simulated gastric fluid indicated development of acid resistance, but to a lesser extent compared to dipping of control samples in lactic acid.

In the present study, the pathogen was not recovered after exposure to simulated gastric fluid at day-10 of frankfurter storage at 10°C as cells were either in exponential phase and as such very sensitive to the acid environment (Samelis et al., 2003) or were still present in insufficient numbers to allow detectable survival. Increases in pathogen

survival during exposure to simulated gastric fluid from day-20 to -40 was associated with higher population levels exposed to simulated gastric fluid. It is important to note that the greater the growth of the pathogen on frankfurters, in the order control > SL > SD, the higher was the subsequent resistance of the pathogen to simulated gastric fluid. In accordance, the highest survival of the pathogen to gastric fluid was noted in the cells present on not-dipped control frankfurters at 40 days, which had grown to the highest numbers, and thus, had attained a high stationary-phase acid resistance at 10°C (Samelis et al. 2003).

Overall, dipping frankfurters in acid solutions resulted in immediate reductions in pathogen populations as well as a delay or inhibition of growth. Formulation with antimicrobials and dipping in 2.5% acetic acid did not allow growth; thus, due to consistently low initial levels exposed to simulated gastric fluid, any inherent resistance or potential development thereof could not be determined. Similarly, inclusion of combinations of antimicrobials in the formulation and dipping in lactic acid did not allow growth of the pathogen during frankfurter storage and acid resistance could not be evaluated. Dipping of frankfurters formulated without antimicrobials in 2.5% acetic or 2.5% lactic acid, as well as dipping of frankfurters formulated with SL or SD in 2.5% lactic acid, allowed growth during storage. Thus, acid tolerance of *L. monocytogenes* populations from those treatments to simulated gastric fluid could be estimated. Populations of *L. monocytogenes* on SL frankfurters dipped in 2.5% lactic acid grew above 4 log CFU/cm^2 at 40 d of storage, and yielded some survivors after exposure to simulated gastric fluid indicating some apparent increase in acid resistance at these levels of growth. Survival of *L. monocytogenes* on frankfurters formulated with SD alone and

not dipped in organic acid solutions seemed to increase during storage, but not to the extent of those dipped in 2.5% lactic acid. The increase in survival of *L. monocytogenes* on the not dipped frankfurters formulated with either SL or SD may have been a result of cell age and the higher initial populations at days 30 and 40. It appeared that pathogen populations reaching > 5 log CFU/cm^2 on not dipped frankfurters containing SL or SD and subjected to prolonged storage may include an acid-resistant sub-population. In contrast, the survival of *L. monocytogenes* in 2.5% lactic acid-dipped frankfurters formulated with SD was higher than that in the corresponding samples that were not dipped, although the initial population level was approximately 1 log lower at days 30 and 40 (Tables VII.6 and VII.7).

There has been limited work involving the exposure of pathogens on or from food sources to simulated gastric fluid (Roering et al., 1999; Uljas and Ingham, 1998; Waterman and Small, 1998). Arnold and Kaspar (1995) tested the acid tolerance of *Escherichia coli* O157:H7 grown to stationary phase or to a state of starvation and found that such cultures were more resistant to the effects of a simulated gastric fluid than exponential phase cells. Roering et al. (1999) found that while *L. monocytogenes* was reduced to near undetectable levels in apple cider within 2 days of storage, its numbers were similarly reduced within 30 min of incubation in simulated gastric fluid (pH 1.5). It was also shown that acid habituated *E. coli* O157:H7 cells in apple juice were more sensitive to subsequent exposure in simulated gastric fluid than non-acid habituated cells (Uljas and Ingham, 1998). The inoculation of acid-sensitive enteric pathogens onto food surfaces has been shown to protect such cultures from acid conditions that approximate gastric acidity (Waterman and Small, 1998).

The fact that on day-20 survival of *L. monocytogenes* during exposure to simulated gastric fluid was observed in 2.5% lactic acid-dipped frankfurters formulated with SD alone may be due to a potential sublethal stressing effect of SD with lactic acid present on the product surface as a residue of dipping or potentially as a product of lactic acid bacteria inherent to the product. Uljas and Ingham (1998) suggested that storage of cells in an environment with lactic acid dramatically sensitized them to simulated gastric fluid. This suggests that *L. monocytogenes* originating from frankfurters with antimicrobials in the formulation and exposed to 2.5% lactic acid during storage could be quite sensitive to simulated gastric fluid. Indeed this was the case, except for those frankfurters formulated with SD only. The increased survival in simulated gastric fluid of *L. monocytogenes* grown on SD-containing frankfurters dipped in lactic acid, but not in those with SD dipped in acetic acid, may have been due to resistance development during storage in the SD-lactic acid environment. This (SD-lactic acid interaction) may have provided microenvironments of pH lower than those provided by SD-acetic acid. Indeed, Waterman and Small (1998) indicated that the pH of the microenvironment occupied by enteric pathogens on the surface of a food affects their survival. More recently, Koutsoumanis and Sofos (2004) reported that previous habituation of *L. monocytogenes*, *E. coli* O157:H7 and *Salmonella* Typhimurium to an acidic environment resulted in maximum acid tolerance of *L. monocytogenes* being induced after habituation at pH 5.5 as compared to pH 5.0 to 4.5 for the other two pathogens. In accordance, since dipping of SD-containing frankfurters in 2.5% lactic acid reduced their pH to approximately 5.5, this might have resulted in more diacetate being in the undissociated form. This may have induced a greater acid tolerance response in *L. monocytogenes* during storage as

compared to not dipped frankfurters with SD, which had a pH of approximately 6.1. Of course, dipping of SD-containing frankfurters in 2.5% acetic acid could have induced a similar response in *L. monocytogenes*; this, however, could not be observed because the numbers of cells exposed to gastric fluid were very low. Even though survivors in SD-containing frankfurters dipped in 2.5% acetic acid were comparable with those on samples dipped in 2.5% lactic acid, the resulting populations would not necessarily have similar responses considering that the pH 5.92 of acetic acid-dipped SD frankfurters may not support the best adaptive response of *L. monocytogenes* as suggested by Koutsoumanis and Sofos (2004). Additional work done in our laboratory supports that the combination of acetic acid with SD resulted in a higher pH than that of lactic acid with SD (unpublished data). This finding is consistent with the potential for an increased induction of acid tolerance on frankfurters by the combination 0.25% SD in the formulation-dipping in 2.5% lactic acid. This increase in acid tolerance may have resulted in protection of *L. monocytogenes* to subsequent low pH synthetic gastric fluid simulated the exposure of *L. monocytogenes* on frankfurters to an extreme acid environment; however, it should be noted that our results are not necessarily representative of real-life effects of the human gastrointestinal environment on the pathogen after consumption.

Table VII.4: Mean log CFU/cm^2 populations (standard deviations) of *Listeria monocytogenes* (on PALCAM agar) on pork frankfurters formulated with antimicrobials and not dipped or dipped in organic acid solutions and later exposed to simulated gastric fluid (pH 1.0) after storage for 0 and 10 days under vacuum at 10°C

		Day-0		Day-10	
		Gastric Fluid Exposure (min)		Gastric Fluid Exposure (min)	
Dipping Solution	Antimicrobial in the Product	0	20[A]	0	20
None	None (Control)	2.3az (0.3)	<0.1*bz	6.6az (0.1)	<0.1bz
	1.8% SL	2.2az (0.2)	<0.1bz	4.2ax (0.1)	<0.1bz
	0.25% SD	1.8ayx (0.2)	<0.1bz	3.1aw (0.2)	<0.1bz
	1.8% SL + 0.125% SD	2.0azy (0.1)	<0.1bz	2.3av (0.3)	<0.1bz
	1.8% SL + 0.25% SD	2.3az (0.4)	<0.1bz	1.9au (0.1)	<0.1bz
2.5% lactic acid	None (Control)	1.2awv (0.2)	<0.1bz	5.4ay (0)	<0.1bz
	1.8% SL	1.5axw (0.3)	<0.1bz	0.6as (0.1)	<0.1bz
	0.25% SD	1.3awv (0.2)	<0.1bz	1.0at (0.1)	<0.1bz
	1.8% SL + 0.125% SD	1.6ayxw (0.5)	<0.1bz	0.8at (0.2)	<0.1bz
	1.8% SL + 0.25% SD	1.3awv (0.2)	<0.1bz	0.7ats (0.1)	<0.1bz
2.5% acetic acid	None (Control)	1.7ayx (0.2)	<0.1bz	2.5av (0.3)	<0.1bz
	1.8% SL	1.3awv (0.1)	<0.1bz	0.4as (0.3)	<0.1bz
	0.25% SD	1.3awv (0.4)	<0.1bz	0.5as (0.2)	<0.1bz
	1.8% SL + 0.125% SD	1.5axw (0.2)	<0.1bz	0.8at (0.4)	<0.1bz
	1.8% SL + 0.25% SD	0.9awv (0.5)	<0.1bz	0.5as (0.2)	<0.1bz

SL – sodium lactate; SD – sodium diacetate
*: below 0.1 log CFU/cm^2, the analysis detection limit for cells attached to frankfurters.
20[A]: Populations remained below 0.1 log CFU/cm^2 throughout 60 min of exposure.
ab - means in the same row with different letters are significantly different ($P<0.05$).
zyxwvuts - means in the same column with different letters are significantly different ($P<0.05$).

Table VII.5: Mean log CFU/cm^2 populations (standard deviations) of *Listeria monocytogenes* (on PALCAM agar) on pork frankfurters formulated with antimicrobials and not dipped or dipped in organic acid solutions and later exposed to simulated gastric fluid (pH 1.0) after storage for 20 days under vacuum at 10°C

Dipping Solution	Antimicrobial in the Product	Gastric Fluid Exposure (min)			
		0	20	40	60
None	None (Control)	8.7az (0.2)	4.5bz (0.3)	4.1bz (1.5)	4.2bz (1.1)
	1.8% SL	6.2ax (0.9)	1.4bx (1.6)	1.2bx (1.6)	<0.1cw
	0.25% SD	4.5aw (0.1)	<0.1* bv	<0.1bw	<0.1bw
	1.8% SL + 0.125% SD	4.2awv (0.7)	<0.1bv	<0.1bw	<0.1bw
	1.8% SL + 0.25% SD	2.6au (0.7)	<0.1bv	<0.1bw	<0.1bw
2.5% lactic acid	None (Control)	7.9ay (0)	3.1by (0.2)	3.1by (0.2)	3.0by (0.2)
	1.8% SL	3.4avu (0.3)	<0.1bv	<0.1bw	<0.1bw
	0.25% SD	3.2au (0.5)	0.8bx (0.7)	0.9bx (0.8)	0.3bx (0.4)
	1.8% SL + 0.125% SD	1.4av (0.7)	<0.1bv	<0.1bw	<0.1bw
	1.8% SL + 0.25% SD	0.9av (0.2)	<0.1bv	<0.1bw	<0.1bw
2.5% acetic acid	None (Control)	4.9aw (0)	0.1bw (0.2)	0.9bx (0.8)	0.3bx (0.4)
	1.8% SL	1.0av (0.1)	<0.1bv	<0.1bw	<0.1bw
	0.25% SD	1.1av (0.2)	<0.1bv	<0.1bw	<0.1bw
	1.8% SL + 0.125% SD	0.8av (0.2)	<0.1bv	<0.1bw	<0.1bw
	1.8% SL + 0.25% SD	1.1av (0.3)	<0.1bv	<0.1bw	<0.1bw

SL – sodium lactate; SD – sodium diacetate
*: below 0.1 log CFU/cm^2, the analysis detection limit for cells attached to frankfurters
abc - means in the same row with different letters are significantly different (P<0.05)
zyxwv - means in the same column with different letters are significantly different (P<0.05)

Table VII.6: Mean log CFU/cm^2 populations (standard deviations) of *Listeria monocytogenes* (on PALCAM agar) on pork frankfurters formulated with antimicrobials and not dipped or dipped in organic acid solutions and later exposed to simulated gastric fluid (pH 1.0) after storage for 30 days under vacuum at 10°C

Dipping Solution	Antimicrobial in the Product	Gastric Fluid Exposure (min)			
		0	20	40	60
None	None (Control)	8.1az (0.2)	4.1by (0.1)	2.9cy (0.2)	3.0cy (0.5)
	1.8% SL	6.6ay (0.9)	1.9bw (1.1)	1.8bx (1.0)	0.8cx (0.6)
	0.25% SD	5.6ax (0.9)	0.6bv (0.6)	0.5bw (0.4)	0.6bx (0.5)
	1.8% SL + 0.125% SD	3.9aw (0.8)	<0.1bu	<0.1bv	<0.1bw
	1.8% SL + 0.25% SD	1.9av (0.2)	<0.1*bu	<0.1bv	<0.1bw
2.5% lactic acid	None (Control)	7.7az (0.2)	5.5bz (0.2	5.4bz (0.2)	5.3bz (0.1)
	1.8% SL	3.7aw (0.5)	0.2bv (0.2	<0.1cv	<0.1cw
	0.25% SD	3.8aw (0.9)	2.5bxw (1.0)	2.6byx (1.1)	2.6by (0.8)
	1.8% SL + 0.125% SD	0.5au (0.3)	<0.1bu	<0.1bv	<0.1bw
	1.8% SL + 0.25% SD	0.4au (0.2)	<0.1bu	<0.1bv	<0.1bw
2.5% acetic acid	None (Control)	6.5ay (0.1)	3.1bx (0.4)	2.5byx (1.3)	2.5by (1.3)
	1.8% SL	0.6au (0.3)	<0.1bu	<0.1bv	<0.1bw
	0.25% SD	1.3av (0.1)	<0.1bu	<0.1bv	<0.1bw
	1.8% SL + 0.125% SD	0.5au (0.3)	<0.1bu	<0.1bv	<0.1bw
	1.8% SL + 0.25% SD	0.5au (0.2)	<0.1bu	<0.1bv	<0.1bw

SL – sodium lactate; SD – sodium diacetate
*: below 0.1 log CFU/cm^2, the analysis detection limit for cells attached to frankfurters
abc - means in the same row with different letters are significantly different (P<0.05)
zyxwv - means in the same column with different letters are significantly different (P<0.05)

Table VII.7: Mean log CFU/cm^2 populations (standard deviations) of *Listeria monocytogenes* (on PALCAM agar) on pork frankfurters formulated with antimicrobials and not dipped or dipped in organic acid solutions and later exposed to simulated gastric fluid (pH 1.0) after storage for 40 days under vacuum at 10°C

Dipping Solution	Antimicrobial in the Product	Gastric Fluid Exposure (min)			
		0	20	40	60
None	None (Control)	8.7az (0.1)	6.3bz (0.3)	6.1bz (0.3)	6.0bz (0.3)
	1.8% SL	7.6ay (0.4)	3.4bx (0.2)	3.2bx (0.2)	3.0bx (0.1)
	0.25% SD	6.4ax (0.9)	1.5bw (1.0)	1.2bw (0.7)	1.4bw (0.5)
	1.8% SL + 0.125% SD	3.6av (0.5)	0.7bv (0.4)	0.2cv (0.2)	<0.1du
	1.8% SL + 0.25% SD	2.1au (0.4)	0.3bv (0.2)	0.2bv (0.2)	0.2bv (0.3)
2.5% lactic acid	None (Control)	8.1az (0.3)	4.9by (0.7)	4.9by (0.8)	4.8by (0.8)
	1.8% SL	4.4awv (1.2)	0.3bv (0.3)	0.2bv (0.2)	<0.1cu
	0.25% SD	4.8aw (0.9)	3.4bx (0.7)	3.5bx (0.8)	3.1bx (0.2)
	1.8% SL + 0.125% SD	0.5at (0.2)	<0.1bu	<0.1bu	<0.1bu
	1.8% SL + 0.25% SD	0.6at (0.2)	<0.1*bu	<0.1bu	<0.1bu
2.5% acetic acid	None (Control)	7.6ay (0.1)	3.6bx (1.0)	3.4bx (0.7)	3.6bx (0.7)
	1.8% SL	1.0at (0.1)	<0.1bu	<0.1bu	<0.1bu
	0.25% SD	1.1at (1.1)	<0.1bu	<0.1bu	<0.1bu
	1.8% SL + 0.125% SD	0.5at (0.2)	<0.1bu	<0.1bu	<0.1bu
	1.8% SL + 0.25% SD	0.6at (0.3)	<0.1bu	<0.1bu	<0.1bu

SL – sodium lactate; SD – sodium diacetate
*: below 0.1 log CFU/cm^2, the analysis detection limit for cells attached to frankfurters
abc - means in the same row with different letters are significantly different (P<0.05)
zyxwv - means in the same column with different letters are significantly different (P<0.05)

CONCLUSIONS

In conclusion, growth of *L. monocytogenes* on frankfurters formulated with antimicrobials was inhibited in the order control < SL < 0.25%SD < SL+0.125%SD < SL+0.25% SD. Survival of *L. monocytogenes* on frankfurters exposed to simulated gastric fluid was higher on those frankfurters allowing more growth. Inclusion of single antimicrobials in the formulation of not dipped frankfurters appeared to increase the resistance of *L. monocytogenes* to simulated gastric fluid provided the initial level of exposure was higher than 5 logs. Although, the combination of SL and SD in the formulation of not dipped frankfurters inhibited growth of the low initial contamination

level of *L. monocytogenes*, storage for 40 d resulted in minor, but detectable, induced resistance to simulated gastric fluid. Frankfurter formulation with only 0.25% SD and dipping in 2.5% lactic acid appeared to increase resistance of *L. monocytogenes* to simulated gastric fluid following growth during prolonged storage of at least 20 d. Growth of *L. monocytogenes* on frankfurters containing antimicrobials in the formulation was inhibited when dipped in 2.5% acetic acid and no detectable survivors were recovered after exposure to simulated gastric fluid. As such, any inherent resistance or potential resistance development of the pathogen on acetic acid-dipped SD frankfurters could not be determined. The results suggest that in certain treatments, resistance of *L. monocytogenes* to simulated gastric fluid may be dependent on adequate initial levels of the pathogen and sufficient time to develop resistance while other treatments may lead to potential resistance due to chemical interventions.

SUMMARY OF DISSERTATION

The overall goal of the studies included in this dissertation was to elucidate the response of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on fresh beef and *Listeria monocytogenes* on RTE meat, respectively, subjected to adverse conditions during processing and/or preparation of such products for consumption. The first four studies were conducted with *E. coli* O157:H7 on fresh beef tissue, while the last study was conducted with *L. monocytogenes* on ready-to-eat pork frankfurters.

The first study (Chapter III) evaluated the use of chemicals as single or sequential interventions to reduce *E. coli* O157:H7 and *S.* Typhimurium populations on beef tissue. Samples (5x2.5x1 cm) of beef tissue were inoculated (approximately 10^5 CFU/cm^2) with four-strain composites of *E. coli* O157:H7 or *S.* Typhimurium and dipped (5 pieces in 1.5 L for 30 s at 23°C, unless otherwise indicated) either singly or in all possible combinations of two of the following solutions: acidic oxidative water (0.005%, AOW – pH 2.67); basic oxidative water (BOW – pH 11.21); lactic acid (2.5%, LA - pH 2.12 at 55°C); distilled water (W, pH 7.01), ammonium hydroxide (0.1%, AH - pH 10.89); or, sodium metasilicate (4%, SM – pH 12.35 at 82°C) to evaluate the effectiveness of such solutions in reducing contamination. In another experiment, an approach incorporating sequential treatments that may be applied in multiple-hurdles systems was evaluated. The multiple-hurdles system included sequential dipping in 1% SM (82°C) followed by hot (82°C) water followed by 5% LA (55°C) and lastly by 5% LA (55°C) after 48 h spray-chilling (at -3°C for 10 h and 1°C for 38 h). Additional systems ranged from no dipping steps to four sequential dipping steps using combinations of 1% SM (82°C), 5% LA

(55°C), warm (55°C) or hot (82°C) water. Individual treatments or combinations among W, AOW, BOW, or AH had minimal (0.1-0.4 log CFU/cm^2) decontaminating effects on fresh beef tissue compared to the higher reductions achieved with individual applications or combinations of treatments using 2.5% LA at 55°C and especially 4% SM at 82°C. In general, it appeared that pathogen reductions by the multiple-hurdles system were separated into two groups based on efficacy in increasing order: those treatments with one or more hot (82°C) application or a single 5% LA application < those combining two 5% LA applications or at least one hot (82°C) and one 5% LA application.

The second study (Chapter IV) simulates a spray-chilling process that may be applied commercially to chill beef carcasses and the effect of including chemicals in such a process. Samples (10x20x2.5 cm) of beef carcass tissue were inoculated (10^4-10^5 CFU/cm^2) with *E. coli* O157:H7 which was either nonacid-habituated (prepared by incubating at 15°C for 48 h in inoculated filter-sterilized composite [1:1] of hot and cold water meat decontamination runoff fluids – W; pH 6.05) or acid-habituated (prepared in inoculated W fluids mixed with filter-sterilized 2% lactic acid runoff fluids in a proportion of 1/99 [vol/vol] – LA/W; pH 4.12). The inoculated surfaces were exposed to conditions simulating carcass chilling (-3°C for 10 h followed by 38 h at 1°C). Treatments applied to samples (between 0 and 10 h) during chilling included: (i) no spraying (NT), or spraying (for 30 s every 30 min) with (ii) water (W), (iii) cetylpyridinium chloride (CPC; 0.1 or 0.5%), (iv) ammonium hydroxide (AH; 0.05%), (v) lactic acid (LA; 2%), (vi) acidified sodium chlorite (ASC; 0.12%), (vii) peroxyacetic acid (PAA; 0.02%), (viii) sodium hydroxide (SH; 0.01%), or (ix) sodium hypochlorite (SC; 0.005%) solutions of 4°C. Samples were taken at 0, 10, 24, 36 and 48 h of the chilling process to determine

changes in *E. coli* O157:H7 populations. Phase I tested W, SH, PAA, LA and 0.5% CPC on meat inoculated with nonacid-habituated pathogen populations, while phase II tested W, SC, AH, ASC, LA and 0.1% CPC on meat inoculated with acid- and nonacid-habituated populations. Reductions in nonacid-habituated *E. coli* O157:H7 populations from phase I increased in the order NT = W = SH < PAA < LA < CPC. Reductions from phase II for acid-habituated cells increased in the order NT=W= SC < ASC=LA= AH < CPC, while on nonacid-habituated cells the order observed was NT=W=SC < AH=ASC < LA < CPC. Previous acid-habituation of *E. coli* O157:H7 inocula rendered the cells more resistant to the effects of spray-chilling, especially with acid; however, the trend of reduction remained spray-chilling with water = nonspray-chilling < spray-chilling with chemical solutions.

Based on previous studies and the knowledge that *E. coli* O157:H7 contamination in fresh beef plants may consist of single or mixed strains, the objective of the third study (Chapter V) was to evaluate the effect of strain interaction on the acid tolerance of this pathogen in meat decontamination runoff fluids and on meat. This study assessed the acid tolerance response of stationary phase, acid-adapted (grown in TSB+1% glucose) or nonadapted (grown in glucose-free TSB) *E. coli* O157:H7 strains (ATCC43889, ATCC43895, ATCC51658 and EO139) grown individually or in a mixed culture during acid-adaptation and inoculated on beef or into meat decontamination runoff (washings) fluids (acidic [pH 4.95] or nonacidic washings [pH 7.01]). The beef was untreated or treated by dipping for 30 s in hot water (75°C) followed by 2% lactic acid (55°C). Inoculated beef samples and washings were stored aerobically at 4 or 15°C for 6 d, and at set intervals (0, 2, and 6 d) were exposed (for 0, 60, 120, and 180 min) to pH 3.5

(adjusted with lactic acid) TSBYE. Overall, there were no significant ($P \geq 0.05$) differences in growth or survival after acid exposure of strains grown as a mixture and those grown individually and mixed prior to inoculation. Populations on treated meat were consistently lower than those on untreated meat during storage and subsequent exposure to acid. At day-0 in washings, acid-adapted populations were more resistant to the effects of acid than nonacid-adapted counterparts and remained as such at 4°C, but not at 15°C, throughout storage. Although at day-0, there were no significant ($P \geq 0.05$) differences in acid resistance between acid-adapted and nonacid-adapted populations on meat; acid-adapted cells displayed consistently higher resistance through day-6. This suggests that meat contaminated with previously acid-adapted *E. coli* O157:H7 and exposed to refrigeration (4°C) temperatures such as a chill room may result in populations resistant to subsequent lactic acid exposure.

The fourth study (Chapter VI) with *E. coli* O157:H7 considered the evaluation of overall stress responses associated with production of fresh beef as well as the preparation (i.e., cooking) and consumption thereof. This study evaluated stress responses of different *E. coli* O157:H7 inocula on untreated or decontaminated (sequential hot water and warm acid dipping) beef stored under vacuum at different temperatures and periodically transferred to aerobic storage conditions prior to exposure to consumer-induced stresses simulating incomplete cooking and consumption. Lean beef tissue samples inoculated with *E. coli* O157:H7 cultures prepared in TSB, meat decontamination runoff fluids (WASH), or dattached from moist biofilms (WETB) or dried biofilms (DRYB) were left untreated or sequentially decontaminated by dipping for 30 s in hot (75°C) water and lactic acid (2%) (decontaminated). Samples were vacuum-

packaged, stored at 4 (7, 14, and 28 d) or 12°C (4 and 8 d), and periodically transferred to aerobic storage (7°C for 5 d). During storage, samples were exposed to sequential heat (55°C for up to 20 min) and synthetic gastric fluid (adjusted to pH 1.0 with HCl for up to 90 min) simulating consumption of undercooked beef. Under the conditions of this study, planktonic pathogen cells prepared in liquid media were, in general, more resistant to heat and acid stresses than those grown as biofilms and detached prior to inoculation. Decontamination of fresh beef resulted in injury that prevented subsequent growth of the surviving populations at 12°C, as well as in decreases of the pathogen's resistance to subsequent heat and acid conditions. The shift of pathogen cells on beef stored under vacuum at 4°C to aerobic storage did not affect cell populations nor subsequent survival after sequential exposure to heat and simulated gastric fluid. The transfer of meat stored under vacuum at 12°C to aerobic storage, however, resulted in reduction in the pathogen during storage and sensitization to the effects of sequential heat and acid exposure and since this sensitization did not occur at 4°C it appeared to be storage temperature-dependent.

Much like the fourth study (Chapter VI), the fifth study (Chapter VII) also evaluated the survival of a pathogen, in this case *L. monocytogenes*, *in-situ*, on RTE pork frankfurters exposed to a simulated gastric fluid. The effect of a simulated gastric fluid (adjusted to pH 1.0 with HCl) on *L. monocytogenes*, inoculated postprocessing on pork frankfurters formulated with sodium lactate (SL) and sodium diacetate (SD) and not dipped (ND) or dipped in solutions of lactic acid or acetic acid, was evaluated during storage of the frankfurters at 10°C for 40 d. Pork frankfurters containing 1.8% SL, 0.25% SD, 1.8% SL+0.125% SD, or 1.8% SL+0.25% SD were inoculated with 10^2-10^3

CFU/cm^2 of a 10-strain preparation of *L. monocytogenes* and were not dipped or dipped for 2 min in solutions of 2.5% lactic or acetic acid before they were vacuum-packaged and stored. Survival of *L. monocytogenes* was determined after exposure of frankfurters for 0, 20, 40 and 60 min to the simulated gastric fluid after storage for 0, 10, 20, 30 or 40 days. Growth of *L. monocytogenes* on frankfurters formulated with antimicrobials was inhibited in the order control < SL < 0.25%SD < SL+0.125%SD < SL+0.25% SD. Survival of *L. monocytogenes* on frankfurters exposed to simulated gastric fluid was higher on those frankfurters allowing more growth. Antimicrobials in the formulation of not dipped frankfurters did not appear to increase resistance of *L. monocytogenes* survivors to simulated gastric fluid; however, dipping frankfurters formulated with 0.25% SD in 2.5% lactic acid appeared to increase resistance of *L. monocytogenes* to simulated gastric fluid following growth during prolonged storage. Use of 2.5% acetic acid dipping combined with antimicrobials in the formulation inhibited growth of *L. monocytogenes* on frankfurters and sensitized the pathogen to subsequent simulated gastric fluid exposure. *L. monocytogenes* inoculated on freshly processed frankfurters may undergo rapid declines when exposed to simulated gastric fluid, but after prolonged storage an acid-resistant sub-population may arise when the total population exceeds 4 log CFU/cm^2.

REFERENCES

Abee, T. and J.A. Wouters. 1999. Microbial stress response in minimal processing. Int. J. Food Microbiol. 50:5-91.

Aberle, E.D., J.C. Forrest, D.E. Gerrard, and E.W. Mills. 2001. Storage and preservation of meat. pp. 178-226. In: Principles of meat science, 4th Edition, E.D. Aberle, J.C. Forrest, D.E. Gerrard, and E.W. Mills (Eds.). Kendall/Hunt Publishing Co., Dubuque, IA.

Acheson, D. and G. Keusch. 1995. *Shigella* and enteroinvasive *Escherichia coli*. pp. 763-784. In: Infections of gastrointestinal tract, M.J. Blaser, P.D. Smith, J.I. Ravdin, H.B. Greenberg, and R.L. Guerrant (Eds.). Raven Press, New York, NY.

Achtman, M., A. Mercer, B. Kusecek, A. Pohl, M. Heuzenroeder, W. Aaronson, A. Sutton, and R.P. Silver. 1983. Six widespread bacterial clones among *Escherichia coli* K1 isolates. Infect. Immun. 39:315-335.

Allen, D.M., M.C. Hunt, A. Luchiari Filho, R.J. Danler, and S.J. Goll. 1987. Effects of spray chilling and carcass spacing on beef carcass cooler shrink and grade factors. J. Anim. Sci. 64:165-170.

Altschul, S.F., W. Gish, W. Miller, E.W. Myers, and D.J. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403-410.

Altuvia, S., M. Almirón, G. Huisman, R. Kolter, and G. Storz. 1994. The *dps* promoter is activated by OxyR during growth and by IHF and σ^S in stationary phase. Mol. Microbiol. 13:265-272.

American Meat Institute (AMI). 1996. Putting the food handling issue on the table: the pressing need for food safety education. American Meat Institute, Washington, DC.

AMIF (American Meat Institute Foundation). 2000a. AMIF study shows pathogen interventions in beef plants are effective against *E. coli* O157:H7. American Meat Institute Foundation, Washington, DC. AMIF News. 2:1-6.

AMIF (American Meat Institute Foundation). 2000b. New technologies in meat processing and packing. American Meat Institute Foundation, Washington, DC.

Angelidis, A.S. and G.M. Smith. 2003. Role of glycine, betaine and carnitine transporters in adaptation of *Listeria monocytogenes* to chill stress in defined medium. Appl. Environ. Microbiol. 69:7492-7498.

Arnold, K.W. and C.W. Kaspar. 1995. Starvation- and stationary-phase-induced acid tolerance in *Escherichia coli* O157:H7. Appl. Environ. Microbiol. 61:2037-2039.

Avery, S.M. and S. Buncic. 1997. Differences in pathogenicity for chick embryos and growth kinetics at 37°C between clinical and meat isolates of *Listeria monocytogenes* previously stored at 4°C. Int. J. Food Microbiol. 34:319-327.

Azadian, B.S., G.T. Finnerty, and A.D. Pearson. 1989. Cheese-borne *Listeria* meningitis in immunocompetent patient. Lancet 333:322-323.

Bacon, R.T. and J.N. Sofos. 2003. Characteristics of biological hazards in foods. pp. 155-193. In: R.H. Schmidt and G.E. Rodrick. (Eds.), Food Safety Handbook. John Wiley and Sons, New York, NY.

Bacon, R.T., K.E. Belk, J.N. Sofos, and G.C. Smith. 2000a. Incidence of *Escherichia coli* O157:H7 on hide, carcass and beef trimmings samples collected from United States packing plants. Executive Summary to AMIF.

Bacon, R.T., K.E. Belk, J.N. Sofos, R.P. Clayton, J.O. Reagan, and G.C Smith. 2000b. Microbial populations on animal hides and beef carcasses at different stages of slaughter in plants employing multiple-sequential interventions for decontamination. J. Food Prot. 63:1080-1086.

Bae, W., P.G. Jones, and M. Inouye. 1997. CspA, the major cold-shock protein of *Escherichia coli*, negatively regulates its own gene expression. J. Bacteriol. 179:7081-7088.

Bailey, C. 1986. Current issues affecting meat chilling and distribution. pp. 18-20. In: Recent advances and developments in the refrigeration of meat by chilling. Int. Inst. Refrig. Paris.

Bailey, C. and R.P. Cox. 1976. The chilling of beef carcasses. Proc. Inst. Refrig. 72:76-90.

Barkate, M.L., G.R. Acuff, L. Lucia, and D.S. Hale. 1993. Hot water decontamination of beef carcasses for reduction of initial bacteria numbers. Meat Sci. 35:397-401.

Barkocy-Gallagher, G.A., D.H. Kang and M. Koohmaraie. 2002. Fate of field-isolated *Escherichia coli* O157 in ground beef at different storage temperatures. J. Food Prot. 65:1106-1109.

Barkocy-Gallagher, G.A., T.M. Arthur, M. Rivera-Betancourt, X. Nou, S.D. Shackelford, T.L. Wheeler, and M. Koohmaraie. 2003. Seasonal prevalence of shiga toxin-producing *Escherichia coli*, including O157:H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. J. Food Prot. 66:1978-1986.

Beachey, E.H. 1981. Bacterial adherence: adhesion-receptor interactions mediating the attachment of bacteria to mucosal surfaces. J. Infect. Dis. 143:325-345.

Beales, N. 2004. Adaptation of microorganisms to cold temperatures, weak acid preservatives, low pH, and osmotic stress: a review. Comp. Rev. Food Sci. Food Safety 3:1-20.

Bearson, S., B.L. Bearson, and J.W. Foster. 1997. Acid stress in enterobacteria. FEMS Microbiol. Lett. 147:173-180.

Beaudry, M., C. Zhu, J.M. Fairbrother, and J. Harel. 1996. Genotypic and phenotypic characterization of *Escherichia coli* isolates from dogs manifesting attaching and effacing lesions. J. Clin. Microbiol. 34:144-148.

Bedie, G.K., J. Samelis, J.N. Sofos, K.E. Belk, J.A. Scanga, and G.C. Smith. 2001. Antimicrobials in the formulation to control *Listeria monocytogenes* postprocessing contamination on frankfurters stored at 4°C in vacuum packages. J. Food Prot. 64:1949-1955.

Bell, R.G. 1997. Distribution and sources of microbial contamination on beef carcasses. J. Appl. Microbiol. 82:292-300.

Bell, B.P., M. Goldcroft, P.M. Griffin, M.A. Davis, D.C. Gordon, P.I. Tarr, C.A. Bartleson, J.H. Lewis, T.J. Barrett, J.G. Wells, R. Baron, and J. Kobayaski. 1994. A multistate outbreak of *Escherichia coli* O157:H7 – Associated bloody diarrhea and hemolytic uremic syndrome from hamburgers. The Washington Experience. JAMA 272:1349-1353.

Bendall, J.R. 1974. The snags and snares of freezing rapidly after slaughter. In: Meat freezing: Why and how?, C.L. Cutting (Ed.). Meat research symposium, No.3, 7.1-7.8.

Benito, Y., C. Pin, M.L. Marin, M.L. Garcia, M.D. Selgas, and C. Casas. 1997. Cell surface hydrophobicity and attachment of pathogenic and spoilage bacteria to meat surfaces. Meat Sci. 45:419-425.

Benjamin, M.M. and A.R. Datta. 1995. Acid tolerance of enterohemorrhagic *Escherichia coli.* Appl. Environ. Microbiol. 61:1669-1672.

Bernard, D.T. and V.N. Scott. 1999. *Listeria monocytogenes* in meats: new strategies are needed. Food Technol. 53:124.

Berry, E.D. and C.N. Cutter. 2000. Effects of acid adaptation of *Escherichia coli* O157:H7 on efficacy of acetic acid spray washes to decontaminate beef carcass tissue. Appl. Environ. Microbiol. 66:1493-1498.

Berry, E.D. and P.M. Foegeding. 1997. Cold temperature adaptation and growth of microorganisms. J. Food. Prot. 60:1583-1594.

Berry, E.D., M.B. Liewen, R.W. Mandigo, and R.W. Hutkins. 1990. Inhibition of *Listeria monocytogenes* by bacteriocin-producing *Pediococcus* during the manufacture of fermented semidry sausage. J. Food Prot. 53:194-197.

Berry, E.D., R.W. Hutkins, and R.W. Mandigo. 1991. The use of bacteriocin-producing *Pediococcus acidilactici* to control postprocessing *Listeria monocytogenes* contamination of frankfurters. J. Food Prot. 54:681-686.

Bettelheim, K.A. 2000. Role of non-O157 VTEC. J. Appl. Microbiol. 88:38S-50S.

Beumer, R.R., J. De Vries, and F.M. Rombouts, 1992. *Campylobacter jejuni* non-culturable coccoid cells. Int. J. Food Microbiol. 15:153-163.

Biss, M.E. and S.C. Hathaway. 1996. Micorbiological contamination of ovine carcasses associated with the presence of wool and fecal material. J. Appl. Bacteriol. 81:594-596.

Bjerklie, S. 2001. Meeting pathogens on their own terms. Meat Process. (February) 28-33.

Blanco, J., M. Blanco, J.E. Blanco, A. Alonso, E. Gonzalez, and M.I. Fernandez. 2001. Epidemiology of verocytotoxigenic *Escherichia coli* (VTEC) in ruminants. pp. 120. In: Verotoxigenic *E. coli*, G. Duffy, P. Garvey, D.A. McDowell (Eds.). Food Science and Nutrition Press, Trumbull, CT.

Blom H., E. Nerbrink, R. Dainty, T. Hagtvedt, E. Borch, H. Nissen, and T. Nesbakken. 1997. Addition of 2.5% lactate and 0.25% acetate controls growth of *Listeria monocytogenes* in vacuum-packed, sensory-acceptable servelat sausage and cooked ham stored at 4°C. Int. J. Food Microbiol. 38:71-76.

Blum, P., M. Velligan, N. Lin, and A. Matin. 1992. Disaggregation and solubilization of human growth hormone inclusion bodies by DnaK. Biotech. 10:301-304.

Bolton, F.J., L. Crozier, and J.K. Williamson. 1996. Isolation of *Escherichia coli* O157 from raw meat products. Lett. Appl. Microbiol. 23:317-321.

Booth, I. R. 1999. The regulation of intracellular pH in bacteria. pp. 19-27. In: Bacterial response to pH, J.C. Derek and G. Gardener (Eds.). John Wiley and Sons, Ltd., Chichester, England.

Bower, C.K. and M.A. Daeschel. 1999. Resistance responses of microorganisms in food environments. Int. J. Food. Microbiol. 50:33-44.

Bowling, R. and R.P. Clayton. 1992. Method for dehairing animals. U.S. Patent 5,149,295.

Brandi, A., P. Pietroni, C.O. Gualerzi, and C.L. Pon. 1996. Post-transcriptional regulation of CspA expression in *Escherichia coli*. Mol. Microbiol. 19:231-240.

Breen, P.J., C.M. Compadre, E.K. Fifer, H. Salari, C. Serbus, and D.L. Lattin. 1995. Quaternary ammonium compounds inhibit and reduce the attachment of viable *Salmonella* Typhimurium to poultry tissues. J. Food Sci. 60:1191-1196.

Brown, W.L. 1991. Designing *Listeria monocytogenes* thermal inactivation studies for extended shelf life refrigerated foods. Food Tech. 45:134-138.

Brown, T., K.N. Chourouzidis, and A.J. Gigiel. 1993. Spray chilling of lamb carcasses. Meat Sci. 34:311-325.

Brudzinski, L. and M.A. Harrison. 1998. Influence of incubation conditions on survival and acid tolerance response of *Escherichia coli* O157:H7 and non-O157:H7 isolates exposed to acetic acid. J. Food Prot. 61:542-546.

Brul, S. and P. Coote. 1999. Preservative agents in foods: mode of action and microbial resistance mechanisms. Int. J. Food Microbiol. 50:1-17.

Buchanan, R. L. and S. G. Edelson. 1996. Culturing enterohemorrhagic *Escherichia coli* in the presence and absence of glucose as a simple means of evaluating the acid tolerance of stationary-phase cells. Appl. Environ. Microbiol. 62:4009-4013.

Buchanan, R.L. and S.G. Edelson. 1999. Effect of pH-dependent, stationary phase acid resistance on the thermal tolerance of *Escherichia coli* O157:H7. Food Microbiol. 16:447-458.

Buchanan, R.L., S.G. Edelson, and G. Boyd. 1999. Effects of pH and acid resistance on the radiation resistance of enterohemorrhagic *Escherichia coli*. J. Food Prot. 62:219-228.

Bukau, B. 1993. Regulation of the *Escherichia coli* heat-shock response. Mol. Microbiol. 9:671-680.

Buncic, S., C.M. Fitzgerald, R.G. Bell, and J.A. Hudson. 1995. Individual and combined listericidal effects of sodium lactate, potassium sorbate, nisin, and curing salts at refrigeration temperature. J. Food Safety 15:247-264.

Butler, J.L., J.C. Stewart, C. Vanderzant, Z.L. Carpenter, and G.C. Smith. 1979. Attachment of microorganisms to pork skin and surfaces of beef and lamb carcasses. J. Food Prot. 42:401-406.

Buzby, J.C., T. Roberts, C.T.J. Lin, and J. McDonald. 1996. Bacterial foodborne disease: Medical costs and productivity losses. Agricultutal Economics Report No. 741. Economic Research Service. U.S. Department of Agriculture. Available at: http://www.ers.usda.gov/publications/AER741/.

Byrne, C.M., D.J. Bolton, J.J. Sheridan, D.A. McDowell, and I.S. Blair. 2000. The effects of preslaughter washing on the reduction of *Escherichia coli* O157:H7 transfer from cattle hides to carcasses during slaughter. Lett. Appl. Microbiol. 30:142-145.

Cabedo, L., J.N. Sofos, and G.C. Smith. 1996. Removal of bacteria from beef tissue by spray washing after different times of exposure to fecal material. J. Food Prot. 59:1284-1287.

Callaway, T.R., R.O. Elder, J.E. Keen, R.C. Anderson, and D.J. Nisbet. 2003. Forage feeding to reduce preharvest *Escherichia coli* populations in cattle, a review. J. Dairy Sci. 86:852-860.

Campanini, M., I. Pedrazzoni, S. Barbuti, and P. Baldini. 1993. Behaviour of *Listeria monocytogenes* during the maturation of naturally and artificially contaminated salami: effect of lactic-acid bacteria starter cultures. Int. J. Food Microbiol. 20:169-175.

Cassens, R.G. 1994. Meat preservation: preventing losses and assuring safety. Food and Nutrition Press, Inc., Trumbull, CN.

Castillo, A., L.M. Lucia, K.J. Goodson, J.W. Savell, and G.R. Acuff. 1998. Comparison of water wash, trimming, and combined hot water and lactic acid treatments for reducing bacteria of fecal origin on beef carcasses. J. Food Prot. 61:823-828.

Castillo, A., L.M. Lucia, G.K. Kemp, and G.R. Acuff. 1999. Reduction *of Escherichia coli* O157:H7 and *Salmonella* Typhimurium on beef carcass surfaces using acidified sodium chlorite. J. Food Prot. 62:580-584.

Castillo, A., L.M. Lucia, I. Mercado, and G.R. Acuff. 2001a. In-plant evaluation of a lactic acid treatment for the reduction of bacteria on chilled beef carcasses. J. Food Prot. 64 :738-740.

Castillo, A., L.M. Lucia, D.B. Roberson, T.H. Stevenson, I. Mercado, and G.R. Acuff. 2001b. Lactic acid sprays reduce bacterial pathogens on cold beef carcass surfaces and in subsequently produced ground beef. J. Food Prot. 64:58-62.

Catani, C.F., A. Yamada, M.C. Vidotto, and T. Yano. 1996. Adhesion of bovine enterotoxigenic *Escherichia coli* (ETEC) by type 1-like fimbriae. FEMS Microbiol. Lett. 137:241-245.

CDC (Centers for Disease Control and Prevention). 1998. Multistate outbreak of listeriosis, United States, 1998. MMWR 47:1085-1086.

CDC (Centers for Disease Control and Prevention). 1999. Update: multistate outbreak of listeriosis, United States, 1998-1999. MMWR 47:1117-1118.

CDC (Centers for Disease Control and Prevention). 2000a. Multistate outbreak of listeriosis, United States, 2000. MMWR 49:1129-1130.

CDC (Centers for Disease Control and Prevention). 2000b. Surveillance for foodborne disease outbreaks – United States, 1993-1997. MMWR 49:1-51.

CDC (Centers for Disease Control and Prevention). 2001. Preliminary FoodNet data on the incidence of foodborne illness – selected sites, United States 2000. MMWR 50:241-246.

CDC (Centers for Disease Control and Prevention). 2002. Public health dispatch: Outbreak of listeriosis –Northeastern United States, 2002. MMWR 51:950-951.

CDC (Centers for Disease Control and Prevention). 2003. Preliminary FoodNet data on the incidence of foodborne illnesses - selected sites, United States, 2002. MMWR 52:340-343.

Chang, E.B., M. Sitrin, and D.D. Black. 1996. Gastrointestinal, hepatobiliary and nutritional physiology. Lippincott-Raven, Philadelphia, PA.

Chen, Y., W.H. Ross, V.N. Scott, and D.E. Gombas. 2003. *Listeria monocytogenes*: low levels equal low risk. J. Food Prot. 66:570-577.

Cheng, H.-Y., H.-Y. Yang, and C.-C. Chou. 2002. Influence of acid adaptation on the tolerance of *Escherichia coli* O157:H7 to some subsequent stresses. J. Food Prot. 65:260-265.

Cheville, A.M., K.W. Arnold, C. Buchrieser, C.-M. Cheng, and C.W. Kaspar. 1996. *rpoS* regulation of acid, heat and salt tolerance in *Escherichia coli* O157:H7. Appl. Environ. Microbiol. 62:1822-1824.

China, B., V. Pirson and, J. Mainil. 1996. Typing of bovine attaching and effacing *Escherichia coli* by multiplex in vitro amplification of virulence-associated genes. Appl. Environ. Microbiol. 62:3462-3465.

Choi, S.H. and K.B. Chin. 2003. Evaluation of sodium lactate as a replacement for conventional chemical preservatives in comminuted sausages inoculated with *Listeria monocytogenes*. Meat Sci. 65:531-537.

Christie, R., N.E. Atkins, and E. Munch-Petersen. 1944. A note on a lytic phenomenon shown by group B streptococci. Aust. J. Exp. Biol. Med. Sci. 22:197-200.

Christman, M.F., R.W. Morgan, F.S. Jacobson, and B.N. Ames. 1985. Positive control of a regulon for defenses against oxidative stress and some heat-shock proteins in *Salmonella typhimurium*. Cell 41:753-762.

Chung, K.J., J.S. Dickson, and J.D. Crouse. 1989. Attachment and proliferation of bacteria on meat. J. Food Prot. 52:173-177.

Clavero, M.R., L.R. Beuchat, and M.P. Doyle. 1998. Thermal inactivation of *Escherichia coli* O157:H7 isolated from ground beef and bovine feces, and suitabilityof media for enumeration. J. Food Prot. 61:285-289.

Cobeljic, M., B. Milijkovic-Selimovic, D. Paunovic-Todosijevic, Z. Velickovic, Z. Lepsanovic, N. Zec, D. Savic, R. Illic, S. Kostantinovic, B. Jovanovic and V. Kostic. 1996. Enteroaggregative *Escherichia coli* associated with an outbreak of diarrhea in a neonatal nursery ward. Epidemiol. Infect. 117:11-16.

Collins, J.E. 1997. Impact of changing consumer lifestyles on the emergence/reemergence of foodborne pathogens. Emerg. Infect. Dis. 3:471-479.

Conlan, J.W. 1997. Neutrophils and tumor necrosis factor-alpha are important for controlling early gastrointestinal stages of experimental murine listeriosis. J.Med.. Microbiol. 46:239-250.

Conner, D.E. and J.S. Kotrola. 1995. Growth and survival of *Escherichia coli* O157:H7 under acidic conditions. Appl. Environ. Microbiol. 61:382-385.

Conner, D.E., J.S. Kotrola, W.B. Mikel, and K.C. Tamblyn. 1997. Effects of acetic and lactic acid treatments applied to beef trim on populations of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in ground beef. J. Food Prot. 60:1560-1563.

Cooksey, K., B.P. Klein, F.K. McKeith, and H.P. Blaschek. 1993a. Post-packaging pasteurization reduces *Clostridium perfringens* and other bacteria in precooked vacuum-packaged beef loin chunks. J. Food Sci. 58:239-241.

Cooksey, K., B.P. Klein, F.K. McKeith, and H.P. Blaschek. 1993b. Reduction of *Listeria monocytogenes* in precooked vacuum-packaged beef using postpackaging pasteurization. J. Food Prot. 56:1034-1038.

Costerton, J.W., Z. Lewandowski, D. Debeer, D. Caldwell, D. Korber, and G. James. 1994. Biofilms, the customized microniche. J. Bacteriol. 176:2137-2142.

Costerton, J.W., Z. Lewandowski, D.E. Caldwell, D.R. Korber, and H.M. Lappin-Scott. 1995. "Microbial biofilms". Ann. Rev. Microbiol. 49:711-745.

Cotter, P.D., N. Emerson, C.G.M. Gahan, and C. Hill. 1999. Identification and disruption of *lisRK*, a genetic locus encoding a two-component signal transduction system involved in stress tolerance and virulence in *Listeria monocytogenes*. J. Bacteriol. 181:6840-6843.

Cronan, J.E., Jr. and C.O. Rock. 1987. Biosynthesis of membrane lipids. pp. 474-497. In: *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology (Vol. I). F.C. Neidhardt, J.L. Ingraham, K.B. Low, B. Magasanik, M. Schaechter, and H.E. Umbarger (Eds.). American Society for Microbiology, Washington, D.C.

Csonka, L.N. 1989. Physiological and genetic responses of bacteria to osmotic stress. Microbiol. Rev. 53:121-147.

Cutter, C.N., W.J. Dorsa, and G.R. Siragusa. 1997. Parameters affecting the efficacy of spray washes against *Escherichia coli* O157:H7 and fecal contamination of beef. J. Food Prot. 60:614-618.

Cutter, C.N., W.J. Dorsa, A. Handie, S. Rodriguez-Morales, X. Zhou, P.J. Breen, and C.M. Compadre. 2000. Antimicrobial activity of cetylpyridinium chloride washes against pathogenic bacteria on beef surfaces. J. Food Prot. 63:593-600.

Cygnarowicz-Provost, M., R.C. Whiting, and J.C. Craig, Jr. 1994. Steam surface pasteurisation of beef frankfurters. J. Food Sci. 59:1-5.

Czuprynski, C.J., N.G. Faith, and H. Steinberg. 2002. Ability of the *Listeria monocytogenes* strain Scott A to cause systemic infection in mice infected by the intragastric route. Appl. Environ. Microbiol. 68:2893-2900.

Datta, A.R. and M.M. Benjamin. 1999. Cell density dependent acid sensitivity in stationary phase cultures of enterohemorrhagic *Escherichia coli* O157:H7. FEMS Microbiol. Lett. 181: 289-295.

Daudin, J.D. and R.J.M. van Gerwen. 1996. Methods to assess chilling kinetics in industrial chillers. pp. 7-15. In: New developments in meat refrigeration, J.D. Daudin (Ed.). Utrecht: EC/CE/AMST-I11.

Davidson, P.M. 2001. Chemical preservatives and natural antimicrobial compounds. pp. 593-627. In: Food microbiology: fundamentals and frontiers, 2nd Edition, M.P. Doyle, L.R. Beuchat, and T.J. Montville. ASM Press, Washington, D.C.

Davidson, P.M. and M.A. Harrison. 2002. Resistance and adaptation to food antimicrobials, sanitizers, and other process controls. Food Technol. 56:69-78.

Delmore, R.J., Jr., J.N. Sofos, G.R. Schmidt, K.E. Belk, W.R. Lloyd, and G.C. Smith. 2000. Interventions to reduce microbiological contamination of beef variety meats. J. Food Prot. 63:44-50.

De Mendoza, D. and J.E. Cronan, Jr. 1983. Thermal regulation of membrane lipid fluidity in bacteria. Trends Biochem. Sci. 8:49-52.

Deumier, F. and A. Collignan. 2003. The effects of sodium lactate and starter cultures on pH, lactic acid bacteria, *Listeria monocytogenes* and *Salmonella* spp. levels in pure chicken dry fermented sausage. Meat Sci. 65:1165-1174.

Deza, M.A., M. Araujo, and M.J. Garrido. 2003. Inactivation of *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Listeria monocytogenes* on the surface of tomatoes by neutral electrolyzed water. Lett. Appl. Microbiol. 37:482-487.

Dickson, J.S. 1991. Control of *Salmonella typhimurium*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 on beef in a model spray chilling system. J. Food Sci. 56:191-193.

Dickson, J.S. and M.E. Anderson. 1992. Microbiological decontamination of food animal carcasses by washing and sanitizing systems: a review. J. Food Prot. 55:133-140.

Dickson, J.S. and M. Koohmaraie. 1989. Cell surface charge characteristics and their relationship to bacterial attachment to meat surfaces. Appl. Environ. Microbiol. 55:832-836.

Dickson, J.S., M.H. Hardin, and G.R. Acuff. 1997. Organic acid rinses. Proc. Recip. Meat Conf. 49:129-131.

Diez-Gonzalez, F. and J.B. Russell. 1997. The ability of *Escherichia coli* O157:H7 to decrease its intracellular pH and resist the toxicity of acetic acid. Microbiol. 143:1175-1180.

Diez-Gonzalez, F. and J.B. Russell. 1999. Factors affecting the extreme acid resistance of *Escherichia coli* O157:H7. Food Microbiol. 16:367-374.

Diez-Gonzalez, F., T.R. Callaway, M.G. Kizoulis, and J.B. Russell. 1998. Grain feeding and the dissemination of acid-resistant *Escherichia coli* from cattle. Sci. 281:1666-1668.

Dorman, C.J. 1996. Flexible response: DNA supercoiling, transcription and bacterial adaptation to environmental stress. Trends Microbiol. 4:214-216.

Dorsa, W.J., C.N. Cutter, and G.R. Siragusa. 1997. Effects of steam vacuuming and hot water spray wash on the microflora of refrigerated beef carcass surface tissue inoculated with *Escherichia coli* O157:H7, *Listeria innocua* and *Clostridium sporogenes*. J. Food Prot. 60:114-119.

Dorsa, W.J., C.N. Cutter, and G.R. Siragusa. 1998a. Long-term effect of alkaline, organic acid, or hot water washes on the microbial profile of refrigerated beef contaminated with bacterial pathogens after washing. J. Food Prot. 61:300-306.

Dorsa, W.J., C.N. Cutter, and G.R. Siragusa. 1998b. Long-term bacterial profile of refrigerated ground beef made from carcass tissue, experimentally contaminated with

pathogens and spoilage bacteria after hot water, alkaline, or organic acid washes. J. Food Prot. 61:1615-1622.

Doyle, M.P. 1991. A new generation of foodborne pathogens. Contemp. Nutr. 16:1-2.

Doyle, M.P. and V.V. Padhye. 1989. *Escherichia coli*. pp. 236-282. In: Foodborne bacterial pathogens. M.P. Doyle (Ed.). Marcel Decker, New York, NY.

Doyle, M.P. and N.V Padhye. 1992. *Escherichia coli* O157:H7: epidemiology, pathogenesis, and methods for detection in food. J. Food Prot. 55:555-565.

Doyle, M.P., T. Zhao, J. Meng, and S. Zhao. 1997. *Escherichia coli* O157:H7. pp. 171-191. In: Food Microbiology – fundamentals and frontiers, M. Doyle, L. Beuchat, and T. Montville (Eds.). ASM Press, Washington, DC.

Drovers Journal. 2000. Consumers prioritize food safety. January:18.

Drumm, B.M., B.M. McKenna, and R.L. Joseph. 1992. Line chilling of beef 2: the effect on carcass temperature, weight loss and roughness. J. Food Eng. 15:285-312.

Duffy, G., D.C.R. Riordan, J.J. Sheridan, J.E. Call, R.C. Whiting, I.S. Blair, and D.A. McDowell. 2000. Effect of pH on survival, thermotolerance, and verotoxin production of *Escherichia coli* O157:H7 during simulated fermentation and storage. J. Food Prot. 63:12-18.

Dykes, G.A. and S.M. Moorhead. 2000. Survival of osmotic and acid stress by *Listeria monocytogenes* strains of clinical or meat origin. Int. J. Food Microbiol. 56:161-166.

Ebel, F., C. Deibel, A. Kresse, C.A. Guzman, and T. Chakraborty. 1996. Temeprature and medium-dependent secretion of proteins by Shiga toxin-producing *Escherichia coli*. Infect. Immun. 64:4472-4479.

Elder, R.O., J.E. Keen, G.R. Siragusa, G.A. Barkocy-Gallagher, M. Koohmarie, and W.W. Laegreid. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevelance in feces, hides, and carcasses of beef cattle during processing. Proc. Natl. Acad. Sci. 97:2999-3003.

El-Kest, S. and E. Marth. 1988. Inactivation of *Listeria monocytogenes* by chlorine. J. Food Prot. 51: 520-524.

Ephros, M.D. Cohen, M. Yavzori, N. Rotman, B. Novics, and S. Ashkenazi. 1996. Encephalopathy associated with Enteroinvasive *Escherichia coli* O144:NM infection. J. Clin. Microbiol. 34:2432-2434.

Etchegaray, J.P., P.G. Jones, and M. Inouye. 1996. Differential thermoregulation of two highly homologous cold-shock genes, *cspA* and *cspB*, of *Escherichia coli*. Genes Cells 1:171-178.

Fabrizio, K.A., R.R. Sharma, A. Demirci, and C.N. Cutter. 2002. Comparison of electrolyzed oxidizing water with various antimicrobial interventions to reduce *Salmonella* species on poultry. Poult. Sci. 81:1598-1605.

Fabrizio, K.A. and C.N. Cutter. 2003. Stability of electrolyzed oxidizing water and its efficacy against cell suspensions of *Salmonella* Typhimurium and *Listeria monocytogenes*. J. Food Prot. 66:1379-1384.

Fang, L., W. Jiang, W. Bae, and M. Inouye. 1997. Promoter-independent cold-shock induction of *cspA* and its depression at 37°C by mRNA stabilization. Mol. Microbiol. 23:355-364.

Farber, J.M. and F. Pagotto. 1992. The effect of acid shock on the heat resistance of *Listeria monocytogenes*. Lett. Appl. Microbiol. 15:197-201.

Farber, J.M. and P.I. Peterkin. 1991. *Listeria monocytogenes*, a food-borne pathogen. Microbiol. Rev. 55:476-511.

Farber, J.M. and P.I. Peterkin. 1999. Incidence and behavior of *Listeria monocytogenes* in meat products. pp. 505-564. In: *Listeria*, listeriosis and food safety, E.T. Ryser and E.H. Marth (Eds.). Marcel Dekker Inc., New York, NY.

Farrell, B.L., A.B. Ronner, and A.C.L. Wong. 1998. Attachment of *Escherichia coli* O157:H7 in ground beef to meat grinders and survival after sanitation with chlorine and peroxyacetic acid. J. Food Prot. 61:817-822.

FDA/FSIS (Food and Drug Administration/Food Safety and Inspection Service). 2001. Draft relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods. FDA Docket No.99N-1168 and FSIS Docket No. 00-048N. Available at: http://www.foodsafety.gov/~dms/lmrisk.html.

Ferguson, N., R. Anderson, and S. Gupta. 1999. The effect of antibody-dependent enhancement on the transmission dynamics and persistence of multi-strain pathogens. Proc. Natl. Acad. Sci. 96:790-794.

Fischer, P.L., P.J. Jooste, and J.C. Novello. 1986. Evaluation of rapid plate counting techniques for the enumeration of psychrotrophic bacteria in raw milk. South African J. Dairy Sci. 18:137-141.

FMI (Food Marketing Institute). 2000. Trends in the United States – Consumer attitudes and the supermarket 2000. Food Marketing Institute, Washington, DC.

Foegeding, P.M., A.B. Thomas, D.H. Pilkington, and T.R. Klaenhammer. 1992. Enhanced control of *Listeria monocytogenes* by *in situ*-produced pediocin during dry fermented sausage production. Appl. Environ. Microbiol. 58:884-890.

Foster, J.W. 1995. Microbial responses to acid stress. pp. 99-115. In: Bacterial stress responses, G. Storz and R. Hengge-Aronis (Eds.). ASM Press, Washington, DC.

Foster, J.W. 1999. When protons attack: microbial strategies of acid adaptation. Curr. Opin. Microbiol. 2:170-174.

Frank, J. F. and R. A. Koffi. 1990. Surface-adherent growth of *Listeria monocytogenes* is associated with increased resistance to surfactant sanitizers and heat. J. Food Prot. 53:550-554.

Gadzella, T.A. and S.C. Ingham. 1994. Heat shock, anaerobic jar incubation and fluid thioglycollate medium have contrasting effects on D-values of *Escherichia coli*. J. Food Prot. 57:671-673.

Gande, N. and P. Muriana. 2003. Prepackage surface pasteurization of ready-to-eat meats with a radiant heat oven for reduction of *Listeria monocytogenes*. J. Food Prot. 66:1623-1630.

Gannon, V.P.J. 1999. Control of *Escherichia coli* O157:H7 at slaughter. In: *Escherichia coli* O157 in farm animals, C.S. Stewart and H. J. Flint (Eds.). CAB International, Wallingford, UK.

Garren, D.M., M.A. Harrison, and S.M. Russell. 1997. Retention of acid tolerance and acid shock responses of *Escherichia coli* O157:H7 and non-O157:H7 isolates. J. Food Prot. 60:1478-1482.

Garren, D.M., M.A. Harrison, and S.M. Russell. 1998. Acid tolerance and acid shock response of *Escherichia coli* O157:H7 and non-O157:H7 isolates provide cross protection to sodium lactate and sodium chloride. J. Food Prot. 61:158-161.

García-Martínez, J., A.J. Martínez-Murcia, F. Rodríguez-Valera, and A. Zorraquino. 1996. Molecular evidence supporting the existence of two major groups in uropathogenic *Escherichia coli*. FEMS Immunol. Med. Microbiol. 14:231-244.

Gardner, J.D., A.A. Ciociola, and M. Robinson. 2002. Measurement of meal-stimulated gastric acid secretion by in vivo gastric autotitration. J. Appl. Physiol. 92:427-434.

Gellin, B.G. and C.V. Broome. 1989. Listeriosis. J Amer. Medic. Assoc. 261:1313-1320.

George, S.M. and M.W. Peck. 1998. Redox potential affects the measured heat resistance of *Escherichia coli* O157:H7 independently of oxygen concentration. Lett. Appl. Microbiol. 27:313-317.

George, S.M., L.C.C. Richardson, I.E. Pol, and M.W. Peck. 1998. Effect of oxygen concentration and redox potential on the recovery of sublethally heat-damaged cells of *Escherichia coli* O157:H7, *Salmonella* Enteritidis and *Listeria monocytogenes*. J. Appl. Microbiol. 84:903-909.

Germani, Y., E. Begaud, P. Duval and C. Le Bouguenec. 1996. Prevalence of enteropathogenic, enteroaggregative, and diffusely adherent *Escherichia coli* among isolates from children with diarrhea in New Caledonia. Dis. 174:1124-1126.

Giammanco, A., M. Maggio, G. Giammanco, R. Morelli, F. Minelli, F. Scheutz, and A. Caprioli. 1996. Characteristics of *Escherichia coli* strains belonging to enteropathogenic *E. coli* serogroups isolated in Italy from children with diarrhea. J. Clin. Microbiol. 34:689-694.

Gibson, A.M., N. Bratchell, and T.A. Roberts. 1988. Predicting microbial growth: growth responses of salmonellae in a laboratory medium as affected by pH, sodium chloride and storage temperature. Int. J. Food Microbiol. 6:155-178.

Gigiel, A.J., P. Collett, and S.J. James. 1989a. Fast and slow beef chilling in a commercial chiller and the effects of operational factors on weight loss. Int. J. Refrig. 12:338-349.

Gigiel, A.J., F. Butler, and W.R. Hudson. 1989b. Alternative methods for pig chilling. Meat Sci. 26:67-83.

Gill, C.O. 1998. Microbial contamination during slaughter. pp. 118-157. In: The microbiology of meat and poultry, A. Davies and R. Board (Eds.). Blackie Academic and Professional, London, UK.

Gill, C.O. 2000. HACCP in primary processing: red meat. pp. 81-122. In: HACCP in the meat industry, M.H. Brown (Ed.). Woodhead Publishing Limited, CRC Press LLC, Boca Raton, FL.

Gill, C.O. 2002. Primary chilling of red meat. pp. 99-136. In: Meat refrigeration, S.J. James and C. James (Ed.). Woodhead Publishing Limited, CRC Press LLC, Boca Raton, FL.

Gill, C.O. and M. Badoni. 2004. Effects of peroxyacetic acid, acidified sodium chlorite or lactic acid solutions on the microflora of chilled beef carcasses. Int. J. Food Microbiol. 91:43-50.

Gill, C.O. and J. Bryant. 1997. Assessment of the hygienic performances of two beef carcass cooling processes from product temperature history data or enumeration of bacteria on carcass surfaces. Food Microbiol. 14:593-602.

Gill, A.O. and R.A. Holley. 2000. Surface application of lysozyme, nisin, and EDTA to inhibit spoilage and pathogenic bacteria on ham and bologna. J. Food Prot. 63:1338-1346.

Gill, C.O. and T. Jones. 1997. Assessment of the hygienic performances of an air cooling process for lamb carcasses and a spray cooling process for pig carcasses. Int. J. Food Microbiol. 38:85-93.

Gill, C.O. and C. Landers. 2003. Effects of spray-cooling processes on the microbiological conditions of decontaminated beef carcasses. J. Food Prot. 66:1247-1252.

Gill, C.O. and N. Penney. 1977. Penetration of bacteria into meat. Appl. Environ. Microbiol. 33:1284-1286.

Gill, C.O., N.G. Leet, and N. Penney. 1984. Structural changes developing with rigor that facilitate bacterial invasion of muscle tissue. Meat Sci. 10:265-274.

Gill, C.O., J.C. McGinnis, and M. Badoni. 1996. Use of total of *Escherichia coli* counts to assess the hygienic characteristics of a beef carcass dressing process. Int. J. Food Microbiol. 31:181-196.

Gill, C.O., J.C. McGinnis, and J. Bryant. 1998. Microbial contamination of meat during the skinning of beef carcass hindquarters at three slaughtering plants. Int. J. Food Microbiol. 42:175-184.

Gill, A.O., P. Delaquis, P. Russo and R.A. Holley. 2002. Evaluation of antilisterial action of cilantro oil on vacuum packed ham. Int. J. Food Microbiol. 73:83-92.

Glass, K.A., J.M. Loeffelholz, J.P. Ford, and M.P. Doyle. 1992. Fate of *Escherichia coli* O157:H7 as affected by pH or sodium chloride and in fermented, dry sausage. Appl. Environ. Microbiol. 58:2513-2516.

Glass, K.A., D.A. Granberg, A.L. Smith, A.M. McNamara, M. Hardin, J. Mattias, K. Ladwig, and E.A Johnson. 2002. Inhibition of *Listeria monocytogenes* by sodium diacetate and sodium lactate on wieners and cooked bratwurst. J. Food Prot. 65:116-123.

Goddard, B.L., W.B. Mikel, D.E. Conner, and W.R. Jones. 1996. Use of organic acids to improve the chemical, physical, and microbiological attributes of beef strip loins stored at -1°C for 112 days. J. Food Prot. 59:849-853.

Goldstein, J., N.S. Pollitt, and M. Inouye. 1990. Major cold shock protein of *Escherichia coli*. Proc. Natl. Acad. Sci. USA 87:283-287.

Goldenberg, D., I. Azar, and A.B. Oppenheim. 1996. Differential mRNA stability of the *cspA* gene in the cold-shock response of *Escherichia coli*. Mol. Microbiol. 19:241-248.

Goldenberg, D., I. Azar, A.B. Oppenheim, A. Brandi, C.O. Gualerzi, and C.L. Pon. 1997. Role of *Escherichia coli cspA* promoter sequence and translational apparatus adaptation in the cold shock response. Mol. Gen. Genet. 256:282-290.

Gombas D.E., Y. Chen, R.S. Clavero, and V.N. Scott. 2003. Survey of *Listeria monocytogenes* in ready-to-eat foods. J. Food Prot. 66:559-569.

Goodson, M. and R.J. Rowbury. 1989. Resistance of acid-habituated *Escherichia coli* to organic acids and its medical and applied significance. Lett. Appl. Microbiol. 8:211-214.

Gorman, B.M., J.B. Morgan, J.N. Sofos, and G.C. Smith. 1995. Microbiological and visual effects of trimming and/or spray washing for removal of fecal material from beef. J. Food Prot. 58:984-989.

Gorman, B.M., S.L. Kochevar, J.N. Sofos, J.B. Morgan, G.R. Schmidt, and G.C. Smith. 1997. Changes on beef adipose tissue following decontamination with chemical solutions or water of 35 or 74°C. J. Muscle Foods 8:185-197.

Gottesman, S. 1996. Proteases and their targets in *Escherichia coli*. Annu. Rev. Genet. 30:465-506.

Goulet, V. and P. Marchetti. 1996. Listeriosis in 225 non-pregnant patients in 1992: clinical aspects and outcome in relation to predisposing conditions. Scand. J. Infect. Dis. 28:367-374.

Gracey, J.F. and D.S. Collins. 1992. Meat Hygiene. WB Saunders, London, England.

Graves Delmore, L.R., J.N. Sofos, J.O. Reagan, and G.C. Smith. 1997a. Hot water rinsing and trimming/washing of beef carcasses to reduce physical and microbiological contamination. J. Food Sci. 61:373-376.

Graves Delmore, L.R., J.N. Sofos, G.R. Schmidt, and G. C. Smith. 1997b. Inactivation of pathogenic bacteria by the chemical dehairing process proposed for use on beef carcasses before slaughter. Presented at the 50th Reciprocal Meat Conference, June-July, Iowa State University, Ames, Iowa. American Meat Science Association, Chicago, IL.

Graves Delmore, L.R., J.N. Sofos, G.R. Schmidt, and G.C. Smith. 1998. Decontamination of inoculated beef with sequential spraying treatments. J. Food Sci. 63:890-893.

Greer, G.G., S.D.M. Jones, B.D. Dilts, and W.M. Robertson. 1990. Effect of spray-chilling on the quality, bacteriology and case life of aged carcasses and vacuum packaged beef. Can. Inst. Food Sci. Technol. J. 23:82-86.

Griffin, P.M. 1995. *Escherichia coli* O157:H7 and other enterohemorrhagic *Escherichia coli*. pp. 739-761. In: Infections of gastrointestinal tract, M.J. Blaser, P.D. Smith, J.I. Ravdin, H.B. Greenberg, and R.L. Guerrant (Eds.). Raven Press, New York, NY.

Griffin, P.M. 1998. Epidemiology of shiga toxin-producing *Escherichia coli* infections in humans in the United States. pp. 15-22. In: *Escherichia coli* O157:H7 and other shiga toxin-producing *E. coli* strains. J.B. Kaper and A.D. O'Brien (Eds.). ASM Press, Washington, DC.

Gross, C.A. 1996. Function and regulation of the heat shock proteins. pp. 1382-1399. In: *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd Edition (Vol. I), F.C. Neidhardt, R. Curtiss, III, J.L. Ingraham, E.C.C. Lin, K.B. Low, B. Magasanik, W.S. Reznikoff, M. Riley, M. Schaechter, and H.E. Umbarger (Eds.). American Society for Microbiology, Washington, D.C.

Goulet, V. and P. Marchetti. 1996. Listeriosis in 225 non-pregnant patients in 1992: clinical aspects and outcome in relation to predisposing conditions. Scand. J. Infect. Dis. 28:367-374.

Hagen, C.J., E.R. Singleton, K.S. Kreuzer, E.M. Sloan, and J.N. Sofos. 1998. Isolation of catalase-negative *Listeria monocytogenes* from foods. DFES 18:424-426.

Hagiwara, S.-I., K. Kawai, A. Anri, and H. Nagahata. 2003. Lactoferrin concentrations in milk from normal and subclinical mastitic cows. J. Vet. Med. Sci. 65:319-323.

Hall, H.K. and J.W. Foster. 1996. The role of Fur in the acid tolerance response of *Salmonella typhimurium* is physiologically and genetically separable from its role in iron acquisition. J. Bacteriol. 178:5683-5691.

Hall, H. K., K. Karem, and J. W. Foster. 1995. Molecular responses of microbes to environmental pH stress. Adv. Microb. Physiol. 37:229-272.

Hamby, P.L., J.W. Savell, G.R. Acuff, C. Vanderzant, and H.R. Cross. 1987. Spray-chilling and carcass decontamination systems using lactic and acetic acid. Meat Sci. 21:1-14.

Hancock, D., T. Besser and D. Rice. 1998. Ecology of *Escherichia coli* O157:H7 in cattle and impact of management practices. pp. 85-91. In: *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* strains, J. Kaper and A. O'Brien (Eds.).ASM Press, Washington, DC.

Hansen, E.B. 2002. Commercial bacterial starter cultures for fermented foods of the future. Int. J. Food Microbiol. 78:119-131.

Hardin, M.D., S.E. Williams, and M.A. Harrison. 1993. Survival of *Listeria monocytogenes* in postpasteurized precooked beef roasts. J. Food Prot. 56:655-660.

Hardin, M.D., G.R. Acuff, L.M. Lucia, and J.W. Savell. 1995. Compariosn of methods for contamination removal from beef carcass surfaces. J. Food Prot. 58:368-374.

Hartl, F.U. 1996. Molecular chaperones in cellular protein folding. Nature 381:571-579.

Harvey, R.J. 1970. Metabolic regulation in glucose-limited chemostat cultures of *Escherichia coli*. J. Bacteriol. 104:698-706.

Havell, E.A., G.R. Beretich, and P.B. Carter. 1999. The mucosal phase of *Listeria* infection. Immunol. 201:164-177.

Hedberg, C.W., S.J. Savarino, J.M. Besser, C.J. Paulus, V.M. Thelen, L.J. Myers, D.N. Cameron, T.J. Barrett, J.B. Kaper, and M.T. Osterholm. 1997. An outbreak of foodborne illness caused by *Escherichia coli* O39:NM, an agent not fitting into the existing scheme for classifying diarrheogenic *E. coli*. J. Infect. Dis. 176:1625-1628.

Heitter, E.F. 1975. Chlor-chill. pp. 31-32. In: Proceedings meat industry research conference. American Meat Industry Foundation, Arlington, VA.

Hengge-Aronis, R. 1996. Regulation of gene expression during entry into stationary phase. pp. 1497-1512. In: *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology F.C. Neidhardt (Ed.). American Society for Microbiology, Washington, D.C.

Hengge-Aronis, R. 1999. Interplay of global regulators and cell physiology in the general stress response of *Escherichia coli*. Curr. Opin. Microbiol. 2:148-152.

Hengge-Aronis, R., W. Klein, R. Lange, M. Rimmele, and W. Boos. 1991. Trehalose synthesis genes are controlled by the putative sigma factor encoded by *rpoS* and are involved in stationary phase thermotolerance in *Escherichia coli*. J. Bacteriol. 173:7918-7924.

Heyde, M. and R. Portalier. 1990. Acid shock proteins of *Escherichia coli*. FEMS Microbiol. Lett. 69:19-26.

Hicks, S., D.C.A. Candy, and A.D. Phillips. 1996. Adhesion of enteroaggregative *Escherichia coli* to pediatric intestinal mucosa in vitro. Infect. Immun. 64:4751-4760.

Hill, C., B. O'Driscoll, and I. Booth. 1995. Acid adaptation and food poisoning microorganisms. Int. J. Food Microbiol. 28:245-254.

Hippe, C.L., R.A. Field, B. Ray, and W.C. Russell. 1991. Effect of spray-chilling on quality of beef from lean and fatter carcasses. J Anim Sci. 69:178-183.

Hochochka, P.W. and G.N. Somero. 1984. Biochemical adaptation. Princeton University Press, Princeton, NJ.

Houtsma, P.C., M.L. Kant-Muermans, F.M. Rombouts, and M.H Zwietering. 1996. Model for the combined effects of temperature, pH, and sodium lactate on growth rates of

Listeria innocua in broth and bologna-type sausages. Appl. Environ. Microbiol. 62:1616-1622.

Hu, K.H., E. Liu, K. Dean, M. Gingras, W. DeGraff, and N.J. Trun. 1996. Overproduction of three genes leads to camphor resistance and chromosome condensation in *Escherichia coli*. Genetics 143:1521-1532.

Huffman, R.D. 2002. Current and future technologies for the decontamination of carcasses and fresh meat. Meat Sci. 62:285-294.

Hugas, M., M. Garriga, M.T. Aymerich, and J. M. Monfort. 1995. Inhibition of *Listeria* in dry fermented sausages by the bacteriocinogenic *Lactobacillus sake* CTC494. J. Appl. Bacteriol. 79:322-330.

Iijima, H., I. Takahashi, and H. Kiyono. 2001. Mucosal immune network in the gut for the control of infectious diseases. Rev. Med. Virol. 11:117-133.

Ikeda, J.S., J. Samelis, P.A. Kendall, G.C. Smith, and J.N. Sofos. 2003. Acid adaptation does not promote survival or growth of *Listeria monocytogenes* on fresh beef following acid and nonacid decontamination treatments. J. Food Prot. 66:985-992.

Islam, M., J. Chen, M.P. Doyle, and M. Chinnan. 2002a. Control of *Listeria monocytogenes* on turkey frankfurters by generally-recognized-as-safe preservatives. J. Food Prot. 65:1411-1416.

Islam, M., J. Chen, M.P. Doyle, and M. Chinnan. 2002b. Effect of selected generally recognized as safe preservative sprays on growth of *Listeria monocytogenes* on chicken luncheon meat. J. Food Prot. 65:794-798.

James, S.J. 1987. Efficient systems for rapid cooling of carcasses and hot-boned meat. pp. 49-56. In: Accelerated processing of meat, A. Romita, C. Valin, and A.A. Taylor (Eds.). Elsevier Applied Science Publishers, London, UK.

James, S.J. and C. Bailey. 1989. Process design data for beef chilling. Int. J. Refrig. 12:42-49.

James, S.J. and C. Bailey. 1990. Chilling of beef carcasses. pp. 159-181. In: Chilled food: the state of the art, T.R. Gromley (Ed.). Applied Science Publishers, New York, NY.

Janes, M.E., T. Cobbs, S. Kooshesh, and M.G. Johnson. 2002. Survival differences of *Escherichia coli* O157:H7 strains in apples of three varieties stored at various temperatures. J. Food Prot. 65:1075-1080.

Jay, J.M. 2000. Modern food microbiology, 6th Edition. APAC Publisher Services, Singapore.

Jenkins, D.E., J.E. Schultz, and A. Matin. 1988. Starvation-induced cross protection against heat or H_2O_2 challenge in *Escherichia coli*. J. Bacteriol. 170:3910-3914.

Jenkins, D.E., S.A. Chaisson, and A. Matin. 1990. Starvation-induced cross protection against osmotic challenge in *Escherichia coli*. J. Bacteriol. 172:2779-2781.

Jericho, K.W.F., J.A. Bradley, V.P. Gannon, and G.C. Kozub. 1993. Visual demerit and microbiology evaluation of beef carcasses: microbiology. J. Food Prot. 56:114-119.

Jericho, K.W.F., J.A. Bradley, and G.C. Kozub. 1994. Bacteriological evaluation of groups of beef carcasses before the wash at six Alberta abattoirs. J. Appl. Bact. 77:631-634.

Jiang, W., Y. Hou, and M. Inouye. 1997. CspA, the major cold-shock protein of *Escherichia coli*, is an RNA chaperone. J. Biol. Chem. 272:196-202.

Johnson, L.R. and T.A. Gerwin. 2001. Gastrointestinal physiology. Mosby, Inc./JEMS Publishing, Carlsbad, CA.

Johnson, R.D., M.C. Hunt, D.M. Allen, C.L. Kastner, R.J. Danler, and C.C. Shrock. 1988. Moisture uptake during washing and spray chilling of Holstein and beef-type steer carcasses. J. Anim. Sci. 66:2180-2184.

Johnson, R.P., R.C. Clarke, J.B. Wilson, S.C. Read, K. Rahn, S.S. Renwick, K.A. Sandhu, D. Alves, M. Karmali, H. Lior, S.A. McEwen, J.S. Spika, and C.L. Gyles. 1996. Growing concerns and recent outbreaks involving non-O157:H7 serotypes of verotoxigenic *Escherichia coli*. J. Food Prot. 59:1112-1122.

Jones, P.G. and M. Inouye. 1994. The cold shock response – a hot topic. Mol. Microbiol. 11:811-818.

Jones, P.G. and M. Inouye. 1996. RbfA, a 30S-ribosomal binding factor, is a cold-shock protein whose absence triggers the cold shock response. Mol. Microbiol. 21:1207-1218.

Jones, S.D.M and W.M. Robertson. 1988. The effects of spray-chilling on the shrinkage and quality of beef. Meat Sci. 24:177-188.

Jones, P.G., R.A. VanBogelen, and F.C. Neidhardt. 1987. Induction of proteins in response to low temperature in *Escherichia coli*. J. Bacteriol. 169:2092-2095.

Jones, P.G., R. Krah, S.R. Tafuri, and A.P. Wolffe. 1992. DNA gyrase, CS7.4, and the cold shock response in *Escherichia coli*. J. Bacteriol. 174:5798-5802.

Jones, E.M., A. Smart, G. Bloomberg, L. Burgess, and M.R. Millar. 1994. Lactoferricin, a new antimicrobial peptide. J. Appl. Bacteriol. 77:208-214.

Jordan, D. 1998. Pre-slaughter control of beef carcass contamination with *Escherichia coli* O157:H7: a risk assessment approach. PhD thesis. University of Guelph, Guelph, Canada. pp. 236.

Joseph, R.L. 1996. Very fast chilling of beef and tenderness. Meat Sci. 43:S217-227.

Juncher, D., C.S. Vestergaard, J. Soltoft-Jensen, C.J. Weber, G. Bertelsen, and L.H. Skibsted. 2000. Effects of chemical hurdles on microbiological and oxidative stability of a cooked cured emulsion type meat product. Meat Sci. 55:483-491.

Juneja, V.K., O.P. Snyder, A.C. Williams, and B.S. Marmer. 1997. Thermal destruction of *Escherichia coli* O157:H7 in hamburger. J. Food Prot. 60:1163-1166.

Junttila, R.J., S.I. Niemela, and J. Hirn. 1988. Minimum growth temperatures of *Listeria monocytogenes* and nonhaemolytic listeria. J. Appl. Bacteriol. 65:321-327.

Kandor, O. and A.L. Goldberg. 1997. Trigger factor is induced upon cold shock and enhances viability of *Escherichia coli* at low temperatures. Proc. Natl. Acad. Sci. USA 94:4978-4981.

Kaper, J.B. 1994. Molecular pathogenesis of enteropathogenic *Escherichia coli*. pp. 173-194. In: Molecular genetics of bacterial pathogenesis, V.L. Miller, J.B. Kaper, D.A. Portnoy, and R.R. Isberg (Eds.). ASM Press, Washington, DC.

Kassenborg, H., C. Hedberg, M. Evans, G. Chin, T. Fiorentino, D. Vugia, M. Bardsley, L. Slutsker, and P. Griffin. 1998. Case-Control Study of Sporadic *Escherichia coli* O157:H7 Infections in 5 FoodNet Sites (CA, CN, GA, MN, OR). 1st International Conference on Emerging Infectious Diseases. Atlanta, GA, March 1998.

Kastner, C.L. 1981. Livestock and meat: carcasses, primal and subprimals. In: CRC Handbook of Transportation and Marketing in Agriculture, Volume1: Food commodities, E.E. Finney (Ed.). CRC Press Inc., Boca Raton, FL.

Kathariou, S. and L. Pine. 1991. The type strain(s) of *Listeria monocytogenes*: a source of continuing difficulties. Int. J. Syst. Bacteriol. 41:328-330.

Katla, T., K. Naterstad, M. Vancanneyt, J. Swings, and L. Axelsson. 2003. Differences in susceptibility of *Listeria monocytogenes* strains to sakacin P, sakacin A, pediocin PA-1, and nisin. Appl. Environ. Microbiol. 69:4431-4437.

Kay, S. 2003. $2.7 billion: The cost of *E. coli* O157:H7. Meat and Poultry February 2003:26-34.

Kim, J.W. and M.F. Slavik. 1996. Cetylpyridinium chloride (CPC) treatment on poultry skin to reduce attached *Salmonella*. J. Food Prot. 59:332-326.

Kim, J., J. Nietfeldt, and A.K. Benson. 1999. Octamer-based genome scanning distinguishes a unique subpopulation of *Escherichia coli* O157:H7 strains in cattle. Proc. Natl. Acad. Sci. USA 96:13288-13293.

Kim, C., Y.C. Hung, and R.E. Brackett. 2000. Efficacy of electrolyzed oxidizing (EO) and chemically modified water on different types of foodborne pathogens. Int. J. Food Microbiol. 61:199-207.

Knabel, S.J. 1995. Foodborne Illness – Role of home food handling practices. Food Technol. 49:119-131.

Knutton, S. 1995. Cellular responses to enteropathogenic *Escherichia coli* infection. Bioscience Rep. 15:469-479.

Ko, R., L.T. Smith, and G.M. Smith. 1994. Glycine betaine confers enhanced osmotolerance and cryotolerance on *Listeria monocytogenes*. J. Bacteriol. 176:426-431.

Kochevar, S.L., J.N. Sofos, R.R. Bolin, J.O. Reagan, and G.C. Smith. 1997. Steam vacuuming as a pre-evisceration intervention to decontaminate beef carcasses. J. Food Prot. 60:107-113.

Kolter, R., D.A. Siegele, and A. Torno. 1993. The stationary phase of the bacterial life cycle. Ann. Rev. Microbiol. 47:855-874.

Kos, B., J. Suskovic, J. Goreta, and S. Matosic. 2000. Effect of protectors on the viability of *Lactobacillus acidophilus* M92 in simulated gastrointestinal conditions. Food Technol. Biotechnol. 38:121-127.

Koseki, S., K. Yoshida, Y. Kamitani, and K. Itoh. 2003. Influence of inoculation method, spot inoculation site, and inoculation size on the efficacy of acidic electrolyzed water against pathogens on lettuce. J. Food Prot. 66:2010-2016.

Koutsoumanis, K.P. and J.N. Sofos. 2004. Comparative acid stress response of *Listeria monocytogenes, Escherichia coli* O157:H7 and *Salmonella* Typhimurium after habituation at different pH conditions. Lett. Appl. Microbiol. 38:321-326.

Kozempel, M., N. Goldberg, E.R. Radewonuk, O.J. Scullen, and J.C. Craig, Jr. 2000. Rapid hot dog surface pasteurization using cycles of vacuum and steam to kill *Listeria innocua*. J. Food Prot. 63:457-461.

Kroll, R.G. and I.R. Booth. 1981. The role of potassium transport in the generation of a pH gradient in *Escherichia coli*. Biochem. J. 198:691-698.

Kuntz, L.A. 1999. Ingredients to raise the microbial bar. Food Prod. Design April, 84-99.

Lange, R. and R. Hengge-Aronis. 1991. Growth-phase regulated expression of bola and morphology of stationary-phase *Escherichia coli* cells are controlled by the novel sigma factor σ^s. J. Bacteriol. 173:4474-4481.

La Teana, A., A. Brandi, M. Falconi, R. Spurio, C.L. Pon, and C.O. Gualerzi. 1991. Identification of a cold shock transcriptional enhancer of the *Escherichia coli* gene encoding nucleoid protein H-NS. Proc. Natl. Acad. Sci. USA 88:10907-10911.

Lazim, Z., T.J. Humphrey, and R.J. Rowbury. 1996. Induction of the PhoE porin by NaCl as the basis for salt-induced acid sensitivity in *Escherichia coli*. Lett. Appl. Microbiol. 23:269-272.

Lee, I.S., J.L. Slonczewski, and J.W. Foster. 1994a. A low-pH-inducible, stationary-phase acid tolerance response in *Salmonella typhimurium*. J. Bacteriol. 176:1422-1426.

Lee, S.J., A. Xie, W. Jiang, J.-P. Etchegaray, P.G. Jones, and M. Inouye. 1994b. Family of the major cold-shock protein, CspA (CS7.4), of *Escherichia coli*, whose members show a high sequence similarity with the eukaryotic Y-box bonding proteins. Mol. Microbiol. 833-837.

Leistner, L. 1995. Principles of hurdle technology. pp. 1-21. In: New Methods of Food Preservation, G.W. Gould, (Ed.). Blackie Academic and Professional, London, England.

Leistner, L. 2000. Basic aspects of food preservation by hurdle technology. Int. J. Food Microbiol. 55:181-186.

Leistner, L. 2002. Hurdle technology. pp. 493-508. In: Control of foodborne microorganisms, V.K. Juneja and J.N. Sofos (Eds.). Marcel Dekker AG, New York, NY.

Leistner, L. and L.G.M. Gorris. 1995. Food preservation by hurdle technology. Trends Food Sci. Technol. 6:41-46.

Lelivelt, M.J. and T.H. Kawula. 1995. Hsc66, an Hsp70 homolog in *Escherichia coli*, is induced by cold shock but not by heat shock. J. Bacteriol. 177:4900-4907.

Letellier, A., S. Messier, and S. Quessy. 1999. Prevalence of *Salmonella* spp. and *Yersinia enterocolitica* in finishing swine at Canadian abattoirs. J. Food Prot. 62:22-25.

Levay, P.F. and M. Viljoen. 1995. Lactoferrin: a general review. Haematologica 80:252-267.

Levine, P., B. Rose, S. Green, G. Ransom, and W. Hill. 2001. Pathogen testing of ready-to-eat meat and poultry products collected at federally inspected establishments in the United States, 1990 to 1999. J. Food Prot. 64:1188-1193.

Levy, O. 1996. Antibiotic proteins of polymorphonuclear leukocytes. Eur. J. Haematol. 56:263-277.

Leyer, G.J. and E.A. Johnson. 1993. Acid adaptation induces cross-protection against environmental stresses in *Salmonella typhimurium*. Appl. Environ. Microbiol. 59:1842-1847.

Leyer, G.J., L.-L. Wang, and E.A. Johnson. 1995. Acid adaptation of *Escherichia coli* O157:H7 increases survival in acidic foods. Appl. Environ. Microbiol. 61:3752-3755.

Lim, K. and A. Mustapha. 2004. Effects of cetylpyridinium chloride, acidified sodium chlorite, and potassium sorbate on populations of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Staphylococcus aureus* on fresh beef. J. Food Prot. 67:310-315.

Lindquist, S. and E.A. Craig. 1988. The heat shock proteins. Annu. Rev. Genet. 22:631-677.

Linnan, M.J., L. Mascola, X.D. Lou, V. Goulet, S. May, C. Salminen, D.W. Hird, M.L. Yonekura, P. Hayes, R. Weaver, A. Audurier, B.D. Plikaytis, S.L. Fannin, A. Kleks, and C.V. Broome. 1988. Epidemic listeriosis associated with Mexican-style cheese. New Eng. J. Med. 319:823-828.

Locker, R.H. 1985. Cold induced toughness of meat. pp. 1-43. In: Advances in meat research, volume 1, A.M. Pearson and T.R. Dutson (Eds.). AVI Publishing, Westport, CT.

Locker, R.H. and C.J. Hagyard. 1963. A cold shortening effect in beef muscles. J. Sci. Food Agric. 14:787.

Loewen, P.C. and R. Hengge-Aronis. 1994. The role of sigma factor sigma-S (KatF) in bacterial global regulation. Annu. Rev. Microbiol. 48:53, 80.

Lönnerdal, B. and S. Iyer. 1995. Lactoferrin: Molecular structure and biological function. Annu. Rev. Nutr. 15:93-110.

Lorber, B. 1997. Listeriosis. Clin. Infect. Dis. 23:1-11.

Lou, Y. and A.E. Yousef. 1997. Adaptation to sublethal environmental stresses protects *Listeria monocytogenes* against lethal preservation factors. Appl. Environ. Microbiol. 63:1252-1255.

Luchansky, J.B., K.A. Glass, K.D. Harsono, A. Degnan, N.G. Faith, and B. Cauvin. 1992. Genomic analysis of *Pediococcus* starter cultures to control *Listeria monocytogenes* in turkey summer sausage. Appl. Environ. Microbiol. 58:3053-3059.

Lundeen, T. 2000. Prevalence of *E. coli* greater in summer; lower after processing. Feedstuffs. April:9.

Maeda, T., J. Okazaki, H. Nagune, Y. Manabe, and H. Kourai. 1998. Bactericidal action of 4,4'-(α,ω-polymethylenedithio)bis-(1-alkyl-pyridinium iodides). Biol. Pharm. Bull. 21:1057-1061.

Magee, D.F. and A.F. Dalley. 1986. Digestion and the structure and function of the gut (Karger Continuing Education Series, Volume 8). S. Karger Publishing, Basel, Germany.

Mallikarjunan, P. and G.S. Mittal. 1995. Optimum conditions for beef carcass chilling. Meat Sci. 39:215-223.

Mallikarjunan, P. and G.S. Mittal. 1996. Selection criteria for beef carcass chilling. Food Res. Int. 29:661-666.

Marechal, P.A., I. Martinez De Marnanon, I. Poirier, and P. Gervais. 1999. The importance of the kinetics of application of physical stresses on the viability of microorganisms: significance for minimal food processing. Trends Food Sci. Tech. 10:15-20.

Marteau, P., P. Marteau, P. Seksik, and R. Jian. 2002. Probiotics and intestinal health effects: a clinical perspective. Br. J. Nutr. 88:S51-57.

Masson, P.L. and J.F. Heremans. 1971. Lactoferrin in milk from different species. Comp. Biochem. Physiol. 39:119-129.

Masters, C.I., J.A. Shallcross, and B.M. Mackey. 1994. Effect of stress treatments on the detection of *Listeria monocytogenes* and enterotoxigenic *Escherichia coli* by the polymerase chain reaction. J. Appl. Bacteriol. 77:73-79.

Matin, A. 1996. Role of alternate sigma factors in starvation protein synthesis – novel mechanisms of catabolite repression. pp. 494-505, 14th Forum in Microbiology.

Mattick, K.L., R.A. Bailey, F. Jorgensen, and T.J. Humphrey. 2002. The prevalence and number of *Salmonella* in sausages and their destruction by frying, grilling or barbecuing. J. Appl. Microbiol. 93:541-547.

Mazur, P. 1966. Physical and chemical basis of injury in single-celled microorganisms subjected to freezing and thawing. pp. 213. In: Cryobiology, H.T. Meryman (Ed.). Academic Press, San Diego, CA.

Mazzotta, A.S. 2001. Thermal inactivation of stationary-phase and acid-adapted *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* in fruit juices. J. Food Prot. 64:315-320.

Mbandi, E. and L. A. Shelef. 2001. Enhanced inhibition of *Listeria monocytogenes* and *Salmonella* Enteritidis in meat by combinations of sodium lactate and diacetate. J. Food Prot. 64:640-644.

Mbandi, E. and L.A. Shelef. 2002. Enhanced antimicrobial effects of combination of lactate and diacetate on *Listeria monocytogenes* and *Salmonella* spp. in beef bologna. Int. J. Food Microbiol. 76:191-198.

McCann, M.P., J.P. Kidwell, and A. Matin. 1991. The putative σ factor KatF has a central role in the development of starvation-mediated general resistance in *Escherichia coli*. J. Bacteriol. 173:4188-4194.

McCarthy, S.A. 1990. *Listeria* in the environment. pp. 25-29. In: Foodborne listeriosis. A.J. Miller, J.L. Smith, and G.A. Somkuti (Eds.). Elsevier Science Publishing Inc., New York, NY.

McKee, M.L. and A.D. O'Brien. 1996. Truncated Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 intimin (EaeaA) fusion proteins promote adherence of EHEC strains to HEp-2 cells. Infect. Immun. 64:2225-2233.

McLauchlin, J. 1991. Epidemiology of listeriosis in Britain. pp. 38-47. In: Proceedings of the international conference on *Listeria* and food safety, A. Amgar (Ed.). Aseptic Processing Association, Laval, France.

Mead, P.S. and P.M. Griffin. 1998. *Escherichia coli* O157:H7. Lancet 352:1207-1212.

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.

Mendonca, A.F., Amoroso, T.L., and S.J. Knabel. 1994. Destruction of gram-negative food-borne pathogens by high pH involves disruption of the cytoplasmic membrane. Appl. Environ. Microbiol. 60:4009-4014.

Merrell, D.S. and A. Camilli. 2002. Acid tolerance of gastrointestinal pathogens. Curr. Opin. Microbiol. 5:51-55.

Miller, S.I., A.M. Kukral, and J.J. Mekalanos. 1989. A two-component regulatory system (*phoPphoQ*) controls *Salmonella typhimurium* virulence. Proc. Natl. Acad. Sci. USA. 86:5054-5058.

Miller, M.A., M.J. Skeen, and H.K. Ziegler. 1995. Nonviable bacterial antigens administered with IL-2 generate antigen-specific T cell responses and protective immunity against *Listeria monocytogenes*. J. Immunol. 155:4817-4828.

Mirade, P.-S and L. Picgirard. 2001. Assessment of airflow patterns inside six industrial beef carcass chillers. Int. J. Food Sci. Technol. 36:463-475.

Mitta, M., L. Fang, and M. Inouye. 1997. Deletion analysis of *cspA* of *Escherichia coli*: requirement of the AT-rich UP element for *cspA* transcription, and the downstream box in the coding region for its cold-shock induction. Mol. Microbiol. 26:321-325.

Montrose, M.H. 2001. Choosing sides in the battle against gastric acid. J. Clin. Invest. 108:1743-1744.

Morgan, J.B., G.C. Smith, J.A. Sherbeck, S.K. Fitzgerald, and C.C. Kukay. 1995. A foreign market audit of U.S. Beef. The final report of the International Beef Quality Audit—1994. Meat Science Program, Department of Animal Sciences, Colorado State University, Fort Collins.

Morris, C.A., L.M. Lucia, J.W. Savell, and G.R. Acuff. 1997. Trisodium phosphate treatment of pork carcasses. J. Food Sci. 62:402-405.

Muffler, A., M. Barth, C. Marschall, and R. Hengee-Aronis. 1997. Heat shock regulation of σ^s turnover: a role for DnaK and relationship between stress responses mediated by σ^s and σ^{32} in *Escherichia coli*. J. Bacteriol. 179:445-452.

Murano, E.A. and M.D. Pierson. 1992. Effect of heat shock and growth atmosphere on the heat resistance of *Escherichia coli* O157:H7. J. Food Prot. 55:171-175.

Muriana, P.M. 1996. Bacteriocins for control of *Listeria* spp. in food. J. Food Prot. Suppl.:54-63.

Muriana, P.M., W. Quimby, C.A. Davidson, and J. Grooms. 2002. Postpackage pasteurization of ready-to-eat deli meats by submersion heating for reduction of *Listeria monocytogenes*. J. Food Prot. 65:963-969.

Murphy, R.Y., L.K. Duncan, E.R. Johnson, M.D. Davis, R.E. Wolfe, and H.G. Brown. 2001. Thermal lethality of *Salmonella* Senftenberg and *Listeria innocua* in fully cooked and packaged chicken breast strips via steam pasteurization. J. Food Prot. 64:2083-2087.

Murphy, R.Y., L.K. Duncan, J.A. Marcy, M.E. Berrang, and K.H. Driscoll. 2002. Effect of packaging-film thickness on thermal inactivation of *Salmonella* and *Listeria innocua* in fully cooked chicken breast meat. J. Food Sci. 67:3435-3440.

Murphy, R.Y., L.K. Duncan, K.H. Driscoll, and J.A. Marcy. 2003a. Lethality of *Salmonella* and *Listeria innocua* in fully cooked chicken breast meat products during postcook in-package pasteurization. J. Food Prot. 66:242-248.

Murphy, RY., L.K. Duncan, K.H. Driscoll, J.A. Marcy, and B.L. Beard. 2003b. Thermal inactivation of *Listeria monocytogenes* on ready-to-eat turkey breast meat products during postcook in-package pasteurization with hot water. J. Food Prot. 66:1618-1622.

Murphy, R.Y., L.K. Duncan, K.H. Driscoll, B.L. Beard, M.B. Berrang, and J.A. Marcy. 2003c. Determination of thermal lethality of *Listeria monocytogenes* in fully cooked chicken breast fillets and strips during postcook in-package pasteurization. J. Food Prot. 66:578-583.

Murray, E.G.D., R.A. Webb, and M.B.R. Swann. 1926. A disease of rabbits characterized by large mononuclear leucocytosis, caused by a hitherto undescribed bacillus, *Bacterium monocytogenes* (n. sp.). J. Pathol. Bacteriol. 29:407-227.

NACMCF (National Advisory Committee on Microbiological Criteria for Foods). 1991. *Listeria monocytogenes*. Int. J. Food Microbiol. 14:185-246.

NACMCF (National Advisory Committee on Microbiological Criteria in Foods). 1993. Report on generic HACCP for raw beef. Food Microbiol. 10:488-449.

Nagasawa, T., I. Kiyosawa, and K. Kuwahara. 1972. Amounts of lactoferrin in human colostrum and milk. J. Dairy Sci. 55:1651-1659.

Naidu, A.S. 2000. Lactoperoxidase. pp.103-132. In: Natural food antimicrobial systems, A.S. Naidu (Ed.). CRC Press, Boca Raton, FL.

Naidu, A.S. 2002. Activated lactoferrin: A new approach to meat safety. Food Technol. 56: 40-45.

Naidu, A.S., J. Tulpinski, K. Gustilo, R. Nimmagudda, and J.B. Morgan. 2003. Activated lactoferrin part 2: Natural antimicrobial for food safety. Agrofood industry hi-tech May/June:27-31.

Nakashima, K., K. Kanamaru, T. Mizuno, and K. Horikoshi. 1996. A novel member of the *cspA* family of genes that is induced by cold shock in *Escherichia coli*. J. Bacteriol. 178:2994-2997.

Narasimha Rao, D., K.K.S. Nair, and P.Z. Sakhare. 1998. Meat microbiology and spoilage in tropical countries. pp. 220-265. In: The microbiology of meat and poultry, A. Davies and R. Board (Eds.). Blackie Academic and Professional, London, UK.

Nataro, J.P. and J.B. Kaper. 1998. Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. 11:142-201.

Nataro, J.P., J. Seriwatana, A. Fasano, D.R. Maneval, L.D. Guers, F. Noriega, F. Dubovsky, M.M. Levine, and J.G. Morris Jr. 1995. Identification and cloning of a novel plasmid-encoded enterotoxin of enteroinvasive *Escherichia coli* and *Shigella* strains. Infect. Immun. 63:4721-4728.

Newkirk, K., W. Feng, W. Jiang, R. Tejero, S.D. Emerson, M. Inouye, and G.T. Montelione. 1994. Solution NMR structure of the major cold shock protein (CspA) from *Escherichia coli*. Proc. Natl. Acad. Sci. USA 91:5114-5118.

Niebuhr, S.E. and J.S. Dickson. 2003. Impact of pH enhancement on populations of *Salmonella*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in boneless lean beef trimmings. J. Food Prot. 66:874-877.

Nixon, B.T., C.W. Ronson, and F.M. Ausubel. 1986. Two-component regulatory systems responsive to environmental stimuli share strongly conserved domains with the nitrogen assimilation regulatory genes *ntrB* and *ntrC*. Proc. Natl. Acad. Sci. USA. 83:7850-7856.

NMA (National Meat Association). 1999. Risky food handling behaviors commonplace. Lean Trimmings Newsletter. April 12:1.

Nottingham, P.M. 1982. Microbiology of carcass meats. pp. 13-65. In: Meat microbiology, M.H. Brown (Ed.). Applied Science Publishers, London, UK.

Nou, X., M. Rivera-Betancourt, J.M. Bosilevac, T.L. Wheeler, S.D. Shackelford, B.L. Gwartney, J.O. Reagan, and M. Koohmaraie. 2003. Effect of chemical dehairing on the prevalence of *Escherichia coli* O157:H7 and the levels of aerobic bacteria and enterobacteriaceae on carcasses in a commercial beef processing plant. J. Food Prot. 66:2005-2009.

Nutsch, A.L., R.K. Phebus, M.J. Reimann, D.E. Schafer, J.E. Boyer, Jr., R.C. Wilson, J.D. Leising, and C.L. Kastner. 1997. Evaluation of a steam pasteurization process in a commercial beef processing facility. J. Food Prot. 60:485-492.

Nutsch, A.L., R.K. Phebus, M.J. Reimann, J.S. Kotrola, R.C. Wilson, J.E. Boyer, Jr., and T.L. Brown. 1998. Steam pasteurization of commercially slaughtered beef carcasses:

Evaluation of bacterial populations at five anatomical locations. J. Food Prot. 61:571-577.

O'Driscoll, B., C.G.M. Gahan, and C. Hill. 1996. Adaptive acid tolerance response in *Listeria monocytogenes*: isolation of an acid-tolerant mutant which demonstrates increased virulence. Appl. Environ. Microbiol. 62:1693-1698.

Offer, G., G. Mead, and E. Dransfield. 1988. Setting the scene: the effects of chilling on microbial growth and the eating quality of meat. Meat chilling, IFR-BL: Subject Day, 23 February 1988.

Okamoto, M., A. Nakane, and T. Minagawa. 1994. Host resistance to an intragastric infection with *Listeria monocytogenes* in mice depends on cellular immunity and intestinal bacteria flora. Infect. Immunol. 62:3080-3085.

Ørskov, I. and F. Ørskov. 1984. Serotyping of *E. coli*. pp. 43-112. In: Methods in Microbiology, T. Bergan and J.R. Norris (Eds.). Academic Press, New York, NY.

Orta-Ramirez, A., J.F. Price, Y.C. Hsu, G.J. Veeramuthu, J.S. Cherrymerritt, and D.M. Smith. 1997. Thermal inactivation of *Escherichia coli* O157:H7, *Salmonella seftenberg*, and enzymes with potential as time-temperature indicators in ground beef. J. Food Prot. 60:471-475.

Padan, E. 1999. The molecular mechanism of regulation of the NhaA Na^+/H^+ antiporter of *Escherichia coli*, a key transporter in the adaptation to Na^+ and H^+. pp. 183-199. In: Bacterial responses to pH, D. Chadwick and G. Cardew (Eds.). John Wiley and Sons, Ltd., Chichester, England.

Palumbo, S. A. 1991. A review of methods for detection of the psychrotrophic foodborne pathogens *Listeria monocytogenes* and *Aeromonas hydrophila*. J. Food Safety 11:105-122.

Palumbo, S.A. and A.C. Williams. 1994. Control of *Listeria monocytogenes* on the surface of frankfurters by acid treatments. Food Microbiol. 11:293-300.

Parish, M.E. and Higgins, D.P. 1989. Survival of *Listeria monocytogenes* in low pH model broth systems. J. Food Prot. 52:144-147.

Park, H., Y.C. Hung, and C. Kim. 2002a. Effectiveness of electrolyzed water as a sanitizer for treating different surfaces. J. Food Prot. 65:1276-1280.

Park, H., Y.C. Hung, and R.E. Brackett. 2002b. Antimicrobial effect of electrolyzed water for inactivating *Campylobacter jejuni* during poultry washing. Int. J. Food Microbiol. 72:77-83.

Peel, M., W. Donachie, and A. Shaw. 1988. Temperature –dependent expression of flagella of *Listeria monocytogenes* studied by electron microscopy, SDS-PAGE and western blotting. J. Gen. Microbiol. 143:2171-2178.

Perna, N.T., G. Plunkett III, V. Burland, B. Mau, J.D. Glasner, D.J. Rose, G.F. Mayhew, P.S. Evans, J.Gregor, H.A. Kirkpatrick, G. Posfai, J. Hackett, S. Klink, A. Boutin, Y. Shao, L. Miller, E.J. Grotbeck, N.W. Davis, A. Lim, E.T. Dimalanta, K.D. Potamousis, J. Apodaca, T.S. Anantharman, J. Lin, G. Yen, D.C. Schwartz, R.A. Welch, and F.R. Blattner. 2001. Genome sequence of enterohemoorhagic *Escherichia coli* O157:H7. Nature 409:529-533.

Peterson, W.L., P.A. Mackowiak, C.C. Barnett, M. Marling-Cason, and M.L. Haley. 1989. The human gastric bactericidal barrier: mechanisms of action, relative antibacterial activity and dietary influences. J. Infect. Dis. 159:979-983.

Pflug, I.J. and R.G. Holcomb. 1991. Principles of the thermal destruction of microorganisms. pp. 85-128. In: Disinfection, sterilization, and preservation, S.S. Block (Ed.). Lea and Febiger, Philadelphia, PA.

Phebus, R.K., A.L. Nutsch, D.E. Schafer, R.C. Wilson, M.J. Riemann, J.D. Leising, C.L. Kastner, J.R. Wolf, and R.K. Prasai. 1997. Comparison of steam pasteurization and other methods for reduction of pathogens on surfaces of freshly slaughtered beef. J. Food Prot. 60:476-484.

Pichereau, V., A. Hartke, and Y. Auffray. 2000. Starvation and osmotic stress induced multiresistances: influence of extracellular compounds. Int. J. Food Microbiol. 55:19-25.

Pine, L., G.B. Malcolm, J.B. Brooks, and M.I. Daneshvar. 1989. Physiological studies on the growth and utilization of sugars by *Listeria* species. Can. J. Microbiol. 35:245-254.

Pirie, J.H.H. 1927. A new disease of veld rodents. "Tiger River Disease". Publ. S. Afr. Inst. Med. Res. 3:163-186.

Pirie, J.H.H. 1940. The genus *Listerella* Pirie. Science. 91:383.

Porto, A.C.S., B.D.G.M. Franco, E.S. Sant'anna, J.E. Call, A. Piva, and J.B. Luchansky. 2002. Viability of a five-strain mixture of *Listeria monocytogenes* in vacuum-sealed packages of frankfurters, commercially prepared with and without 2.0 or 3.0% added potassium lactate, during extended storage at 4 and 10°C. J. Food Prot. 65:308-315.

Prasai, R.K., R.K. Phebus, C.M. Garcia Zepada, C.L. Kastner, A.E. Boyle, and D.Y.C. Fung. 1995. Effectiveness of trimming and/ or washing on microbiological quality of beef carcasses. J. Food Prot. 58:1114-1117.

Prescott, L.M., J.P. Harley, and D.A. Klein. 1999. In: Microbiology, 4th Edition. WCB McGraw-Hill, New York, NY.

Qvist, S., K. Sehested, and P. Zeuthen. 1994. Growth suppression of *Listeria monocytogenes* in a meat product. Int. J. Food Microbiol. 24:283-293.

Raja, N., M. Goodson, D.G. Smith, and R.J. Rowbury. 1991. Decreased DNA damage by acid and increased repair of acid-damaged DNA in acid-habituated *Escherichia coli*. J. Appl. Bacteriol. 70:507-511.

Ransom, J.R., R.T. Bacon, K.E. Belk, J.N. Sofos, J.A. Scanga, and G.C. Smith. 2002. Comparison of sampling methods for sampling rectal/colonal feces, hides and carcasses for microbial testing. J. Food Prot. 65:621-626.

Ransom, J.R., K.E. Belk, J.N. Sofos, J.D. Stopforth, J.A. Scanga, and G.C. Smith. 2003a. Comparison of intervention technologies for reducing *Escherichia coli* O157:H7 on beef cuts and trimmings. Food Protection Trends 23:24-34.

Ransom, J.R., J.N. Sofos, K.E. Belk, G.A. Dewell, K.S. McCurdy, G.C. Smith, and M. D. Salman. 2003b. Determining the prevalence of *Escherichia coli* O157 in cattle and beef from the feedlot to the cooler. pp.175. In: 90th Annual Meeting of the International Association for Food Protection, New Orleans, LA.

Ravishankar, S. and M.A. Harrison. 1999. Acid adaptation of *Listeria monocytogenes* strains does not offer cross-protection against an activated lactoperoxidase system. J. Food Prot. 62:670-673.

Reagan, J.O., G.R. Acuff, D.R. Buege, M.J .Buyck, J.S. Dickson, C.L. Kastner, J.L. Marsden, J.B. Morgan, R. Nickelson, II, G.C. Smith, and J.N. Sofos. 1996. Trimming and washing of beef carcasses as a method of improving the microbiological quality of meat. J. Food Prot. 59:751-756.

Reed, C.A. 1996. Foodborne illness prevention before slaughter? Yes! JAVMA 208:1366.

Rees, C.E.D., C.E.R. Dodd, P.T. Gibson, I.R. Booth, and G.S.A.B. Stewart. 1995. The significance of bacteria in stationary phase to food microbiology. Int. J. Food Microbiol. 28:263-275.

Richmond, M. 1990. The microbiological safety of foods, Parts I & II. Report of the Committee on the Microbiological Safety of Food, London, HMSO.

Riley, L.W., R.S. Remis, S.D. Helgerson, H.B. McGee, J.G. Wells, B.R. Davis, R.J. Herbert, C.S. Olcott, L.M. Johnson, N.T. Hargrett, P.A. Blake, and M.L. Cohen. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N. Engl. J. Med. 308:681-685.

Riordan, D.C.R., G. Duffy, J.J. Sheridan, R.C. Whiting, I.S. Blair, and D.A. McDowell. 2000. Effects of acid adaptation, product pH, and heating on survival of *Escherichia coli* O157:H7 in pepperoni. Appl. Environ. Microbiol. 66:1726-1729.

Rocourt, J. 1999. The genus *Listeria* and *Listeria monocytogenes*: phylogenetic position, taxonomy, and identification. pp. 1-20. In: *Listeria*, listeriosis and food safety, E.T. Ryser and E.H. Marth (Eds.). Marcel Dekker Inc., New York, NY.

Rocourt, J., P. Boerlin, and F. Grimont. 1992. Assignment of *Listeria grayi* and *Listeria murrayi* to a single species, *Listeria grayi* with a revised description of *Listeria grayi*. Int. J. System. Bacteriol. 42:171-174.

Rocourt, J., C. Jacquet, and A. Reilly. 2000. Epidemiology of human listeriosis and seafoods. Inter. J. Food Microbiol. 62:197-209.

Roe, A.J., D. McLaggan, I. Davidson, C. O'Byrne, and I.R. Booth. 1998. Perturbation of anion balance during inhibition of growth of *Escherichia coli* by weak acids. J. Bacteriol. 180:767-772.

Roels, T.H., M.E. Proctor, L.C. Robinson, K. Hulbert, C.A. Bopp, and J.P. Davis. 1998. Clinical features of infections due to *Escherichia coli* producing heat-stable toxin during an outbreak in Wisconsin: A rarely suspected cause of diarrhea in the United States. Clin Infect. Dis. 26:898-902.

Roering, A.M., R.K. Wierzba, A.M. Ihnot, and J.B. Luchansky. 1998. Pasteurization of vacuum-sealed packages of summer sausage inoculated with *Listeria monocytogenes*. J. Food Safety 18:49-56.

Roering, A.M., J.B. Luchansky, A.M. Ihnot, S.E. Ansay, C.W. Kaspar, and S.C. Ingham. 1999. Comparative survival of *Salmonella typhimurium* DT 104, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in preservative-free apple cider and simulated gastric fluid. Int. J. Food Microbiol. 46:263-269.

Rowbury, R.J., M. Goodson, Z. Lazim, and T.J. Humphrey. 1996. Sensitization to acid induced by sodium ions in *Escherichia coli*: dependence on (p)ppGpp and cAMP and suppression of the *relA*-associated defect by mutations in *envZ*. Microbios 85:161-177.

Rowbury, R.J. 2001. Cross-talk involving extracellular sensors and extracellular alarmones gives early warning to unstressed *Escherichia coli* of impending lethal chemical stress and leads to induction of tolerance responses. J. Appl. Microbiol. 90:677-695.

Rowe, M.T. and R. Kirk. 1999. An investigation into the phenomenon of cross-protection in *Escherichia coli* O157:H7. Food Microbiol. 16:157-164.

Ruiz Chavez, R.R. 1996. Gastric acid. Rev. Gastroenterol Peru. 16:249-253.

Russell, N.J. 1990. Cold adaptation of microorganisms. Phil. Trans. R. Soc. Lond. Ser. 326:595-611.

Russell, A.D. 1991. Mechanisms of bacterial resistance to non-antibiotics: food additives and food and pharmaceutical preservatives. J. Appl. Bacteriol. 71:191-201.

Russell, S.M. 2003. The effect of electrolyzed oxidative water applied using electrostatic spraying on pathogenic and indicator bacteria on the surface of eggs. Poult. Sci. 82:158-162.

Russell, N.J., R.I. Evans, P.F. terSteeg, J. Hellemons, A. Verheaul, and T. Abee. 1995. Membranes as a target for stress adaptation. Int. J. Food Microbiol. 28:255-261.

Ryser, E.T. and E.H. Marth. 1999. *Listeria*, listeriosis and food safety. Marcel Dekker Inc., New York, NY.

Ryu, J.-H. and L.R. Beuchat. 1999. Changes in heat tolerance of *Escherichia coli* O157:H7 after exposure to acidic environments. Food Microbiol. 16:317-324.

Ryu, J.-H., Y. Deng, and L.R. Beuchat. 1998. Influence of acid tolerance responses on survival, growth, and thermal cross-protection of *Escherichia coli* O157:H7 in acidified media and fruit juices. Int. J. Food Microbiol. 45:185-193.

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.

Salyers, A. and D. Whitt. 1994. Bacterial pathogenesis – a molecular approach. ASM Press, Washington, DC.

Samelis, J. and J. Metaxopoulos. 1999. Incidence and principal sources of *Listeria* spp. and *Listeria monocytogenes* contamination in processed meats and a meat processing plant. Food Microbiol. 16:465-477.

Samelis, J. and J.N. Sofos. 2003. Strategies to control stress-adapted pathogens and provide safe foods. pp. 303-351. *In*: Microbial adaptation to stress and safety of new-generation foods, A.E. Yousef and V.K. Juneja (Eds.). CRC Press, Inc. Boca Raton, FL.

Samelis, J., J.N. Sofos, P.A. Kendall, and G.C. Smith. 2001a. Influence of the natural microbial flora on the acid tolerance response of *Listeria monocytogenes* in a model system of fresh meat decontamination fluids. Appl. Environ. Microbiol. 67:2410-2420.

Samelis, J., J.N. Sofos, P.A. Kendall, and G.C. Smith. 2001b. Fate of *Escherichia coli* O157:H7, *Salmonella* Typhimurium DT104, and *Listeria monocytogenes* in fresh meat decontamination fluids at 4 and 10°C. J. Food Prot. 64:950-957.

Samelis, J., J.N. Sofos, M.L. Kain, J.A. Scanga, K.E. Belk, and G.C. Smith. 2001c. Organic acids and their salts as dipping solutions to control *Listeria monocytogenes* inoculated following processing of sliced pork bologna stored at 4°C in vacuum packages. J. Food Prot. 64:1722-1729.

Samelis, J., J.N. Sofos, P.A. Kendall, and G.C. Smith. 2002a. 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.

Samelis, J., J.N. Sofos, J.S. Ikeda, P.A. Kendall, and G.C. Smith. 2002b. Exposure to non-acid fresh meat decontamination fluids sensitizes *Escherichia coli* O157:H7 to organic acids. Lett. Appl. Microbiol. 34:7-12.

Samelis, J., G.K. Bedie, J.N. Sofos, K.E. Belk, J.A. Scanga, and G.C. Smith. 2002c. Control of *Listeria monocytogenes* with combined antimicrobials after postprocess contamination and extended storage of frankfurters at 4°C in vacuum packages. J. Food Prot. 65:299-307.

Samelis, J., G.K. Bedie, J.N. Sofos, K.E. Belk, J.A. Scanga, and G.C. Smith. 2002d. Control of *Listeria monocytogenes* with combined antimicrobials after postprocess contamination and extended storage of frankfurters at 4°C in vacuum packages. J. Food Prot. 65:299-307.

SAS (SAS Institute Inc.). 2002. SAS/STAT ® User's Guide, SAS system, Version 8.2. Cary, NC: SAS Institute Inc.

Schillinger, U., M. Kaya, and F.-K. Lucke. 1991. Behaviour of *Listeria monocytogenes* in meat and its control by a bacteriocin-producing strain of *Lactobacillus sake*. J. Appl. Bacteriol. 70:473-478.

Schindelin, H., M.A. Marahiel and U. Heinemann. 1993. Universal nucleic acid-binding domain revealed by crystal structure of the *B. subtilis* major cold shock protein. Nature 364:164-168.

Schlech, W.F., P.M. Lavigne, R.A. Bortolussi, A.C. Allen, E.V. Haldane, A.J. Won, A.W. Hightower, S.E. Johnson, S.H. King, E.S. Nicholls, and C.V. Broome. 1985. Epidemic listeriosis-evidence for transmission by food. N. Engl. J. Med. 308:203-206.

Schlyter, J.H., A.J. Degnan, J. Loeffelholz, K.A. Glass, and J.B. Luchansky. 1993a. Evaluation of sodium diacetate and ALTA^{TM} 2341 on viability of *Listeria monocytogenes* in turkey slurries. J. Food Prot. 56:808-810.

Schlyter, J. H., K. A. Glass, J. Loeffelholz, A.J. Degnan, and J.B. Luchansky. 1993b. The effects of diacetate with nitrite, lactate, or pediocin on the viability of *Listeria monocytogenes* in turkey slurries. Int. J. Food Microbiol. 19:271-281.

Schnell, T.D., J.N. Sofos, V.G. Littlefield, J.B. Morgan, B.M. Gorman, R.P. Clayton, and G.C. Smith. 1995. Effects of postexsanguination dehairing on the microbial load and visual cleanliness of beef carcasses. J. Food Prot. 58:1297-1302.

Schnuchel, A., R. Wiltscheck, M. Czisch, M. Herrler, G. Willimsky, P. Graumann, M.A. Marahiel, and T.A. Holak. 1993. Structure in solution of the major cold shock protein from *Bacillus subtilis*. Nature 364:169-171.

Schultz, J.E. and A. Matin. 1991. Molecular and functional characterization of a carbon starvation gene of *Escherichia coli*. J. Mol. Biol. 218:129-140.

Scott, W.J. and J.R. Vickery. 1939. Investigations on chilled beef, part II. Cooling and storage at the meat works. Council Sci. Ind. Res. Australia, Bulletin number 129.

Seeliger, H.P.R. and D. Jones. 1987. *Listeria*. pp. 1235-1245. In: Bergey's Manual of Systematic Bacteriology, J.G. Hotl (Ed.). Williams and Wilkins Co., Baltimore, MD.

Selgas, D., M.L. Marin, C. Pin, and C. Casas. 1993. Attachment of bacteria to meat surfaces: a review. Meat Sci. 34:265-273.

Seman, D.L., A.C. Borger, J.D. Meyer, P.A. Hall, and A.L. Milkowski. 2002. Modeling the growth of *Listeria monocytogenes* in cured ready-to-eat processed meat products by manipulation of sodium chloride, sodium diacetate, potassium lactate, and product moisture content. J. Food Prot. 65: 651-658.

Sharma, R.R. and A. Demirci. 2003. Treatment of *Escherichia coli* O157:H7 inoculated alfalfa seeds and sprouts with electrolyzed oxidizing water. Int. J. Food Microbiol. 86:231-237.

Shelef, A. 1994. Antimicrobial effects of lactates: a review. J Food Prot. 57:445-450.

Shelef, L.A. and L. Addala. 1993. Inhibition of *Listeria monocytogenes* and other bacteria by sodium diacetate. J. Food Safety 14:103-115.

Silver, R.P., W. Aaronson, A. Sutton, and R. Schneerson. 1980. Comparative analysis of plasmids and some metabolic characteristics of *Escherichia coli* K1 from diseased and healthy individuals. Infect. Immun. 29:200-206.

Simpson, R.K. and A. Gilmour. 1997. The resistance of *Listeria monocytogenes* to high hydrostatic pressure in foods. Food Microbiol. 14:567-573.

Siragusa, G.R. 1995. The effectiveness of carcass decontamination systems for controlling the presence of pathogens on the surfaces of meat animal carcasses. J. Food Safety 15:229-238.

Slonczewski, J.L. and J.W. Foster. 1996. pH-regulated genes and survival at extreme pH. pp. 1539-1549. In: *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, F.C. Neidhardt, (Ed.). ASM Press, Washington, D.C.

Slutsker, L. and A. Schuchat. 1999. Listeriosis in humans. pp. 75-95. In: *Listeria*, listeriosis and food safety, E.T. Ryser and E.H. Marth (Eds.). Marcel Dekker Inc., New York, NY.

Slutsker, L., A.A. Ries, K. Maloney, J.G. Wells, K.D. Greene, P.M. Griffin and the *Escherichia coli* O157:H7 Study Group. 1998. A nationwide case-control study of *Escherichia coli* O157:H7 infection in the United States. J. Infect. Dis. 177:962-966.

Smith, M.G. 1985. The generation time, lag time and minimum temperature of growth of coliform organisms in meat and the implication for codes of practice in abattoirs. J. Hyg. Camb. 94:289-300.

Smith, M.G. 1995. Survival of *E. coli* and *Salmonella* after chilling and freezing in liquid media. J. Food Sci. 60:509-512.

Smith, J.L. 1998. Foodborne illness in the elderly. J. Food Prot. 61:1229-1239.

Smith, G.C. 2000. Bacteriological/Chemical safety: Meat as a food. Presented at the Minnesota Dietetics Association Annual Conference. Minneapolis, MN.

Smoot, L.M. and M.D. Pierson. 1998. Effect of environmental stress on the ability of *Listeria monocytogenes* Scott A to attach to food contact surfaces. J. Food Prot. 61:1293-1298.

Sofos, J.N. 1984. Antimicrobial effects of sodium and other ions in foods: a review. J. Food Safety 6:45-78.

Sofos, J.N. and G.C. Smith. 1998. Nonacid meat decontamination technologies: Model studies and commercial applications. Int. J. Food Microbiol. 44:171-188.

Sofos, J.N., S.I. Kochevar, G.R. Bellinger, D.R. Buege, D.D. Hancock, S.C. Ingham, J.B. Morgan, J.O. Reagan, and G.C. Smith. 1999a. Sources and extent of microbiological contamination of beef carcasses in seven United States slaughtering plants. J. Food Prot. 62:140-145.

Sofos, J.N., K.E. Belk, and G.C. Smith. 1999b. Processes to reduce contamination with pathogenic microorganisms in meat. Presented at the 45th International Congress of Meat Science and Technology, Yokohama, Japan. 2:596-605.

Sofos, J.N., P. Skandamis, J.D. Stopforth, and R.T. Bacon. 2003. Current research in meatborne pathogenic bacteria. Proceedings of the 56th Annual American Meat Science Association Reciprocal Meat Conference, pp. 33-37.

Sommers, C., M. Kozempel, X. Fan, and E.R. Radewonuk. 2002. Use of vacuum-steam-vacuum and ionising radiation to eliminate *Listeria innocua* from ham. J. Food Prot. 65:1981-1983.

Squires, C. and L. Squires. 1992. The Clp proteins: proteolysis regulators or molecular chaperones? J. Bacteriol. 174:1081-1085.

Stan, S.D. and M.A. Daeschel. 2003. Reduction of *Salmonella enterica* on alfalfa seeds with acidic electrolyzed oxidizing water and enhanced uptake of acidic electrolyzed oxidizing water into seeds by gas exchange. J. Food Prot. 66:2017-2022.

Stein, R. 2000. Know the enemy: understanding the basics of foodborne pathogens. Meat and Poultry 10:46-51,79.

Stekelenburg, F.K. 2003. Enhanced inhibition of *Listeria monocytogenes* in Frankfurter sausage by the addition of potassium lactate and sodium diacetate mixtures. Food Microbiol. 20:133-137.

Stoodley, P., K. Sauer, D.G. Davies, and J.W. Costerton. 2002. Biofilms as complex differentiated communities. Annu Rev Microbiol.56:187-209.

Stopforth, J.D., J. Samelis, J.N. Sofos, P.A. Kendall and G.C. Smith. 2002. Biofilm formation by acid-adapted and nonadapted *Listeria monocytogenes* in fresh beef decontamination washings and its subsequent inactivation with sanitizers. J. Food Prot. 65:1717-1727.

Stopforth, J.D., J. Samelis, J.N. Sofos, P.A. Kendall, and G.C. Smith. 2003a. Influence of organic acid concentration on survival of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in beef carcass wash water and on model equipment surfaces. Food Microbiol. 20:651-660.

Stopforth, J.D., J. Samelis, J.N. Sofos, P.A. Kendall, and G.C. Smith. 2003b. Influence of extended acid stressing in fresh beef decontamination fluids on sanitizer inactivation of acid-adapted *Escherichia coli* O157:H7 biofilms. J. Food Prot. 66:2258-2266.

Strydom, P.E. and E.M. Buys. 1995. The effects of spray-chilling on carcass mass loss and surface associated bacteriology. Meat Sci. 39:265-276.

Stuart, M.R. and P.E. Pease. 1972. A numerical study on the relationship of *Listeria* and *Erysipelothrix*. J. Gen. Microbiol. 73:551-565.

Sutherland, I.W. 2001. The biofilm matrix – an immobilized but dynamic microbial environment. TRENDS in Microbiology 9:222-227.

Swenson, D.L., N.O. Bukanov, D.E. Berg, and R.A. Welch. 1996. Two pathogenicity islands in uropathogenic *Escherichia coli* J96: Cosmid cloning and sample sequencing. Infect. Immun. 64:3736-3743.

Swift and Company. 1973. Carcass chilling process. U.S. Patent 1,380,891.

Tanabe, H., J. Goldstein, M. Yang, and M. Inouye. 1992. Identification of the promoter region of the *Escherichia coli* major cold shock gene, *cspA*. J. Bacteriol. 174:3867-3873.

Taormina, P.J. and L.R. Beuchat. 2001. Survival and heat resistance of *Listeria monocytogenes* after exposure to alkali and chlorine. Appl. Environ. Microbiol. 67:2555-2563.

Tauxe, R.V. 1997. Emerging foodborne diseases: An evolving public health challenge. Emerg. Infect. Dis. 3:425-434.

Tay, H., C. Lowell, and P.C. Stein. 1996. Electrophysiologic monitoring of the effects of soluble virulence factors produced by *Escherichia coli* infection in urine. Urol. 48:389-392.

Thomas, J.G. and F. Baneyx. 1998. Roles of the *Escherichia coli* small heat shock proteins IbpA and IbpB in thermal stress management: Comparison with ClpA, ClpB and HtpG in vivo. J. Bacteriol. 180:5165-5172.

Tian, G., I. Vainberg, W. Tap, S. Lewis, and N. Cowan. 1995. Specificity in chaperonin-mediated protein folding. Nature 375:250-253.

Tilden J., W. Young, A. McNamara, C. Custer, B. Boesel, M. Lambert-Fair, J. Majkowski, D. Vugia, S.B. Werner, J. Hollingsworth, and J.G. Morris. 1996. A new route of transmission for *Escherichia coli*: infection from dry fermented salami. Am. J. Public Health 86:1142-1145.

Tomita, M., M. Takase, H. Wakabayashi, and W. Bellamy. 1994. Antimicrobial peptides of lactoferrin. pp.209-218. In: Lactoferrin: structure and function, T.W. Hutchens (Eds.), Plenum Press, New York, NY.

Tompkin, R.B. 2002. Control of *Listeria monocytogenes* in the food-processing environment. J. Food Prot. 65:709-725.

Tompkin, R.B., V.N. Scott, D.T. Bernard, W.H. Sveum, and K.S. Gombas. 1999. Guidelines to prevent post-processing contamination from *Listeria monocytogenes*. DFES 19:551-562.

Tornberg, E. 1996. Biophysical aspects of meat tenderness. Meat Sci. 43:5175-5191.

Tsai, Y.-W. and S.C. Ingham. 1997. Survival of *Escherichia coli* O157:H7 and *Salmonella* spp. in acidic condiments. J. Food Prot. 60:751-755.

Tse-Dinh, Y.-C., H. Qi, and R. Menzel. 1997. DNA supercoiling and bacterial adaptation: thermotolerance and thermoresistance. Trends Microbiol. 5:323-326.

Uljas, H.E. and S.C. Ingham. 1998. Survival of *Escherichia coli* O157:H7 in synthetic gastric fluid after cold and acid in apple juice or trypticase soy broth acidified with hydrochloric acid or organic acids. J. Food Prot. 61:939-947.

USDA-FDA (United States Department of Agriculture Food and Drug Administration). 1998. Secondary direct food additives permitted in food for human consumption. Code of Federal Register (CFR), Office of the Federal Register, United States Department of Agriculture, FDA, Washington, DC. CFR 63:11118-11119.

USDA-FDA (United States Department of Agriculture Food and Drug Administration). 2003. Secondary direct food additives permitted in food for human consumption. U.S. Department of Agriculture. Food and Drug Administration, Washington, DC. Fed. Regist. 66:48208-48209.

USDA-FSIS (United States Department of Agriculture Food Safety and Inspection Services). 1990. FSIS Directive 10.240.1: Microbiological Monitoring Program: Sampling, Testing Procedures and Actions for *Listeria monocytogenes* and *Salmonella*. U.S. Department of Agriculture.

USDA-FSIS (United States Department of Agriculture Food Safety and Inspection Services). 1993. Immediate actions: cattle clean meat program. FSIS Correlation Packet, Interim Guidelines for Inspectors. Food Safety and Inspection Service, United States Department of Agriculture, Washington, D.C.

USDA-FSIS (United States Department of Agriculture Food Safety and Inspection Services). 1994. Nationwide beef microbiological baseline data collection program: steers and heifers, October, 1992-September, 1993. United States Department of Agriculture, FSIS, Washington, DC.

USDA-FSIS (United States Department of Agriculture Food Safety and Inspection Services). 1995. Comparison of methods for achieving the zero tolerance standard for fecal, ingesta and milk contamination of beef carcasses: Notice of conference. Fed. Register 60:49553-49563.

USDA-FSIS (United States Department of Agriculture Food Safety and Inspection Services). 1996. Pathogen reduction; hazard analysis and critical control point (HACCP) systems; final rule. Fed. Register 61:38806-38989.

USDA-FSIS (United States Department of Agriculture Food Safety and Inspection Services). 1999. Generic HACCP model for beef slaughter. United States Department of Agriculture, FSIS, Washington, DC.

USDA-FSIS (United States Department of Agriculture Food Safety and Inspection Services). 2000. Meat packaging materials. May, 2000. United States Department of Agriculture, FSIS, Washington, DC.

USDA-FSIS (United States Department of Agriculture Food Safety and Inspection Services). 2001. Retained water in raw meat and poultry products; poultry chilling requirements; final rule. Fed. Regist. 66:19713-19714.

USDA-FSIS (United States Department of Agriculture Food Safety and Inspection Service). 2002. Technical report: Comparitive risk assessment for intact (nontenderized) and non-intact (tenderized) beef. Food Safety and Inspection Service, U.S. Department of Agriculture, Washington, D.C. Available at: http://haccpalliance.org/alliance/NonintactBeefTechRpt.pdf

USDA-FSIS (United States Department of Agriculture Food Safety and Inspection Services). 2003a. Control of *Listeria monocytogenes* in ready-to-eat-meat and poultry products; Final Rule. Fed. Regist. 68:34208-34254.

USDA-FSIS (United States Department of Agriculture Food Safety and Inspection Services). 2003b. FSIS Directive 10.240.4: Verification Procedures for the *Listeria monocytogenes* Regulation and Microbial Sampling of Ready-to-Eat (RTE) products for the FSIS Verification Testing Program. U.S. Department of Agriculture.

Uyttendaele, M., E. Jozwik, A. Tutenel, L. De Zutter, J. Uradzinski, D. Pierard, and J. Debevere. 2001. Effect of acid resistance of *Escherichia coli* O157:H7 on efficacy of buffered lactic acid to decontaminate chilled beef tissue and effect of modified atmosphere packaging on survival of *Escherichia coli* O157:H7 on red meat. J. Food Prot. 64:1661-1666.

VanBogelen, R.A., P.M. Kelley, and F.C. Neidhardt. 1987. Differential induction of heat shock, SOS, and oxidative stress regulons and accumulation of nucleotides in *Escherichia coli*. J. Bacteriol. 169:26-32.

Van der Strate, B.W.A., M.C. Harmsen, P. Schäfer, P.J. Swart, T.H. The, G. Jahn, C.P. Speer, D.K.F. Meijer, and K. Hamprecht. 2001. Viral load in breast milk correlates with transmission of human cytomegalavirus to preterm neonates, but lactoferrin concentrations do not. Clin. Diagn. Lab. Immunol. 8:818-821.

Vanderzant, C., M.O. Hanna, J.G. Ehlers, J.W. Savell, D.B. Griffin, D.D. Johnson, G.C. Smith, and D.M. Stiffler. 1985. Methods of chilling and packaging of beef, pork, and lamb variety meats for transoceanic shipment: microbiological characteristics. J. Food Prot. 48:765-769.

Van Duijn, B., D.L. Ypey, J. de Goede, A.A. Verveen, and W. Hekkens. 1989. A model study of the regulation of gastric acid secretion. Am. J. Physiol. 257:G157-168.

Vasquez, F., E.A. Gonzalez, J.I. Garabal, and J. Blanco. 1996. Fimbriae extracts from enterotoxigenic *Escherichia coli* strains of bovine and porcine origin with K99 and/or F41 antigens. Vet Microbiol. 48:231-241.

Veeramuthu, G.J., J.F. Price, C.E. Davis, A.M. Booren, and D.M. Smith. 1998. Thermal inactivation of *Escherichia coli* O157:H7, *Salmonella seftenberg*, and enzymes with potential as time-temperature indicators in ground turkey thigh meat. J. Food Prot. 61:171-175.

Velkov, V.V. 1999. How environmental factors regulate mutagenesis and gene transfer in microorganisms. J. Biosci. 24:529-559.

Venkitanarayanan, K.S., Ezeike, G.O. Hung, Y.C., and M.P. Doyle. 1999a. Efficacy of electrolyzed oxidizing water for inactivating *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes*. Appl. Environ. Microbiol. 65:4276-4279.

Venkitanarayanan, K.S., Ezeike, G.O. Hung, Y.C., and M.P. Doyle. 1999b. Inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on plastic kitchen cutting boards by electrolyzed oxidizing water. J. Food Prot. 62:857-860.

Vialette, M., A. Pinon, E. Chasseignaux, and M. Lange. 2003. Growth kinetics comparison of clinical and seafood Listeria monocytogenes isolates in acid and osmotic environment. Int. J. Food Microbiol. 82:121-131.

Visick, J. and S. Clarke. 1995. Repair, refold, recycle: how bacteria can deal with spontaneous and environmental damage to proteins. Mol. Microbiol. 16:835-845.

Voyle, C.A. 1974. Structural and histological changes associated with the freezing of meat. pp. 6.1-6.6. In: Meat freezing: Why and how?, C.L. Cutting (Ed.). Meat Research Institute Symposium Number 3.

Wai, S.N., A. Takade, and K. Amako. 1995. The release of outer membrane vesicles from the strains of enterotoxigenic *Escherichia coli*. Microbiol. Immunol. 39:451-456.

Wai, S.N., A. Takade, and K. Amako. 1996. The hydrophobic surface protein layer of enteroaggregative *Escherichia coli* starins. FEMS Microbiol. Lett. 135:17-22.

Wallace, F.M., J.E Call, A.C.S. Porto, G.J. Cocoma, The ERRC Special Projects Team, and J.B. Luchansky. 2003. Recovery rate of *Listeria monocytogenes* from commercially prepared frankfurters during extended refrigerated storage. J. Food Prot. 66:584-591.

Wang, G. and M.P. Doyle. 1998. Heat shock response enhances acid tolerance of *Escherichia coli* O157:H7. Lett. Appl. Microbiol. 26:31-34.

Waterman, S.R. and P.L.C. Small. 1998. Acid-sensitive enteric pathogens are protected from killing under extremely acidic conditions of pH 2.5 when they are inoculated onto certain food sources. Appl. Environ. Microbiol. 64:3882-3886.

Weaver, R.A. and Shelef L.A. 1993. Antilisterial activity of sodium, potassium or calcium lactate in pork liver sausage. J. Food Safety 13:133-146.

Wederquist, H.J., J.N. Sofos, and G.R. Schmidt. 1994. *Listeria monocytogenes* inhibition in refrigerated vacuum packaged turkey bologna by chemical additives. J. Food Sci. 59:498-500, 516.

Weis, J. and H.P.R. Seeliger. 1975. Incidence of *Listeria monocytogenes* in nature. Appl. Microbiol. 30:29-32.

Wesley, I.V. 1999. Listeriosis in animals. pp. 39-73. In: *Listeria*, listeriosis and food safety, E.T. Ryser and E.H. Marth (Eds.). Marcel Dekker Inc., New York, NY.

Whittam, T.S., M.L. Wolfe, I.K. Wachsmuth, F. Ørskov, I. Ørskov, and R.A. Wilson. 1993. Clonal relationships among *Escherichia coli* strains that cause hemorrhagic colitis and infantile diarrhea. Infect. Immun. 61:1619-1629.

Willshaw, G.A., T. Cheasty, B. Rowe, H.R. Smith, D.N. Faithfull-Davies, and T.G. Brooks. 1995. Isolation of enterotoxigenic *Escherichia coli* from British troops in Saudi Arabia. Epidemiol. Infect. 115:455-463.

Wistow, G. 1990. Cold shock and DNA binding. Nature 344:823-824.

Wong, A.C.L. 1998. Biofilms in food processing environments. J. Dairy Sci. 81:2765-2770.

Woody, J.-M., R.A. Walsh, S. Doores, W.R. Henning, R.A. Wilson, and S.J. Knabel. 2000. Role of bacterial association and penetration on destruction of Escherichia coli O157:H7 in beef tissue by high pH. J. Food Prot. 63:3-11.

Wooton, A.E. 1986. Factors affecting the chilling rate of beef sides. pp. 115-121. In: Proceedings of the International Institute of Refrigeration, Commission C2, Volume 3. Bristol: I.I.F./I.I.R.

Yamamori, T. and T. Yura. 1982. Genetic control of heat-shock protein synthesis and its bearing on growth and thermal resistance in *Escherichia coli* K-12. Proc. Natl. Acad. Sci. USA 79:860-864.

Yamamoto, S., K. Nakata, K. Yuri, H. Katae, A. Terai, H. Kurazono, Y. Takeda, and O. Yoshida. 1996. Assessment of the significance of virulence factors of uropathogenic

Escherichia coli in experimental urinary tract infection in mice. Microbiol. Immunol. 40:607-610.

Yamanaka, K. and M. Inouye. 1997. Growth-phase-dependent expression of *cspD*, encoding a member of the *cspA* family in *Escherichia coli*. J. Bacteriol. 179:5126-5130.

Yamanaka, K. T. Mitani, T. Ogura, H. Niki, and S. Hiraga. 1994. Cloning, sequencing and characterization of multi-copy suppressors of a *mukB* mutation in *Escherichia coli*. Mol. Microbiol. 13:301-312.

Yamanaka, K., L. Fang and M. Inouye. 1998. The CspA family in *Escherichia coli*: multiple gene duplication for stress adaptation. Mol. Microbiol. 27:247-255.

Yoon, K.S., C.N. Burnette, and R.C. Whiting. 2003. Effects of pH and agitation on the growth of *Listeria monocytogenes* Scott A in Brain Heart Infusion broth containing combined potassium lactate and sodium diacetate during storage at 4 or 10°C. J. Food Prot. 66:1469-1473.

Yousef, A.E. and P.D. Courtney. 2003. Basics of stress adaptation and implications in new-generation foods. pp. 1-31. In: Microbial stress adaptation and food safety, A.E. Yousef and V.K. Juneja (Eds.). CRC Press, New York, NY.

Yousef, A.E., J.B. Luchansky, A.J. Degnan, and M.P. Doyle. 1991. Behavior of *Listeria monocytogenes* in wiener exudates in the presence of *Pediococcus acidilactici* H or pediocin AcH during storage at 4 or 25°C. Appl. Environ. Microbiol. 57:1461-1467.