DISSERTATION

CONTROL OF *ESCHERICHIA COLI* O157:H7 AND *LISTERIA MONOCYTOGENES* IN MEAT AND POULTRY PRODUCTS WITH CHEMICALS AND HEATING TREATMENTS

Submitted by

Cangliang Shen

Department of Animal Sciences

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SURVERVISION BY CANGLIANG SHEN ENTITLED CONTROL OF *ESCHERICHIA COLI* O157:H7 AND *LISTERIA MONOCYTOGENES* IN MEAT AND POULTRY PRODUCTS WITH CHEMICALS AND HEATING TREATMENTS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Patricia Kendall

Keith Belk

John A. Scanga

Advisor John Sofos

Department Head William R. Wailes, Jr.

ABSTRACT OF DISSERTATION

CONTROL OF *ESCHERICHIA COLI* O157:H7 AND *LISTERIA MONOCYTOGENES* IN MEAT AND POULTRY PRODUCTS WITH CHEMICALS AND HEATING TREATMENTS

Objectives of studies included in this dissertation were to evaluate the effects of chemical antimicrobials, such as cetylpyridinium chloride, lactic acid, hops beta acids, commercial salad dressings, and heat treatments including cooking with various appliances and microwave oven heating to control *Escherichia coli* O157:H7 in moisture enhanced noninact beef and *Listeria monocytogenes* on ready-to-eat (RTE) meat and poultry products. In the first study, the effect of different cooking appliances on thermal inactivation of *E. coli* O157:H7 in nonintact beef steaks of different thickness was evaluated. In general, the thicker the steaks, the higher the reduction levels reached, and roasting in a standard kitchen oven showed the best inactivation effect compared to the other cooking appliances. The second study evaluated thermal inactivation of *E. coli* O157:H7 in nonintact beef steaks with pan-broiling or roasting appliances set at different temperatures. Results showed that setting the cooking appliances at higher temperatures resulted in higher reduction levels of *E. coli* O157:H7 cells in nonintact beef steaks compared to the lower ones. The third study involved comparison of inactivation of different types of stress-adapted or unstressed *E. coli* O157:H7 cells, inoculated in moisture enhanced nonintact beef steaks with various brining solutions and cooked by pan-broiling on an electric skillet. It was evident that acid stress-adapted cells were more resistant to heat treatment, while cold or desiccation stress-adapted cells were more

sensitive to heat treatment than controls. The lowest pathogen counts survived during cooking of beef steaks moisture enhanced to include cetylpyridinium chloride or lactic acid for all stressed inocula tested, thus, indicating that cetylpyridinium chloride and lactic acid could be considered as potential antimicrobial agents for use in beef brining solutions. An additional study evaluated the antilisterial activity of hops beta acids (HBA) in broth medium. HBA exhibited promising antilisterial activity in culture broth, and its activity was increased with increasing concentrations (0.5 to 5.0 μg/ml), and when combined with potassium lactate (1%), sodium diacetate (0.25%), or acetic acid (0.1%), at 4°C. In a subsequent study, HBA applied as dipping solutions (0.03 to 0.10%) on frankfurters, inoculated with *L. monosytogenes*, vacuum packaged and stored at 4 or 10°C, inhibited pathogen growth for 30 to 50 (4°C) or 20 to 28 days (10°C). The last two studies were designed to detect the antilisterial effects of commercial salad dressings, oil with vinegar or lemon juice, on artificially inoculated frankfurters, diced ham and turkey breast during simulated home storage, without or with prior microwave heating for 30 s or 45 s. Results indicated that microwave heating followed by immersing in salad dressings, especially oil with vinegar, could potentially contribute to control of *L. monocytogenes* on RTE meat and poultry products in the home environment. The results of all studies in this dissertation indicated that certain chemical antimicrobials and heating treatments could be effectively to control *E. coli* O157:H7 and *L. monocytogenes* in meat and poultry products.

Cangliang Shen
Department of Animal Sciences
Colorado State University
Fort Collins, CO80523
Spring 2010

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TABLE OF CONTENTS

ABSTRACT OF DISSERTATION..... iii

ACKNOWLEDGEMENTS..... v

TABLE OF CONTENTS..... vi

LIST OF TABLES..... xiii

LIST OF FIGURES..... xxi

CHAPTER 1..... 1

INTRODUCTION..... 1

CHAPTER 2..... 8

LITERATURE REVIEW..... 8

2.1. Translocation of surface contamination into inner tissues..... 8

2.2. Inactivation of *E. coli* O157:H7 by cooking..... 10

2.3. Inactivation of *E. coli* O157:H7 by adding antimicrobials in brine solutions..... 13

2.3.1. Cetylpyridinium chloride..... 14

2.3.2. Sodium metasilicate..... 15

2.3.3. Lactic acid..... 16

2.4. Stress resistance, adaptation and cross-protection of microorganisms..... 17

2.4.1. Resistance and adaptation of microorganisms to food processing stresses..... 17

2.4.2. Acid stress..... 17

2.4.3. Cold stress..... 18

2.4.4. Heat stress..... 19

2.4.5. Starvation stress..... 19

2.4.6. Desiccation stress..... 20

2.4.7. Cross-protection of acid and starvation stressed *E. coli* O157:H7 cells..... 21

2.5. Control of *Listeria monocytogenes* by chemical antimicrobials..... 23

2.6. Control of foodborne pathogens by plant extracts including hops beta acids..... 26

2.7. Control of foodborne pathogens by salad dressings..... 29

2.8. Control of foodbrone pathogens by microwaving heating..... 33

CHAPTER 3..... 37

INACTIVATION OF *ESCHERICHIA COLI* O157:H7 IN NONINACT BEEF STEAKS OF DIFFERENT THICKNESS COOKED BY PAN-BROILING, DOUBLE PAN-BROILING OR ROASTING USING FIVE TYPES OF COOKING APPLIANCES..... 37

ABSTRACT..... 37

3.1. INTRODUCTION..... 38

3.2. MATERIALS AND METHODS..... 41

3.2.1. Bacterial strains and inoculum preparation..... 41

3.2.2. Preparation of noninact beef steaks..... 42

3.2.3. Cooking of noninact beef steaks..... 43

3.2.4. Microbiological, physical, and chemical analyses..... 44

3.2.5. Statistical analysis..... 45

3.3. RESULTS AND DISCUSSION..... 46

3.3.1. Temperatures of cooking appliances and steaks..... 46

3.3.2. Cooking losses, pH, water activity, and moisture and fat contents... 49

3.3.3. Cooking inactivation effects on bacterial populations..... 50

3.4. CONCLUSIONS..... 55

CHAPTER 4..... 68

THERMAL INACTIVATION OF *ESCHERICHIA COLI* O157:H7 IN MOISTURE ENHANCED NONINTACT BEEF BY PAN-BROILING OR ROASTING IN VARIOUS COOKING APPLIANCES SET AT DIFFERENT TEMPERATURES..... 68

ABSTRACT..... 68

4.1. INTRODUCTION..... 69

4.2. MATERIALS AND METHODS..... 71

4.2.1. Bacterial strains and inoculum preparation..... 71

4.2.2. Preparation and inoculation of nonintact beef steak..... 72

4.2.3. Cooking of beef steaks..... 72

4.2.4. Physical and chemical analyses..... 73

4.2.5. Microbiological analyses..... 74

4.2.6. Statistical analysis..... 75

4.3. RESULTS AND DISCUSSION..... 75

4.3.1. Temperatures of cooking appliances..... 75

4.3.2. Temperature of steaks..... 76

4.3.3. Cooking losses, pH, water activity, and moisture and fat contents... 79

4.3.4. Cooking inactivation effects on the pathogen..... 79

4.4. CONCLUSIONS..... 81

CHAPTER 5..... 92

THERMAL INACTIVATION OF ACID, COLD, HEAT, STARVATION, AND DESICCATION STRESS-ADAPTED *ESCHERICHIA COLI* O157:H7 IN MOISTURE ENHANCED NONINTACT BEEF..... 92

ABSTRACT..... 92

5.1. INTRODUCTION..... 94

5.2. MATERIALS AND METHODS..... 97

5.2.1. Bacterial strains and stress-adapted inoculum preparation..... 97

5.2.2. Preparation and inoculation of nonintact beef steaks..... 100

5.2.3. Cooking of beef steaks..... 101

5.2.4. Physical and chemical analyses..... 101

5.2.5. Microbiological analyses..... 102

5.2.6. Statistical analysis..... 102

5.3. RESULTS AND DISCUSSION..... 103

5.3.1. Temperatures of electric skillet and steak samples..... 103

5.3.2. Cooking losses, pH, water activity, and moisture and fat contents... 104

5.3.3. Microbial data on unstressed and stress-adapted cultures before cooking..... 106

5.3.4. Cooking inactivation effects on unstressed and stress-adapted cultures..... 107

5.4. CONCLUSIONS..... 111

CHAPTER 6..... 118

ANTILISTERIAL ACTIVITY OF HOPS BETA ACIDS IN BROTH WITH OR WITHOUT OTHER ANTIMICROBIALS..... 118

ABSTRACT..... 118

6.1. INTRODUCTION..... 119

6.2. MATERIALS AND METHODS..... 122

6.2.1. Bacterial strains and inoculum preparation..... 122

6.2.2. Antimicrobial treatments and analyses..... 123

6.2.3. Statistical analysis..... 124

6.3. RESULTS AND DISCUSSION..... 125

6.4. CONCLUSIONS..... 128

CHAPTER 7..... 137

CONTROL OF *LISTERIA MONOCYTOGENES* ON FRANKFURTERS BY DIPPING IN HOPS BETA ACIDS SOLUTIONS..... 137

ABSTRACT..... 137

7.1. INTRODUCTION..... 138

7.2. MATERIALS AND METHODS..... 140

7.2.1. Preparation of frankfurters..... 140

7.2.2. Bacterial strains and inoculum preparation..... 141

7.2.3. Product inoculation and treatment..... 142

7.2.4. Microbiological, chemical and physical analyses..... 143

7.2.5. Data analysis..... 144

7.3. RESULTS AND DISCUSSION..... 145

7.3.1. Physcial and chemical properties..... 145

7.3.2. Reduction of *L. monocytogenes* on frankfurters..... 146

7.3.3. Survival and growth of *L. monocytogenes* on frankfurters dipped in HBA solutions..... 147

7.4. CONCLUSIONS..... 149

CHAPTER 8..... 154

ANTILISTERIAL ACTIVITIES OF SALAD DRESSINGS, WITHOUT OR WITH PRIOR MICROWAVE OVEN HEATING, ON FRANKFURTERS DURING SIMULATED HOME STORAGE..... 154

ABSTRACT..... 154

8.1. INTRODUCTION..... 155

8.2. MATERIALS AND METHODS..... 159

8.2.1. Preparation of frankfurters..... 159

8.2.2. Preparation of inocula and inoculation of frankfurters..... 160

8.2.3. Frankfurters treatment..... 161

8.2.4. Microbiological, chemical and physical analyses of samples..... 162

8.2.5. Statistical analysis..... 163

8.3. RESULTS AND DISCUSSION..... 164

8.3.1. Chemical and physical properties of frankfurters..... 164

8.3.2. Growth of *L. monocytogenes* on frankfurters during aerobic storage at 7°C..... 164

8.3.3. Antilisterial activities of salad dressings without prior microwave heating..... 165

8.3.4. Antilisterial activities of salad dressings with prior microwave heating..... 167

8.4. CONCLUSIONS..... 168

CHAPTER 9..... 174

EVALUATION OF SALAD DRESSINGS FOR CONTROL OF *LISTERIA MONOCYTOGENES* ON DICED HAM AND TURKEY BREAST WITHOUT OR WITH PRIOR MICROWAVE OVEN HEATING..... 174

ABSTRACT..... 174

9.1. INTRODUCTION..... 175

9.2. MATERIALS AND METHODS..... 178

9.2.1. Product inoculation..... 178

9.2.2. Product treatments..... 179

9.2.3. Microbiological, chemical and physical analyses of samples..... 180

9.2.4. Statistical analysis..... 181

9.3. RESULTS AND DISCUSSION..... 182

9.3.1. Physical and chemical properties of ham and turkey breast..... 182

9.3.2. Microbial growth on diced ham and turkey breast during aerobic storage 183

9.3.3. Antilisterial effects of salad dressings without prior microwave heating..... 184

9.3.4. Antilisterial effects of salad dressings with prior microwave heating..... 186

9.4. CONCLUSIONS..... 188

CHAPTER 10..... 204

SUMMARY OF DISSERTATION..... 204

REFERENCES..... 211

APPENDIX..... 256

LIST OF TABLES

Table number	Title	Page
Table 2.1	The conundrum of terminologies of cooking methods.....	13
Table 3.1	Terminologies used to describe various cooking methods, and appliances used for cooking in this study.....	57
Table 3.2	Mean (± standard deviation) of time (min) to reach the internal (geometric center) temperature of 65°C during cooking of nonintact beef steaks of different thickness.....	58
Table 3.3	Mean (± standard deviation) cooking losses (%) of nonintact beef steaks of different thickness cooked to the internal (geometric center) temperature of 65°C with different cooking appliances.....	59
Table 3.4	Mean (± standard deviation) pH and water activity values of nonintact beef steaks of different thickness uncooked or cooked to the internal (geometric center) temperature of 65°C with different cooking appliances.....	60
Table 3.5	Mean (± standard deviation) moisture and fat content (%) of nonintact beef steaks of different thickness uncooked or cooked to the internal (geometric center) temperature of 65°C with different cooking appliances.....	61
Table 3.6	Mean (± standard deviation; log CFU/g) total bacterial and rifampicin-resistant *Escherichia coli* O157:H7 populations, recovered with tryptic soy agar supplemented with sodium pyruvate (0.1%, TSAP) and TSAP plus rifampicin (100 µg/ml), respectively, from nonintact beef steaks of different thickness that were uncooked and cooked with different appliances to the internal (geometric center) temperature of 65°C.....	62
Table 4.1	Mean (± SD) cooking losses (%) of nonintact beef steaks cooked with various cooking appliances set at different temperatures.....	83
Table 4.2	Mean (± SD) pH, water activity, moisture and fat content (%) of uncooked and cooked nonintact beef steaks cooked with various appliances set at different temperatures.....	84
Table 5.1	Mean (± SD) weight losses (%) of nonintact beef steaks moisture enhanced with various brining solutions before and after cooking to 65°C.....	112

Table 5.2 Mean (± SD) pH of nonintact beef steaks moisture enhanced with various brining solutions before and after cooking to 65°C..... 112

Table 5.3 Mean (± SD) water activity of nonintact beef steaks moisture enhanced with various brining solutions before and after cooking to 65°C..... 113

Table 5.4 Mean (± SD) moisture and fat content (%) of nonintact beef steaks moisture enhanced with various brining solutions before and after cooking to 65°C..... 113

Table 5.5 Mean (± SD, log CFU/g, n=6) of total bacterial counts recovered with tryptic soy agar plus pyruvate (0.1%, TSAP) from uncooked and cooked nonintact beef steaks treated with brines containing various antimicrobials and cooked to the internal temperature of 65°C..... 114

Table 6.1 Mean (± standard deviation) pH values of *Listeria monocytogenes* cultures in tryptic soy broth supplemented with 0.6% yeast extract (TSBYE) inoculated with hops beta acid (HBA), potassium lactate (PL), sodium diacetate (SD) or acetic acid (Ac) alone or in combination stored at 4°C for 35 days..... 130

Table 6.2 Mean (± standard deviation) pH values of *Listeria monocytogenes* cultures in tryptic soy broth supplemented with 0.6% yeast extract (TSBYE) with hops beta acid (HBA), potassium lactate (PL), sodium diacetate (SD) or acetic acid (Ac) alone or in combination stored at 10°C for 20 days..... 131

Table 6.3 Mean (± standard deviation) pH values of *Listeria monocytogenes* cultures in tryptic soy broth supplemented with 0.6% yeast extract (TSBYE) with hops beta acid (HBA), potassium lactate (PL), sodium diacetate (SD) or acetic acid (Ac) alone or in combination stored at 25°C for 2 days..... 132

Table 6.4 Growth kinetics of *Listeria monocytogenes* in tryptic soy broth supplemented with 0.6% yeast extract containing hops beta acids (HBA) and stored at 4°C for 35 days, 10°C for 20 days, or 25°C for 2 days; initial inoculum was 2.6-2.8 log CFU/ml..... 133

Table 6.5 Growth kinetics of *Listeria monocytogenes* in tryptic soy broth supplemented with 0.6% yeast extract with hops beta acids (HBA) and potassium lactate (PL) in combination and stored at 4°C for 35 days, 10°C for 20 days, or 25°C

for 2 days; initial inoculum was 2.6-2.8 log CFU/ml..... 134

Table 6.6 Growth kinetics of *Listeria monocytogenes* in tryptic soy broth supplemented with 0.6% yeast extract with hops beta acids (HBA) and sodium diacetate (SD) in combination and stored at 4°C for 35 days, 10°C for 20 days, or 25°C for 2 days; initial inoculum was 2.6-2.8 log CFU/ml..... 135

Table 6.7 Growth kinetics of *Listeria monocytogenes* in tryptic soy broth supplemented with 0.6% yeast extract containing hops beta acids (HBA) and acetic acid (Ac) in combination and stored at 4°C for 35 days, 10°C for 20 days, or 25°C for 2 days; initial inoculum was 2.6-2.8 log CFU/ml..... 136

Table 7.1 Growth kinetics of *Listeria monocytogenes* on the surface of untreated (control) frankfurters, and frankfurters dipped (2 min) in distilled water or hops beta acids (HBA), then vacuum packaged and stored for 90 days at 4°C or 48 days at 10°C..... 151

Table 8.1 Mean pH values of frankfurters inoculated with *Listeria monocytogenes* and stored aerobically at 7°C for 14 days. Samples were untreated (CON) or immersed for 5 or 20 min in distilled water (DW), sunflower oil + lemon juice (SL) or vinegar (SV), extra virgin olive oil + lemon juice (EL) or vinegar (EV), Vinaigrette (V), Ranch (R), Thousand Island (T) or Caesar (C), withoutor with prior microwave heating for 30 s, on days 0, 7, and 14 of storage..... 170

Table 8.2 Mean (± SD) counts (log CFU/cm^2, n=6) of total microbial flora on frankfurters inoculated with *Listeria monocytogenes* and stored aerobically at 7°C for 14 days. Samples were untreated (CON) or immersed for 5 or 20 min in distilled water (DW), sunflower oil + lemon juice (SL) or vinegar (SV), extra virgin olive oil + lemon juice (EL) or vinegar (EV), Vinaigrette (V), Ranch (R), Thousand Island (T) or Caesar (C), without or with prior microwave heating for 30 s, on days 0, 7, and 14 of storage..... 171

Table 9.1 Mean (± SD) pH values of diced ham samples inoculated with *Listeria monocytogenes* and stored (day-0) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s..... 189

Table 9.2 Mean (± SD) pH values of diced ham samples inoculated

with *Listeria monocytogenes* and stored (day-7) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s or 45 s..... 190

Table 9.3 Mean (± SD) pH values of diced ham samples inoculated with *Listeria monocytogenes* and stored (day-14) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s or 45 s..... 191

Table 9.4 Mean (± SD) pH values of diced turkey breast samples inoculated with *Listeria monocytogenes* and stored (day-0) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s..... 192

Table 9.5 Mean (± SD) pH values of diced turkey breast samples inoculated with *Listeria monocytogenes* and stored (day-5) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s or 45 s..... 193

Table 9.6 Mean (± SD) pH values of diced turkey breast samples inoculated with *Listeria monocytogenes* and stored (day-9) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s or 45 s..... 194

Table 9.7 Mean (± SD) counts (log CFU/cm^2, n=6) of total microorganisms on diced ham that were inoculated with *Listeria monocytogenes* and stored (day-0) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s..... 195

Table 9.8 Mean (± SD) counts (log CFU/cm^2, n=6) of total microorganisms on diced ham that were inoculated with *Listeria monocytogenes* and stored (day-7) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin

	olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s or 45 s.....	196
Table 9.9	Mean (± SD) counts (log CFU/cm^2, n=6) of total microorganisms on diced ham that were inoculated with *Listeria monocytogenes* and stored (day-14) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s or 45 s.....	197
Table 9.10	Mean (± SD) counts (log CFU/cm^2, n=6) of total microorganism on diced turkey breasts that that were inoculated with *Listeria monocytogenes* and stored (day-0) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s.....	198
Table 9.11	Mean (± SD) counts (log CFU/cm^2, n=6) of total microorganism on diced turkey breasts that that were inoculated with *Listeria monocytogenes* and stored (day-5) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s or 45 s.....	199
Table 9.12	Mean (± SD) counts (log CFU/cm^2, n=6) of total microorganism on diced turkey breasts that that were inoculated with *Listeria monocytogenes* and stored (day-9) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s or 45 s.....	200
Appendix Table 1 (Figure 3.1)	Cooking time (min) and temperature (°C) for nonintact beef steaks of different thickness cooked by pan-broiling using the Presto® electric skillet.....	257
Appendix Table 2 (Figure 3.2)	Cooking time (min) and temperature (°C) for nonintact beef steak of different thickness cooked by pan-broiling using the Sanyo® grill.....	258
Appendix Table 3 (Figure 3.3)	Cooking time (min) and temperature (°C) for nonintact beef steaks of different thickness cooked by double pan-broiling using the Geogre Forman® grill.....	259
Appendix Table 4	Cooking time (min) and temperature (°C) for nonintact	

(Figure 3.4)	beef steaks of different thicknes cooked by roasting using the Oyster® toaster oven.....	260
Appendix Table 5 (Figure 3.5)	Cooking time (min) and temperature (°C) for nonintact beef steaks of different thickness cooked by roasting using the Magic Chef ®kitchen oven.....	261
Appendix Table 6 (Figure 4.1)	Cooking time (min) and temperature (°C) of pan-broiling and roasting appliances set and maintained at different temperatures setting during cooking of nonintact beef steaks.....	262
Appendix Table 7 (Figure 4.2)	Cooking time (min) and temperature (°C) of nonintact beef steaks cooked by pan-broiling using the Presto® electric skillet setting at 149 or 204°C.....	263
Appendix Table 8 (Figure 4.3)	Cooking time (min) and temperature (°C) of nonintact beef steaks cooked by pan-broiling using the Sanyo® grill setting at 149 or 218°C.....	264
Appendix Table 9 (Figure 4.4)	Cooking time (min) and temperature (°C) for nonintact beef steaks cooked by roasting using the Oyster® toaster oven setting at 149 or 232°C.....	265
Appendix Table 10 (Figure 4.5)	Cooking time (min) and temperature (°C) for nonintact beef steaks cooked by roasting using the Magic Chef® kitchen oven setting at 149, 204, or 260°C.....	266
Appendix Table 11 (Figures 4.6; 4.7)	Mean (± SD, log CFU/g, n=9) of *Escherichia coli* O157:H7 and total microbial counts recovered with tryptic soy agar plus pyruvate (0.1%, TSAP) plus rifampicin (100 µg/ml) and TSAP from uncooked and cooked nonintact beef steaks to the internal temperature of 65°C with a Presto® electric skillet, a Sanyo® grill, an Oyster® toaster oven, and a Magic Chef® kitchen oven set at different temperatures.....	267
Appendix Table 12 (Figure 5.1)	Temperature changes of the surface of Presto® electric skillet and the lower, and upper surface, edge and geometric center of nonintact beef steaks during cooking to the internal geometric center temperature of 65°C.....	268
Appendix Table 13 (Figure 5.2)	Mean (± SD, log CFU/g, n=6) unstressed, acid, cold, heat, starvation or desiccation stress adapted *Escherichia coli* O157:H7 recovered with tryptic soy agar plus pyruvate (0.1%, TSAP) plus rifampicin (100µg/ml) from uncooked and cooked nonintact beef steaks with brines containing different antimicrobials and cooked to internal temperature of 65°C.....	269

Appendix Table 14 (Figure 7.1) Mean pH values of untreated (control) frankfurters, and frankfurters dipped (2 min) in distilled water (DW) or hops beta acids (HBA) and stored for 90 or 48 days under vacuum at 4 (A) or 10°C (B), respectively..... 270

Appendix Table 15 (Figure 7.2) Mean (log CFU/cm^2) *L. monocytogenes* (PALCAM agar; A) and total microbial (TSAYE; B) populations on the surface of untreated (control) frankfurters, and frankfurters dipped (2 min) in distilled water (DW) or hops beta acids (HBA) during storage in vacuum packages at 4°C for 90 days..... 271

Appendix Table 16 (Figure 7.2) Mean (log CFU/cm^2) *L. monocytogenes* (PALCAM agar; A) and total microbial (TSAYE; B) populations on the surface of untreated (control) frankfurters, and frankfurters dipped (2 min) in distilled water (DW) or hops beta acids (HBA) during storage in vacuum packages at 10°C for 48 days..... 272

Appendix Table 17 (Figures 8.1; 8.2) Mean (± SD) populations (log CFU/cm^2, n=6) of *Listeria monocytogenes* on frankfurters inoculated and stored aerobically at 7°C for 14days. Samples were left untreated (CON) or immersed for 5 or 20 min in distilled water (DW), sunflower oil + lemon juice (SL) or vinegar (SV), extra virgin olive oil + lemon juice (EL) or vinegar (EV), Vinaigrette (V), Ranch (R), Thousand Island (T) or Caesar (C), without or with prior microwave heating for 30 s, on days 0, 7 and 14 of storage..... 273

Appendix Table 18 (Figures 9.1; 9.2) Mean (± SD) populations (log CFU/cm^2, n=6) of *Listeria monocytogenes* on diced ham that were inoculated and stored (day-0) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s..... 274

Appendix Table 19 (Figures 9.1- 9.3) Mean (± SD) populations (log CFU/cm^2, n=6) of *Listeria monocytogenes* on diced ham that were inoculated and stored (day-7) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s or 45 s..... 275

Appendix Table 20 (Figures 9.1- 9.3) Mean (± SD) populations (log CFU/cm^2, n=6) of *Listeria monocytogenes* on diced ham that were inoculated and stored (day-14) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon

juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s or 45 s..... 276

Appendix Table 21 (Figures 9.1; 9.2) Mean (± SD) populations (log CFU/cm^2, n=6) of *Listeria monocytogenes* on diced turkey breast that were inoculated and stored (day-0) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s..... 277

Appendix Table 22 (Figures 9.1-9.3) Mean (± SD) populations (log CFU/cm^2, n=6) of *Listeria monocytogenes* on diced turkey breast that were inoculated and stored (day-5) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s or 45 s..... 278

Appendix Table 23 (Figures 9.1-9.3) Mean (± SD) populations (log CFU/cm^2, n=6) of *Listeria monocytogenes* on diced turkey breast that were inoculated and stored (day-9) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s or 45 s..... 279

LIST OF FIGURES

Figure number	Title	Page
Figure 3.1 (Appendix Table 1)	Cooking time and temperature curves for nonintact beef steaks of different thickness cooked by pan-broiling using the Presto® electric skillet. Each point is the average of four determinants.....	63
Figure 3.2 (Appendix Table 2)	Cooking time and temperature curves for nonintact beef steaks of different thickness cooked by pan-broiling using the Sanyo® grill. Each point is the average of four determinants.....	64
Figure 3.3 (Appendix Table 3)	Cooking time and temperature curves for nonintact beef steaks of different thickness cooked by double pan-broiling using the George Foreman® grill. Each point is the average of four determinants.....	65
Figure 3.4 (Appendix Table 4)	Cooking time and temperature curves for nonintact beef steaks of different thickness cooked by roasting using the Oster® toaster oven. Each point is the average of four determinants.....	66
Figure 3.5 (Appendix Table 5)	Cooking time and temperature curves for nonintact beef steaks of different thickness cooked by roasting using the Magic Chef® kitchen oven. Each point is the average of four determinants.....	67
Figure 4.1 (Appendix Table 6)	Temperature curves of pan-broiling and roasting appliances set and maintained at different temperatures settings during cooking of nonintact beef steaks.....	85
Figure 4.2 (Appendix Table 7)	Cooking time (min) and temperature (°C) curves of nonintact beef steaks cooked by pan-broiling using the Presto® electric skillet setting at 149 or 204°C. Each point is the average of four determinants.....	86
Figure 4.3 (Appendix Table 8)	Cooking time (min) and temperature (°C) curves of nonintact beef steaks cooked by pan-broiling using the Sanyo® grill setting at 149 or 218°C. Each point is the average of four determinants.....	87
Figure 4.4 (Appendix Table 9)	Cooking time (min) and temperature (°C) curves of nonintact beef steaks cooked by roasting using the Oster® toaster oven setting at 149 or 232°C. Each point is the average of four determinants.....	88

Figure 4.5 (Appendix Table 10) Cooking time (min) and temperature (°C) curves of nonintact beef steaks cooked by roasting using the Magic Chef® kitchen oven setting at 149, 204 or 260°C. Each point is the average of four determinants..... 89

Figure 4.6 (Appendix Table 11) Mean (± SD, log CFU/g, n=9) of *Escherichia coli* O157:H7 counts recovered with tryptic soy agar plus pyruvate (0.1%, TSAP) plus rifampicin (100 µg/ml) from uncooked and cooked nonintact beef steaks to the internal temperature of 65°C with a Presto® electric skillet, a Sanyo® grill, an Oster® toaster oven, and a Magic Chef® kitchen oven set at different temperatures. a-d: Means with a different letter within a figure are different ($P < 0.05$)..... 90

Figure 4.7 (Appendix Table 11) Mean (± SD, log CFU/g, n=9) of total bacterial counts recovered with tryptic soy agar plus pyruvate (0.1%, TSAP) from uncooked and cooked nonintact beef steaks to the internal temperature of 65°C with a Presto® electric skillet, a Sanyo® grill, an Oster® toaster oven, and a Magic Chef® kitchen oven set at different temperatures. a-d: Means with a different letter within a figure are significantly different ($P < 0.05$)..... 91

Figure 5.1 (Appendix Table 12) Temperature changes of the surface of Presto® electric skillet and lower, and upper surface, edge, and geometric center of nonintact beef steaks during cooking to the internal geometric center temperature of 65°C. Each point is averaged across all brining treatments..... 115

Figure 5.2 (Appendix Table 13) Survivor counts (mean, log CFU/g, n=6) of *Escherichia coli* O157:H7 recovered during cooking (65°C, internal) of non-intact beef steaks, moisture enhanced with variance brining solutions and inoculated with unstressed, acid, cold, heat, starvation and desiccation stress-adapted inocula with tryptic soy agar plus pyruvate plus rifampicin (100 µg/ml)..... 116

Figure 7.1 (Appdendix Table 14) Mean pH values of untreated (control) frankfurters, and frankfurters dipped (2 min) in distilled water (DW) or hops beta acids (HBA) and stored for 90 or 48 days under vacuum at 4 or 10°C, respectively..... 152

Figure 7.2 (Appendix Table 15, 16) Mean (log CFU/cm^2) *L. monocytogenes* (PALCAM agar) and total microbial (TSAYE) populations on the surface of untreated (control) frankfurters, and frankfurters dipped (2 min) in distilled water (DW) or hops beta acids (HBA) during storage in vacuum packages at 4°C for 90 days or 10°C for 48 days..... 153

Figure 8.1 (Appendix Table 17) Mean populations (log CFU/cm^2) of *Listeria monocytogenes* on frankfurters inoculated and stored aerobically at 7°C for 14 days. Samples were left untreated (CON) or immersed for 5 or 20 min in distilled water (DW), sunflower oil + lemon juice (SL) or vinegar (SV), extra virgin olive oil + lemon juice (EL) or vinegar (EV), Vinaigrette (V), Ranch (R), Thousand Island (T) or Caesar (C) on days 0, 7 and 14 of storage..... 172

Figure 8.2 (Appendix Tables 18-23) Mean populations (log CFU/cm^2) of *Listeria monocytogenes* on frankfurters inoculated and store aerobically at 7°C for 14 days. Samples were left untreated (CON) or immersed for 5 or 20 min in distilled water (DW), sunflower oil + lemon juice (SL) or vinegar (SV), extra virgin olive oil + lemon juice (EL) or vinegar (EV), Vinaigrette (V), Ranch (R), Thousand Island (T) or Caesar (C), with prior microwave (MW) heating for 30 s, on days 0, 7 and 14 of storage..... 173

Figure 9.1 (Appendix Tables 18-23) Mean populations (log CFU/cm^2) of *Listeria monocytogenes* inoculated on diced ham or turkey breast inoculated, stored aerobically at 7°C for 14 or 9 days, respectively, and left untreated or immersed for 5 or 10 min in distilled water, sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette or Thousand Island..... 201

Figure 9.2 (Appendix Tables 18-23) Mean populations (log CFU/cm^2) of *Listeria monocytogenes* inoculated on diced ham or turkey breast inoculated, stored aerobically at 7°C for 14 or 9 days, respectively, and left untreated or immersed for 5 or 10 min in distilled water, sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette or Thousand Island, with prior microwave heating (MW) for 30 s..... 202

Figure 9.3 (Appendix Tables 18-23) Mean populations (log CFU/cm^2) of *Listeria monocytogenes* inoculated on diced ham or turkey breast inoculated, stored aerobically at 7°C for 14 or 9 days, respectively, and left untreated or immersed for 5 or 10 min in distilled water, sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette or Thousand Island, with prior microwave heating (MW) for 45 s..... 203

CHAPTER 1

INTRODUCTION

Escherichia coli O157:H7 is a foodborne pathogen which may contaminate foods such as ground meat or nonintact beef products, and has a low infective dose of less than 10 cells (Doyle et al., 1997). Consumption of beef products contaminated with the pathogen by susceptible individuals including children and immunocompromised patients may lead to severe symptoms such as watery or bloody diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (Bacon and Sofos, 2003). It was first identified as a foodborne human pathogen during two hemorrhagic colitis outbreaks associated with consumption of undercooked hamburger patties in the United States in the early 1980s. Following the notable outbreak associated with consumption of undercooked ground beef contaminated by this pathogen in 1992-1993, the USDA-FSIS defined *E. coli* O157:H7 as an adulterant in raw ground beef (USDA-FSIS, 1999) and all nonintact beef products (USDA-FSIS, 1999). An estimated 62,000 cases of symptomatic *E. coli* O157:H7 occur annually in the United States, resulting in approximately 1,800 hospitalizations and 52 deaths (Mead et al., 1999). The United States Department of Agriculture Economic Research Service reported that the estimated cost of foodborne *E. coli* O157:H7 illness totals $ 0.7 billion annually in the United States, including medical costs, productivity losses and premature deaths (available at http://www.ers.usda.gov/briefing/foodborne disease/).

According to the USDA-FSIS, nonintact beef products are those injected with solutions for flavoring or tenderizing; mechanically tenderized by needling, cubing or

pounding devices, and reconstructed into formed entrees like non-intact beef steaks (USDA-FSIS, 2002a; 2002b). Non-intact beef products have been implicated as the source for outbreaks of *E. coli* O157:H7 mainly due to the translocation of pathogen cells from the beef muscle surface to their interior parts by chemical, or mechanical tenderization or restructuring processes (Hajmeer et al., 2000; Ortega-Valenzuela et al., 2001). They have been involved in several relatively recent, from 2000 to 2008, outbreaks of *E. coli* O157:H7 in the United States (USDA-FSIS, 2005a; 2005b; USDA-FSIS 2007a; 2007b; 2007c; 2007d; 2007e; USDA-FSIS, 2008a): (i) Two cases were linked to consumption of needle-tenderized sirloin steaks in Michigan, August, 2000; (ii) Eleven cases in five States were associated with consumption of a boneless beef filet bacon-wrapped steak product injected with marinade, in June, 2003; (iii) Four cases were related to consumption of tenderized and marinated beef steaks in Colorado, in August, 2004; (iv) A total of 116,654 kg of mechanically tenderized and injected beef products was recalled in Pennsylvania, in April, 2007; (v) Approximately 58,050 kg of mechanically tenderized beef products was recalled in Machigan, in March and April, 2007; (vi) A total of approximately 2.57 million kg of both fresh and frozen ground beef products was recalled in California, in April, 2007; (vii) Thirty two illness in nine states were associated with consumption of frozen ground beef hamburgers, and a total of 9.77 million kg of frozen ground beef was recalled in New Jersey, in Spetember, 2007; (viii) A total of approximately 0.6 million kg of prime or subprime cuts of beef was recalled in Nebraska, in August, 2008. In 2007, USDA-FSIS published a recall notice stating that several cases of *E. coli* O157:H7 infection linked to consumption of steaks injected with tenderizers and flavor-enhancing solutions (USDA-FSIS, 2007a). In general, all these outbreaks have been attributed to inadequate cooking (USDA-FSIS, 2005a; 2005b; 2007a) or cooking products directly from the unthawed state (Laine et al., 2005).

The North Carolina State's Division of Environmental Health in the Department of Environment and Natural Resources stated that cooking all muscle beef such as steaks and roasts to an internal temperature of 155°F (68.3°C), as measured by a food thermometer at home, will eliminate the bacteria inside the meat (Division of Environmental Health- Department of Environment and Natural Resources, 2005). The USDA-FSIS indicated that ground beef cooked to an internal temperature of at least 160°F (71.1°C) (USDA-FSIS, 2001), and nonintact blade-tenderized beef steaks, oven-broiled to an internal temperature of 140°F (60°C) or above would not present a great risk to the consumers (USDA-FSIS, 2002a; 2002b). In 2002, a comparative risk assessment for non-intact and intact beef steaks conducted by USDA-FSIS (USDA-FSIS, 2002a; 2002b) indicated that there were insufficient data to determine whether the traditional cooking methods are adequate to destroy *E. coli* O157:H7 translocated during the blade tenderization process. Because proper cooking renders foods microbially safe, it is important to develop effective cooking protocols for non-intact beef products.

However, *E. coli* O157:H7 in non-intact products may survive during cooking and cause illness among consumers if the injected ingredients decrease thermal inactivation effects and protect pathogen cells from heat (Yen et al., 1991, 1992; Calicioglu et al., 2002a). Surviving pathogen cells may grow, or be adapted to the stresses originating in environments representative of fresh beef processing or marination procedures including pH, temperature, osmolarity, starvation, dessication, atmospheric pressure, chemicals, and antimicrobials (Samelis and Sofos, 2003; Yousef and Courtney, 2003). Specifically for *E. coli* O157:H7, cells adapted to sub-lethal levels of heat, acid and osmotic stress may become cross-protected against these stresses. Therefore, it is necessary to evaluate the responses of potentially stress-adapted *E. coli* O157:H7 cells in nonintact beef products representing actual conditions encountered during marination and cooking.

Listeria monocytogenes, the causative agent of listeriosis, is a postprocessing contaminant of ready-to-eat (RTE) meat products and has been associated with multistate outbreaks of listeriosis in the United States through consumption of frankfurters and poultry deli meats (CDC 1998; 1999; 2000; 2002). Although listeriosis outbreaks are not common (3.3 cases per 1,000,000 individuals per year), its fatality rate is high (20-40%) in susceptible individuals including elderly people, pregnant women and neonates, organ transplant recipients, cancer patients, patients with AIDS or diabetics (Goulet and Marchetti, 1996; Jensen et al., 1994). Listeriosis is estimated to cause 2493 cases of illness, 499 deaths and approximately $200 million losses in the United States annually (Mead et al., 1999; CDC, 2002). Contamination of *L. monocytogenes* on RTE meat and poultry products has been an important source of these cases. A cumulative report of Levine et al. (2001) listed 9 different categories of RTE meat and poultry products contaminated by *L. monocytogenes* between 1990 and 1999, including jerky (0.52%), large diameter cooked sausages (1.31%), cooked uncured poultry products (2.12%), roast and corned beef (3.09%), small diameter cooked sausages (3.56%), and sliced ham and luncheon meat (5.16%). The safety concern of *L. monocytogenes* is further highlighted by several multi-state outbreaks of listeriosis from RTE products from 1998 to 2002. During 1998 to 1999, 101 cases and 21 deaths were associated with consumption of frankfurters and deli meats contaminated with *L. monocytogenes* (CDC, 1999). In 2000, a multistate outbreak associated with deli turkey meat resulted in 29 cases, 4 deaths and 3 miscarriages or stillbirths (CDC, 2000). Then in 2002, consumption of sliceable turkey deli meat caused 46 cases, 7 deaths and 3 stillbirth or miscarriages in the northeastern United States (CDC, 2002). Currently, the USDA-FSIS enforces a "zero tolerance" policy and requires the food-processing industry to apply control measures for *L. monocytogenes*

in RTE meat and poultry products, through selection of one of three alternatives: (i) apply processing and antimicrobial interventions to inactivate and inhibit *L. monocytogenes* growth; (ii) use of either processes or antimicrobials to inactivate or control growth; and (iii) employ sanitation measures and environmental testing (USDA-FSIS, 2003).

The addition of antimicrobial compounds generally recognized as safe, such as various organic acids and their salts to product formulations (Samelis et al., 2002a; Barmpalia et al., 2005) and by post-processing product dipping (Samelis et al., 2001; Barmpalia et al., 2004; Geornaras et al., 2005; Geornaras et al., 2006a; 2006b) or spraying (Byelashov et al., 2008) treatments with antimicrobial agents have been demonstrated to be effective for control of *L. monocytogenes* on RTE meat products. However, most antimicrobial agents are synthetic chemical compounds and are not readily accepted by consumers (Sofos et al., 1998). Many consumers prefer to purchase foods containing natural antimicrobials and to use natural antimicrobial systems to maintain food safety in their home environment. Some natural antimicrobials from plants such as lemon, rosemary, grape fruit, and hops have been approved by USDA-FSIS for possible use to reduce pathogenic bacteria in meat and poultry products (USDA-FSIS, 2008b).

Although many *L. monocytogenes* outbreaks have been associated with eating outside the home, research has indicated that foodborne illness is three times more likely to originate in consumer's homes than in commercial cafeterias (Borneff et al., 1988) due to poor food hygiene practices, improper temperature control, and inappropriate refrigerator management (Kennedy et al., 2005; Yang et al., 2006; Kilonzo-Nthenge et al., 2008). *L. monocytogenes* has been found in home kitchen surfaces (Salamina et al., 1996), and it is possible that the presence of raw and processed foods contaminated by *L. monocytogenes* in the refrigerator may cause cross-contamination in the kitchen after the

products are opened (Chen et al., 2001; Gorman et al., 2002; Kusumaningrum et al., 2003). The presence of *L. monocytogenes* in the home food preparation environment, such as the kitchen may be a risk factor for listeriosis and requires development of appropriate risk management strategies for consumers to control *L. monocytogenes* in their home environment.

The overall objective of seven studies included in this dissertation was to evaluate thermal and chemical processes and intervention for control of *E. coli* O157:H7 and *L. monocytogenes* in nonintact reconstructed beef products and on RTE meat and poultry products, respectively. The first three studies were focused on inactivation of *E. coli* O157:H7 in moisture-enhanced reconstructed noninact beef steaks and the last four studies were conducted with *L. monocytogenes* in broth media and on RTE products including frankfurters, ham and turkey breasts. The objective of the first study was to compare thermal inactivation of *E. coli* O157:H7 in moisture-enhanced reconstructed nonintact beef steaks of different thickness, cooked to an internal temperature of 65°C by pan-broiling, double pan-broiling or roasting with five types of different cooking appliances. The objective of the second study was to compare thermal inactivation of *E. coli* O157:H7 in moisture-enhanced reconstructed nonintact beef steaks using pan-broiling or roasting in four types of cooking appliances set at different temperatures to a final internal temperature of 65°C. The objective of the third study was to evaluate the effect of acid, cold, heat, starvation or desiccation stress-adaptation on thermal inactivation of *E. coli* O157:H7 cells in moisture-enhanced reconstructed nonintact beef steaks with different brining solution ingredients cooked by pan-broiling in an electric skillet. The objective of the fourth study was to determine the antilisterial activity of hops beta acids (HBA), alone or in combination with potassium lactate, sodium diacetate or acetic acid, in a culture broth medium stored at 4°C, 10°C or 25°C. The objective of

the fifth study was to evaluate the activity of HBA as dipping solutions against *L. monocytogenes* on frankfurters stored in vacuum-packages at 4°C or 10°C. The objective of the sixth and seventh study was to detect the antilisterial effects of commercial salad dressings and plant oils with lemon juice or vinegar, without or with prior microwave oven heating, on frankfurters, diced ham or turkey breasts during simulated home storage at 7°C for 9 or 14 days.

CHAPTER 2

LITERATURE REVIEW

2.1. Translocation of surface contamination into inner tissues

It is widely recognized that *E. coli* O157:H7 is considered as a biological hazard reasonably likely to occur on the surface of intact meat products. However, a study by Warren (2002) showed that the prevalence of *E. coli* O157:H7 on intact subprimal beef cuts before mechanical tenderization was only 0.2% (2 positive among 1014 samples), and the pathogen counts were less than 0.375 CFU/cm^2 in each of two positive samples. Although the likelihood of potential pathogen cells to be transferred into the inside from the outside of the product is extremely low, due to the very low prevalence of pathogen cells on the product surface, studies with inoculated samples (Sporing, 1999; Lambert et al., 2001; Gill et al. 2005a; 2005b) did show that pathogens would translocate to the inner tissue of the products after they are processed by mechanical tenderization or moisture enhancement. Sporing (1999) reported that approximately 3-4% of surface inoculated *E. coli* O157:H7 was transferred into the geometric center of the steaks during a single-pass blade tenderization process. Gill et al. (2005a) reported that 2.0 and 1.5 log CFU/25g of aerobes and coliforms were recovered from the deep tissue of mechanically tenderized striploins with surface initial counts of 2.8 and 2.0 log CFU/25cm^2 of aerobes and coliforms, respectively. In another study, Gill et al. (2005b) found that the numbers of

aerobic and coliforms recovered from brine injected pork deep tissue were 2.1 and 1 log CFU/25 g, respectively. In a study, inoculating *Salmonella* onto pork, Lambert et al. (2001) found that blade tenderization and needle injection transferred 1-7% and 4-8% of the pathogen cells from the surface into deeper tissue, respectively. The most recent study conducted by Echeverry et al. (2009) showed that 2-4 and 1-3 log CFU/g of *E. coli* O157:H7 and *Salmonella* Typhimurium (an initial inoculation level of 5.0 log CFU/g) were transferred from the surface to the internal muscles after mechanical tenderization and brine needle injection, respectively.

Studies (Sporing, 1999; Thippareddi et al., 2000; Luchansky et al., 2008) also showed that most pathogen cells present on the surface are translocated into places near the surface, and the recovered pathogen numbers decreased with increasing depth of tissue. In the same study, Sporing (1999) found that 6 log CFU/g of *E. coli* O157:H7 recovered from the top 1 cm below the surface of subprimals compared to approximately 3 log CFU/g from the bottom 6 cm depth. In a study, testing penetration of *Salmonella* Typhimurium in pork loins after needle injection, Thippareddi et al. (2000) found that the geometric center harbored 2.0 log CFU/g of the pathogen, which was lower than that on the top 1 cm strip (4.5 log CFU/g). More recently, Luchansky et al. (2008) reported that blade tenderization caused 0.2 to 2.7 log CFU/g of surface inoculated 0.5 to 3.5 log CFU/g of *E. coli* O157:H7 to be transferred into the topmost 1 cm of beef subprimal tissue, and the recovered pathogen levels in deep segments of the tissue ranged from 7 to 34-fold lower than that in the topmost 1 cm.

The potential contamination or subsequent transfer of foodborne pathogen cells into

the inner parts of beef products through mechanical tenderization or moisture enhancement processes, in part, prompted the USDA-FSIS to extend the *E. coli* O157:H7 adulterant policy from ground beef to other noninact products in 1999 (USDA-FSIS, 1999). In 2005, USDA-FSIS informed manufacturers producing mechanically tenderized meat products to reassess their hazard analysis and critical control point (HACCP) plans and take into account the fact that there had been *E. coli* O157:H7 outbreaks associated with consumption of mechanically tenderized beef in the period of 2000-2004 (USDA-FSIS, 2005a).

2.2. Inactivation of *E. coli* O157:H7 by cooking

A series of studies by Gill and his co-workers (Gill and McGinnis, 2004; Gill et al., 2005a; 2005b) have indicated that cooking mechanically tenderized beef or pork products to the internal temperature of medium rare or well done should guarantee safe products. Gill and McGinnis (2004) and Gill et al. (2005a) reported that cooking mechanically tenderized beef or injected moisture-enhanced pork to an internal temperature of 61°C (medium rare) reduced microbial numbers by less than 1 log CFU/10 g in deep tissue of beef samples and allowed survival of only 1.0 log CFU /25 g of aerobic bacteria in deep tissue of pork samples. Another study (Gill et al., 2005b) found that cooking mechanically tenderized striploins to 61°C or 71°C (well done) allowed recovery of aerobes (initial level of 2 log CFU/25 g) only from 2 of 25 samples cooked to each condition. Their most recent study (Gill et al., 2009) was conducted by injecting nonpathogenic *E. coli* and *Listeria innocua* in broth or brining solutions, containing 2% or 5% of sodium chloride and sodium tripolyphosphate that were supplemented with or

without 2% soybean or emulsified sunflower oil, into the centers of 3 cm thick steaks, and cooked to the target temperature of 50 to 70°C. The results showed that supplementing brines with protein or oil did not protect the organisms from injury or heating, and cooking to 60 to 65°C was sufficient to inactivate all bacteria (> 7 log unit) in the meat.

According to the USDA-FSIS, the comparative risk assessment of nonintact and intact beef steaks indicated that oven broiling to an internal temperature of more than 60°C would result in safe blade-tenderized beef steaks (USDA-FSIS 2002a; 2002b), and they also recommended that the starting temperature of the cooking oven should be at 350 °F (176°C) or no lower than 325 °F (162.8°C) when cooking corned beef (USDA-FSIS, 2008c). A cooking study conducted by Sporing (1999) showed that oven-broiled 3.2 cm thick steaks to the internal temperature of 60 to 76.7°C resulted in a 6.5 log reduction, which was greater than that achieved at the internal temperature of 48.9°C or 54.4°C (4.8-log reduction). The most recent cooking study conducted by Luchansky et al. (2009) found that cooking blade-tenderized beef subprimal steaks to internal temperature ranging from 48.8 to 60°C on a commercial open-flame gas grill resulted in 2.6 to 4.6 log CFU/g reductions of surfaced inoculated *E. coli* O157:H7.

Steaks are typically prepared in commercial restaurants or consumer homes using a variety of cooking methods including broiling, grilling, frying and microwaving (Lawrence et al., 2001; Sporing 1999; USDA-FSIS 2002a; 2002b). The survey of food intakes by individuals conducted by the USDA indicated that approximately 30% of consumers grill or broil steaks, while 40% of them fry steaks (USDA-ERS, 1998). Ortega-Valenzuela et al. (2001) determined that broiling was the most effective cooking

method for eliminating *E. coli* O157:H7 compared with gas grilling when the same internal temperature was reached. Sporing (1999) reported approximately 3 to 5, 4 to 6 and 5 to 6 log CFU/g reductions on beef steaks cooked to the internal temperature of 65.6°C using electric skillet frying, gas grilling and oven broiling, respectively; they also determined that cooking effectiveness on pathogen inactivation increased in the order broiling > grilling > frying. Studies of Mukherjee et al. (2007) also showed that broiling was more effective than grilling or frying when reaching the same internal temperature of 60 or 65°C. The possible explanation is that broiling causes more even distribution of heat surrounding the samples, takes longer time to reach the final internal temperature, and allows the temperature of the product near the surface to increase significantly more than by grilling or frying (Sporing, 1999).

In addition to the cooking methods, the thickness of the steaks is another factor to influence the heat transfer through the product and consequently the elimination rates of pathogens inside the steaks. Sporing (1999) reported that thick steaks (3.2 cm) compared to thin ones (1.3 cm) had more reduction of *E. coli* O157:H7 when cooked to the same internal temperature by broiling in a standard kitchen oven. Thippareddi et al. (2000) also found more reduction of *Salmonella* at 71.1°C in nonintact pork loin chops of 2.5 cm thickness compared to 1.25 cm thickness. A possible reason for this is that the thicker the product, the longer cooking time taken to reach the same internal temperature, resulting in overheating of products of the samples, and in higher microbial inactivation levels than in thinner samples.

According to the guidelines of cooking by the American Meat Science Association

(American Meat Science Association, 1995), cooking methods include roasting, broiling and pan-broiling. Roasting is a method transmitting heat to the meat by convection in a closed preheated oven; broiling cooks directly through radiant heat from one direction; and, pan-broiling is used for cooking thin patties by direct heat of conduction. The terminologies of the cooking methods were also described by other names in different research studies, which are shown in Table 2.1.

Table 2.1. The conundrum of terminologies of cooking methods

Heat transferring	What is stated by other studies	What is stated by AMSA	Appliances
Conduction	Grilling (Mukherjee et al., 2007)	Double pan-broiling	George Foreman® grill
Conduction	Frying (Mukherjee et al., 2007); (Sporing, 1999)	Pan-broiling	Presto® electric skillet Sanyo® grill
Convection	Broiling (Mukherjee et al., 2007); (Sporing, 1999); (Kendall et al., 1974)	Roasting	Oster® toaster oven Magic Chef ® kitchen oven

2.3. Inactivation of *E. coli* O157:H7 by adding antimicrobials in brine solutions

Primary brine components injected into moisture-enhanced nonintact beef products are sodium chloride plus sodium polyphosphate or other polyphosphates (Uttaro and Aalhus, 2007). Sodium chloride helps in tenderizing meat by expanding the myofilament lattice within the constraint of actomyosin cross-bridges, while phosphates act as tenderizers by dissociating the actin/myosin complex starting at the edge of the A-band.

The microbial risks associated with needle injection of brine solutions into moisture-enhanced noninact beef products may be controlled through potential use of chemical antimicrobials in brine solutions. The potential antimicrobials include cetylpyridinium chloride, sodium metasilicate, and lactic acid.

2.3.1. Cetylpyridinium chloride

Cetylpyridinium chloride (CPC, 1-hexaadecylpyridium chloride) is a water soluble, colorless, and odorless quaternary ammonium compound (Mastler, 1996). Its mechanisms of killing bacteria include the interaction of cetylpyridinium ions with acid groups of bacteria to form weakly ionized compounds inhibiting bacterial metabolism, and its positively charged cetyl radicals binding on negative charges of bacterial cell walls to destroy cell walls and membranes (Huyck, 1944; Kourai et al., 1985). CPC has been approved by USDA-FSIS as an antimicrobial to treat the surface of raw poultry carcasses prior to immersion in a chiller (USDA-FSIS, 2008b) and has been shown to have good antimicrobial activities (Kim and Slavik, 1995; Cutter et al., 2000; Ransom et al., 2003; Singh et al., 2005; Lim and Mustapha, 2007). Kim and Slavik (1995) reported that spraying 0.1% CPC on chicken skin reduced *Salmonella* Typhimurium by 0.9-1.7 log units. Cutter et al. (2000) found that spraying 1% CPC on lean or adipose beef surfaces immediately reduced *E. coli* O157:H7 and *Salmonella* Typhimurium by as high as 5-6 log CFU/cm^2. Similarly on beef carcass surfaces, Ransom et al. (2003) reported that 0.5% CPC reduced *E. coli* O157:H7 by 2.1-4.8 log CFU/cm^2 and was found to be more effective than other treatments including 2% lactic or acetic acid. Singh et al. (2005) reported that immersing cooked roast beef in 1% CPC solution for 1 min reduced aerobic

and lactic acid bacteria populations by 1 to 1.5 log CFU/cm^2 after 42 days of storage at 0 or 4°C. A study with sliced roast beef conducted by Lim and Mustapha (2007) found that *L. monocytogenes* and *Staphylococcus aureus* were reduced to undetectable levels in 2 h after spraying with 0.5% CPC.

2.3.2. Sodium metasilicate

Sodium metasilicate (SM) is a strong alkali, and the pH of its 0.1-1.0% (wt/wt) solutions ranged from 11.3 to 12.7 (Weber et al., 2004). It is currently approved by FDA (US-FDA, 2003) and USDA-FSIS (USDA-FSIS, 2008b) as a generally recognized as safe (GRAS) ingredient used for decontamination in fruits, vegetables, nuts, as an antimicrobial component of marinades for raw meat and poultry products, and on raw beef carcasses, subprimals, and trimmings. The antimicrobial activities of SM have been well documented in water (Weber et al., 2004), ground beef (Pohlman et al., 2009), and beef tissues (Stopforth et al., 2005). Weber et al. (2004) reported that exposure of *E. coli* O157:H7 (6 log CFU/ml) to SM (0.6%) for 5 to 10 s reducing pathogen cell numbers to undetectable levels. Pohlman et al. (2009) reported that 4% SM reduced *E. coli* O157:H7 counts in ground beef by approximately 1.2 log CFU/g without side sensory effects. A study (Stopforth et al., 2005) conducted in our laboratory showed that dipping beef tissue samples (inoculation level of 5 log CFU/cm^2) into 4% SM solutions for 30 s reduced *Salmonella* Typhimurium by 1.1-1.5 log CFU/cm^2 and *E. coli* O157:H7 by 1.6-2.0 log CFU/cm^2. The antimicrobial effect of SM is possibly attributed to its high pH destroying gram-negative bacteria cell membranes and causing leakage of the cell internal contents (Mendonca et al., 1994).

2.3.3. Lactic acid

In most commercial beef processing plants of the United States, lactic acid has become the most commonly used organic acid to decontaminate beef carcasses during the postevisceration process (Bosilevac et al., 2006). It is usually used as 2% solutions via an online spray cabinet that warms the lactic acid to 55°C (Huffman, 2002). Numerous studies (Cutter et al., 1997; Dorsa et al., 1997; Castillo et al., 1998; 2001; Bacon et al., 2000; Ransom et al., 2003) have reported on the efficacy of lactic acid for sanitizing whole beef carcasses or tissues, which prompted USDA-FSIS to approve use of 5% lactic at a warm temperature of 55°C to decontaminate chilled beef carcasses prior to fabrication (USDA-FSIS, 2008b). In addition to its application on beef carcasses, a recent study (Mukherjee et al., 2009) in our laboratory found that lactic acid (0.27%) enhanced *E. coli* O157:H7 reduction during cooking of ground beef samples to the internal temperature of 65°C.

The results of these studies (Kim and Slavik, 1995; Cutter et al., 1997; Dorsa et al., 1997; Castillo et al., 1998; 2001; Bacon et al., 2000; Cutter et al., 2000; Ransom et al., 2003; Singh et al., 2005; Stopforth et al., 2005; Lim and Mustapha, 2007) indicated that using antimicrobials, such as cetylpyridinium chloride, sodium metasilicate or lactic acid, as a spraying or dipping solution on poultry or beef carcasses before mechanical or chemical tenderization could effectively reduce surface contamination and the translocation of foodborne pathogen into the inner tissue. However, additional studies are needed to detect the antimicrobial effects of these ingredients in noninact beef products inoculated with stress adapted or unstressed cells during cooking.

2.4. Stress resistance, adaptation and cross-protection of microorganisms

2.4.1. Resistance and adaptation of microorganisms to food processing stresses

During processing, including marination, or storage of meat products, food related stresses such as acid, heat, salt and preservatives may occur or may be applied purposefully to control pathogen growth (Archer, 1996; Bower and Daeschel, 1999). For example, the acid washes for carcasses can lead to acid stress (Abee and Wouters, 1999; Dickson and Siragusa, 1994); low nutrient or nutrient competitive environments including equipment surfaces, walls, and floors, can cause starvation stress (Dickson and Frank, 1993; Lou and Yousef, 1996; Matin, 1991); rapid dehydration and exposure to high salt concentrations can cause osmotic injury (Bremer and Kramer, 2000); applying certain sanitizers can lead to oxidative stress (Yousef and Courtney, 2003); refrigerated foods are subjected to cold stress; and shifting food from lower to higher temperatures results in heat shock (Bunning et al., 1990; Farber and Brown, 1990). To survive and grow in these stressful environments, bacterial cells must maintain their cell integrity and homeostatic balance, by increasing cell membrane fluidity (cold stress) (Sofos, 1989), expelling protons and regulating pH membrane gradients (acid or alkaline stress) (Gould, 1995; Sofos and Busta, 1999), and by water efflux from the cell (osmotic stress) (Gould 1995; Pichereau et al., 2000).

2.4.2. Acid stress

Acid stress usually occurs during fermentation of foods, addition of organic acids as preservatives in food processing, or applying organic acids (usually 1-2.5% lactic acid) to decontaminate carcasses (Smith and Palumbo, 1978; Abee and Wouters, 1999; Dickson

and Siragusa, 1994;). The mechanisms of microbial inactivation by organic acids include interfering with the ribosome and protein synthesis, disrupting the proton-motive force, altering the protein profile of the outer membrane (gram-negative cells) or cytoplasmic membrane (gram-positive cells), and damaging the DNA or RNA of microbial cells. The strategies used by microorganisms to resist acid stress include pH homeostasis, changes in membrane structure by alteration of protein permeability, internal buffering ability, and the pH stability of essential proteins (Slonczewski and Foster, 1996). Specifically for *E. coli* cells, three acid resistance systems have been identified in its stationary phase, which include an oxidative system and two fermentative systems associated with a glutamate decarboxylase and an arginine decarboxylase (Lin et al., 1995; 1996).

2.4.3. Cold stress

Cold stress usually occurs when growing bacteria are exposed to a sudden temperature decrease of more than 10°C, which induces cold shock in susceptible microorganisms (Jones et al., 1996). The sensitivity of microbial cells to cold stress varies greatly according to the microbial population density, temperature of growth, rate of cooling, and the temperature differences after cooling occurs (Postgate and Hunter, 1963; Mackey, 1984). The mechanisms developed by microorganisms to cope with cold stress include modifications of the cell membrane to maintain membrane fluidity and the maintenance of macromolecular structural integrity in proteins and ribosomes (Jaenicke 1991; Berry and Foegeding, 1997), and synthesis of cold shock proteins (Jiang et al., 1997). A study conduced by Jones et al. (1987) showed that at least 15 different cold shock proteins observed in cold stress adapted *E. coli* cells, and these proteins play a

variety of functions including transcription, translation, synthesis of proteins, degradation of mRNA, and recombination (Jones et al., 1992; Jones and Inouye, 1994; Jones et al., 1996).

2.4.4. Heat stress

Microbial cells can become heat stress adapted during sublethal thermal processing, such as low temperature pasteurization of eggs, slow roasting of meat products, and long time low temperature heating of sous-vide products. Studies conducted with a fermented beef-pork sausage homogenate have shown that the heat shock response occurred at temperatures as low as 42-48°C in *E. coli* O157:H7 (Murano and Pierson, 1992), *Campylobacter jejuni* (Palumbo, 1984), *Salmonella* Typhimurium (Mackey and Derrick, 1986; Bunning et al., 1990), and *L. monocytogenes* (Bunning et al., 1990). In heat stress adapted cells, heat induced stress proteins prevent disruption of microbial cellular metabolism through repairing, destroying or replacing damaged components (Yousef and Courtney, 2003). In general, microbial cells become resistant to heat stress by synthesis of heat shock proteins (HSP) (Lindquist and Craig, 1988). HSP, mainly the HSP70 and HSP90 gene families, are involved in various cellular processes including proteolysis, cell wall synthesis, cell division, and plasmid replication to assist cells to grow at high temperature (Yura et al., 1993; Georgopoulos et al., 1994; Gross, 1996). A number of HSP have been identified in *E. coli* heat stressed cells, such as Dnak (HSP70), Dnal, GrpE, CroEL (HSP 90), and GroES (Cross, 1996; Missiakas et al., 1996).

2.4.5. Starvation stress

Starvation stress adapted cells usually appear on equipment surfaces, walls, and floors,

where there is adequate oxygen but inadequate available nutritients for microbial growing (Dickson and Frank, 1993). Nutrient depletion may cause a series of changes in the cell envelop, membrane composition, and DNA structure of microorganisms during the stationary phase of growth (Hengge-Aronis, 1993). It also leads to other physiological changes in microbial cells including decreases of cell size and membrane fluidity, and increasing protein turnover (Lou and Yousef, 1996). Similar to other stresses, starvation stress induces starvation and stationary-phase proteins into cells, which increase their resistance capability by using alternative growth substrates or energy through stabilizing ribosomes against degradation (Tolker-Nielsen and Molin, 1996), changing morphological transformation into spherical conformations (Givskov et al., 1994), and enhancing microorganism metabolic potential (Matin, 1991). In *E. coli* cells, starvation proteins were encoded by two groups of genes, including *cst* genes controlled by carbon starvation and *pex* genes controlled by carbon, nitrogen or phosphorus starvation (Martin, 1991).

2.4.6. Desiccation stress

Desiccation stress, an extreme form of osmotic stress, usually occurs in dry environments or the drying process involved in processing of foods such as beef jerky. In general, bacteria protect themselves and become adapted to osmolarity by rapid sccumulating ions, such as K^+, as well as other compatible solutes including proline, glycine betaine and trehalose (Kempf and Bremer, 1988). There are two groups of transport systems for betain and proline found in *E. coli* cells, which was ProU induced when betaine was absent or at lower concentrations (Ames et al., 1990) and ProP a

constitutive ion-motive force-driven transporter (Culham et al., 1993). In order to survive drying stress, the cells need to maintain their biological integrity in the absence of liquids (Potts, 1994), and they usually use polyhydroxyl compounds such as trehalose to replace the water shell around macromolecules to prevent cell damage (Leslie et al., 1995).

2.4.7. Cross-protection of acid and starvation stressed *E. coli* O157:H7 cells

Evidence has indicated that if a microbial population is adapted to a particular stress it may also exhibit a greater resistance when subsequently it is exposed to a similar or unrelated stress, and this phenomenon is termed as cross-protection (Blackburn and Davies, 1994). According to Cheville et al. (1996), cross-protection has been shown to be, at least partly, under the control of the *rpoS* gene. Their study showed that stationary phase acid, heat and salt tolerance was significantly reduced, and starvation-induced acid tolerance did not develop in an *rpoS* mutant of *E. coli* O157:H7 strain ATCC 43895.

Specifically *E. coli* O157:H7 is well known for its acid resistance as some of its strains have been isolated from acidic foods such as mayonnaise (Zhao and Doyle, 1994) and apple cider (Zhao et al., 1993). Benjamin and Datta (1995) tested the survival ability of *E. coli* O157:H7 strains in acid at pH of 2.5 or 3.0 at 37°C, and found that no death of *E. coli* O157:H7 strain ATCC 43895 for at least 5 h, and that 60-80% *E. coli* O157:H7 isolates were acid tolerant. Certain strains of *E. coli* O157:H7 have been shown to exhibit significant cross-protection when subjected to an acid stress followed by an acid, salt or heat stress. Berry and Cutter (2000) reported that acid adaptation of *E. coli* O157:H7 strain ATCC 43895 and ATCC 43889 decreased the ability of 2% acetic acid spray washing to reduce populations of this pathogen on beef carcass surfaces by 1.5-2.5 log

CFU/cm^2. Rowe and Kirk (1999) found that *E. coli* O157:H7 strain NCTC 12079 showed a remarkable resistance to salt (20% w/v) when pre-stressed in acid (pH 3.5-4.5) compared with the control (pH 7.0); this strain also exhibited resistance to subsequent heating (56°C) for 80 min, after it was pre-stressed at pH 4.0. It was reported by Ryu and Beuchat (1999) that D-values of 24-h acid adapted cells (100.2 min at 52°C and 28.3 min at 54°C) were significantly higher than those of unadapted ones (13.6 min at 52°C and 9.2 min at 54°C). The study of Cheng et al. (2002) indicated that after exposure to heat (52°C) for 100 min, acid adapted cells of *E. coli* O157:H7 strain ATCC 43895 showed a 10 fold increase in survival compared to unadapted cells. They also found that the percentage of surviving acid-adapted cells of strain 933 (16.3%) was about 20-fold higher compared to that of the non-adapted cells (0.3%), when they were exposed to 10% sodium chloride solutions at ambient temperature for 8 days.

In addition to the acid resistance, starvation is another stress of *E. coli* O157:H7 studied. Starvation also can induce cross-protection and is related to the food processing environment because water, used for cleaning or rinsing food contact surfaces, is generally recognized as of low nutrient status (Rowe and Kirk, 2000). In the Rowe and Kirk study, starvation (24 h in distilled water at 37°C) was found to significantly increase the heat resistance of *E. coli* O157:H7 strains NCTC 12079 and ATCC 43889 (Rowe and Kirk, 2000), which is in agreement with the study of Leenanon and Drake (2001), which indicated that starvation increased heat resistance of *E. coli* O157:H7 strain ATCC 43895. It was also reported by Lisle et al. (1998) that *E. coli* O157:H7 adapted to starvation by developing a chlorine or deoxycholate resistance phenotype when exposed to starvation

followed by chlorine or deoxycholate treatment. The above studies of cross-protection are particularly important, because of the low infective dose of *E. coli* O157:H7, less than 10 organisms (Doyle et al., 1997), which means that mere survival rather than multiplication could cause an unacceptable risk (Rowe and Kirk, 1999).

Not all of the stressed-adapted cells are cross-protected when exposed to subsequent stresses. For example, in a recent study by Shaker et al. (2008), it was shown that desiccation; heat and cold stressed *Enterobacter* (Cronobacter) *sakazakii* cells had increased thermal inactivation in rehydrated infant milk formula. Similarly, Leenanon and Drake (2001) reported that the D-values of three *E. coli* O157:H7 strains at 56°C in a broth system for 50 min decreased (2-3 min) after exposure to cold stress. The possible reason for this is that exposure of the cells to cold and heat stresses may induce cold shock proteins and the repression of heat shock proteins, and these two proteins can be incorporated with unsaturated fatty acids of the microbial cell membrane and reduce melting points of the pathogen cells (Beales, 2004).

2.5. Control of *Listeria monocytogenes* by chemical antimicrobials

From 2000 to 2003, *L. monocytogenes* has been associated with several multistate outbreaks in the United States due to consumption of contaminated frankfurters and poultry deli products (CDC, 1998; 1999; 2000; 2002). These outbreaks, in part, prompted USDA-FSIS to establish an interim final rule to control *L. monocytogenes* in RTE meat and poultry products (USDA-FSIS, 2003), based on three alternative approaches. Alternative 1 and 2 require use of a postlethality treatments and/or antimicrobial agents to reduce and control the pathogen, whereas alternative 3 relies only on sanitation and

testing to control the pathogen in the postlethality enviroment (USDA-FSIS, 2003). Thus, food industry needs the information of antilisterial effects of various antimicrobials to be best suited to their processing enviroment and meet the requirement of USDA-FSIS. Use of generally recognized as safe (GRAS) chemical antimicrobial agents as ingredients of formulation, dipping or spraying treatments can effectively control *L. monocytogenes* in RTE meat and poultry products.

Numerous studies have shown the antilisterial activities of GRAS antimicrobial agents added in the formulation of RTE meat and poultry products. Wederquist et al. (1994) found that inclusion of 0.5% sodium acetate in the formulation of turkey bologna effectively inhibited the growth of *L. monocytogenes*, followed by sodium lactate (2%) or potassium sorbate (0.26%). Bedie et al. (2001) reported that adding sodium diacetate (0.25%), sodium acetate (0.25-0.5%) or sodium lactate (3%) in frankfurter formulations inhibited *L. monocytogenes* growth at 4°C for 20, 50 and 70 days, respectively. Blom et al. (1997) reported that vacuum-packaged servelat sausage or cooked ham formulated with 0.25% sodium acetate inhibited growth of *L. monocytogenes* for up to 35 days at 4°C. A study conducted by Samelis et al. (2002a) found that sodium lactate (3%) inhibited *L. monocytogenes* growth on vacuum-packaged frankfurters for 50 days (4°C), and its combination with 0.25% of sodium acetate, sodium diacetate, or glucono-δ-lactone extended the growth inhibition to up to 120 days. In a similar study, Barmpalia et al. (2004) reported that pork bologna formulated with the combination of 1.8% sodium lactate and 0.25% sodium diacetate allowed the lowest *L. monocytogenes* growth rate compared to that of control and other treatments.

Dipping or spraying antimicrobials, including acids (acetic, lactic or citric) and salts (potassium sorbate or benzoate, sodium benzoate, propionate, acetate diacetate or lactate), onto RTE meat and porltry products as post-processing treatments has been shown to be effective against *L. monocytogenes*. Palumbo and Williams (1994) reported that dipping surface inoculated frankfurters into a combination of acetic and citric acid (at 2.5% each) inhibited growth of *L. monocytogenes* for up to 90 days at 5°C. Samelis et al. (2001) reported that dipping slices of pork bologna in acetic acid (2.5 or 5%), sodium diacetate (5%), or potassium benzoate (5%) did not permit *L. monocytogenes* growth during 120 days of storage at 4°C. In a similar study with frankfurters, Barmpalia et al. (2004) found that frankfurters formulated with 1.8% sodium lactate or 0.25% sodium diacetate and dipped in 2.5% solutions of lactic or acetic acid completely inhibited *L. monocytogenes* growth for over 28 days. Geornaras et al. (2006a) found that dipping frankfurters in a 2.5% aqueous solution of lactic acid reduced the initial level of *L. monocytogenes* by 1.8 log CFU/cm^2, increased the lag phase, and decreased the pathogen growth rate during storage in vacuum packages at 10°C for 48 days. In another study of Geornaras et al. (2006b), it was found that dipping commercial smoked sausage in acetic acid (2.5%), lactic acid (2.5%) or potassium benzoate (5%) alone reduced initial *L. monocytogenes* populations by 0.4 to 1.5 log CFU/cm^2. In the limited studies evaluating application of antimicrobials by spraying, Islam et al. (2002) found that *L. monocytogenes* populations of chicken lunchen meat sprayed with sodium benzoate, sodium propionate, potassium sorbate, or sodium diacetate (each at 15, 20, or 25%) were 1.5 to 3.0 log CFU/g less than those on the untreated samples. Byelashov et al. (2008) reported that lactic acid (5%) and sodium

lauryl sulfate (0.5%) sprayed before or after inoculation reduced *L. monocytogenes* populations on frankfurters by 1.8 and 2.8 log CFU/cm^2, respectively.

2.6. Control of foodborne pathogens by plant extracts including hops beta acids

Much attention has been given to plant extracts, due to their antimicrobial properties and the preference of consumers for "fresh", "minimally processed" foods containing natural ingredients. The antimicrobial activities of many plant extracts such as rosemary, sage, clove, *Ginkgo biloba* leaf and lemon, have been studied extensively. Shelef et al. (1980) reported that 0.3% of sage or rosemary extracts in nutrient agar or trypticase soy agar inhibited growth of 21 gram-positive organisms including nine pathogenic ones. In another study, Pandit and Shelef (1994) found that rosemary (≥0.5%, w/v) and cloves (≥0.1%, w/v) were listericidal in brain heart infusion broth, and the inhibitory concentrations of rosemary oil were 10% in broth against *L. monocytogenes*. Xie et al. (2003) found strong antilisterial activity by *Ginkgo biloba* leaf extract in brain heart infusion broth during storage at 4, 25 or 37°C. More recently, Conte et al. (2007) reported that lemon extract (100-150 ppm) inhibited growth of vegetative cells and spores of food spoilage microorganisms including yeasts, *Bacillus* species and lactic acid bacteria.

The antimicrobial activities of plant extracts are attributed to the presence of compounds such as phenolics, terpenoids, essential oils, alkaloids, lectins, polypeptides, and polyacetylenes. These compounds inhibit microbial growth through various mechanisms including membrane disruption, adhesion binding, protein and cell wall binding, enzyme inactivation, and intercalation into cell wall and/or DNA (Cowan, 1999).

However, many studies examining the antimicrobial activity of plant extracts have

been conducted only with microbiological media, and limited information exists regarding their effects in food products. In a study conducted by Hao et al. (1998a), nine plant extracts were evaluated for their ability to inhibit the growth of *Aeromonas hydrophila* and *L. monocytogenes* in refrigerated cooked beef, but only eugenol (clove extract) and pimento extract showed inhibitory effects. Their study on refrigerated, cooked poultry (Hao et al., 1998b), also indicated that eugenol and pimento extract were most effective in inhibiting growth of the above two pathogens, especially with 4 log CFU/g less growth of *A. hydrophila* occurring on eugenol-treated breast meat than on control samples. Ahn et al. (2004) reported that 1% pycnogenol (pine bark extract) reduced *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* Typhimurium by 1.08, 1.24 and 1.33 log CFU/g, respectively, in raw ground beef after 9 days of refrigerated storage. They also found that ActiVin (grape seed extract) and oleoresin rosemary resulted in about 1-log CFU/g reduction of those three pathogens after 9 days of storage. Cutter (2000) observed that herb extract dispersed in sodium citrate or sodium chloride reduced *L. monocytogenes* and *Salmonella* Typhimurium by 1.3 and 1.8-1.9 log CFU/cm^2 in beef, respectively.

Although published studies have verified the antimicrobial efficiency of plant extracts, there are some practical restrictions for their application in food products, including the high cost, high concentration for antimicrobial effectiveness, and negative sensory qualities in foods (Sofos et al., 1998). A possible approach to minimize these potential problems is using them in combination with chemical antimicrobial agents in accordance with the "multiple hurdle" concept (Leistner, 2000). Specifically, plant extracts in

combination with other antimicrobials may allow minimization of the concentrations used in food products, thus limiting the side sensory effects on foods. For example, Sivarooban et al. (2007) reported that the combination of grape seed extract and nisin reduced *L. monocytogenes* on TSBYE and turkey frankfurters to undetectable levels after 15 h and 21 days of storage, respectively. Xie et al. (2003) also found that the antilisterial activity of *Ginkgo biloba* leaf extract in BHI broth was enhanced when it was used in combination with sodium ethylenediaminetetraacetic acid (EDTA).

Among the plants known to have components with antimicrobial properties is the hop plant (*Humulus lupulus*), which belongs to the family of *Cannabinaceae*. Hops have been used to preserve beer wort and provide flavor in beer since the 12th century in Germany and since the 15th century in England (Srinivasan et al., 2004). Their antimicrobial properties are mainly due to the natural hops acids. The hops beta acids (HBA) are major constituents of hops acids, and their primary components are lupulone ($C_{27}H_{38}O_4$), colupulone ($C_{26}H_{37}O_4$), and adlupulone ($C_{27}H_{38}O_4$) (US-FDA, 2001). They are active against Gram-positive bacteria (Bhattacharya et al., 2003; Haas and Barsoumian, 1994), and have no activity against most Gram-negative microorganisms (Bhattacharya et al., 2003). Hops beta acids are reported to affect bacterial cells by causing leakage in the cytoplasmic membrane, which in turn inhibits the active transport of sugars and amino acids (Teuber and Schmalreck, 1973). The possible reason for the resistance of Gram-negative bacteria to hops beta acids may be the lipopolysaccharide containing outer membrane, since lupulone and humulone of hops beta acids are inactivated by serum phosphatides (Sacks and Humphreys, 1951). Recently, the US-FDA (US-FDA, 2001) and

USDA-FSIS (USDA-FSIS, 2008b) approved HBA as generally recognized as safe for use as antimicrobial agents in the amount of 4.4 mg/kg on cooked meat and 5.6 mg/kg in casings for meat products.

To date, there are limited published studies on the antimicrobial activity of HBA. Srinivasan et al. (2004) found that HBA had wide anti-protozoa effects. Bhattacharya et al. (2003) showed that the minimum inhibitory concentration of HBA against *Streptococcus mutans* ranged from 2 to 10 μg/ml. Larson et al. (1996) showed that hops extracts (containing 60% beta acids) exhibited 100% inhibition against *L. monocytogenes* in brain heart infusion broth, coleslaw and skim milk, when the concentration was 10-30 μg/ml, 100-1000 μg/ml, and 1000 μg/ml, respectively. The above studies, however, used the paper disc diffusion essay or optical density measurements to determine inhibition. They did not test the antimicrobial activity of HBA in broth media or on meat products using the agar plating method, and did not compare it to other chemical antimicrobial agents used in the food industry. Thus, it is necessary to elucidate their antilisterial activities in broth media and on the surface of RTE meat products.

2.7 Control of foodborne pathogens by salad dressings

In the United States, commercially produced salad dressings are widely used and maintain a good safety record (Smittle, 2000). Vinegar or lemon juice may be used alone, or as a mixture, as natural flavoring and acidifying liquids in some vegetable salads (Sengun and Karapinar, 2005) or in home-made egg mayonnaise (Lock and Board, 1995). Food oils of plant origin are used widely in the processing of foods and in homemade dishes (Medina et al., 2007). Mixtures of oil with vinegar are used as ingredients in

prepared foods (Rodrigo et al., 1999). In Greece, olive oil with oregano and lemon juice is used as a sauce on cooked fish (Tassou et al., 1995) and vegetable salads. These salad dressings may provide a harsh environment for foodborne pathogens such as *Salmonella*, *E. coli* O157:H7, *L. monocytogenes*, or *Staphylococcus aureus* to survive, due to the presence of acetic, lactic or citric acids as low pH ingredients, salt, and preservatives such as sorbic and/or benzoic acid (Beuchat et al., 2006).

Antimicrobial effects of salad dressings, including Ranch, Thousand Island, olive oil, lemon juice and vinegar have been well documented. Beuchat et al. (2006) reported that the shelf-stable, dairy-based, pourable Ranch and blue cheese salad dressings stored at 25°C did not support growth of *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes*, and that cells of these three pathogens were undetectable by enrichment in the two salad dressings within 1 day, between 1 and 8 days, and 2 and 8 days, respectively. The study of Weagant et al. (1994) showed that 8 log CFU/g *E. coli* O157:H7 inoculated into blue cheese and Thousand Island dressings decreased to 5 and 3 log CFU/g, respectively, after 35 days of storage at 5°C. Medina et al. (2006) examined the antimicrobial activities of different olive oils, and found that virgin olive oil, olive oil, and pomace olive oil showed strong bactericidal effects against *S. aureus*, *L. monocytogenes*, and *Yersinia* sp. Radford et al. (1991) observed a faster death rate of *Salmonella enterica* in mayonnaise made with virgin olive oil than in that prepared with sunflower oil. Ciafardini et al. (2002) indicated that the decrease in the number of yeasts inoculated into extra virgin olive oil may be attributed to the partial mechanical removal of the suspended particles in the oil and the concentration of polyphenols in the oil. Castillo et al. (2000) reported that diluted and

undiluted lemon juice produced an 8-log reduction of *Vibrio cholerae* after exposure for 30 min. Entani el al. (1998) found that vinegar (2.5% or 10% acetic acid) had a bactericidal effect on *E. coli* O157:H7, and this effect increased with the increasing storage temperature (10-50°C). The same bactericidal effect of vinegar or lemon juice on 5 strains of *E. coli* in tryptic soy broth was described in a study by Vijayakumar and Wolf-Hall (2002a), who reported that the minimum bactericidal concentrations were 16.7-20% for white vinegar, 2.7% for apple cider vinegar, and 6.7% for lemon juice, respectively. All these research results clearly elucidate the strong antimicrobial effectiveness of salad dressings, which led to the hypothesis that exposing foods to salad dressings prior to consumption may control transmission of foodborne pathogens.

According to Sengun and Karapinar (2004; 2005), lemon juice or vinegar alone caused a significant reduction of *Salmonella* Typhimurium on rocket leaves (between 1.23-4.17 and 1.32-3.12 log CFU/g, respectively) and carrots (between 0.79-3.95 and 1.57-3.58 log CFU/g, respectively), and dipping in a lemon juice-vinegar mixture (1:1) for 15 or 30 min reduced the pathogen numbers to an undetectable level on rocket leaves (Sengun and Karapinar, 2005) or carrots (Sengun and Karapinar, 2004), respectively. They also reported that lemon juice, vinegar and their mixture caused 0.87-2.93, 0.66-2.92 and 0.86-3.24 log CFU/g reductions of *Salmonella* Typhimurium on spring onions (Sengun and Karapinar, 2005), respectively. A similar study conducted by Vijayakumar and Wolf-Hall (2002b) found a 2.6 and 5.4 log CFU/g reduction of *E. coli* O157:H7 CDC1993, when inoculated iceberg lettuce was immersed in 13% lemon juice or 35% white vinegar for 5 or 10 min, respectively. Chang and Fang (2007) observed that

dipping inoculated lettuce in commercial vinegar (5% acetic acid) for 5 min at 25°C reduced *E. coli* O157:H7 by 3 log CFU/g. Karapinar and Gonul (1992) reported a 5 log CFU/g reduction in *Yersinia enterocolitica* when parsley leaves were treated with 40% (v/v) vinegar for 15 min. A recent study conducted by Kisla (2007) showed the maximum reduction of 0.78 log CFU/g of *Salmonella* Typhimurium in stuffed mussels after immersing in lemon dressing for 15 min.

Similar to lemon juice and vinegar, combinations of oil with vinegar or lemon juice also showed strong bactericidal activity against foodborne pathogens in mayonnaise and vegetable salads. Medina et al. (2007) reported that combination of virgin olive oil with lemon juice or vinegar decreased *Salmonella* Enteritidis and *L. monocytogenes* populations below the detection limit after 30 min of immersing in egg mayonnaise or milk mayonnaise, respectively. In their experiments, slight reductions of *L. monocytogenes* on lettuce seasoned with sunflower oil and vinegar or lemon juice alone were observed, and *L. monocytogenes* were killed after 30 min when virgin olive oil was used with either lemon juice or vinegar added. Tassou et al. (1995) found that olive oil and lemon juice had bacteriostatic and bactericidal effects on *S. aureus* and *S.* Enteritidis treated Mediterranean gilt-head seabream stored under a modified atmosphere of 40% CO_2, 30% O_2, and 30% N_2 or air at 0 ± 1°C.

The results of the above studies suggest that salad dressings such as Ranch, Thousand island, lemon juice, vinegar, and olive oil, used alone or as mixtures, may be considered as potential antimicrobial agents for use by consumers to reduce the risk of foodborne illness in the home environment. However, the above studies were limited to vegetables

or seafood and there is no study investigating their antimicrobial effects on RTE meat or poultry products.

2.8 Control of foodborne pathogens by microwaving heating

Microwave heating of foods was introduced as early as 1945 by Percy Spencer of Raytheon Corporation as a radar technology during World War II (Buffler, 1993). It was first used in food service in 1947 and the use of microwave ovens in consumer's home started in 1955. Currently, the use of microwave ovens has become remarkably popular for heating or reheating foods (Fung and Cunningham, 1980), defrosting, tempering, or thawing products (Decareau, 1985), baking, pasteurizing or sterilizing (Goldblith, 1966; Olsen, 1965; Kenyon et al., 1971), and evaporating and drying (Sunderland, 1982). The USDA-FSIS reported that over 90% of homes in America have at least one microwave oven (USDA-FSIS, 2008d) due to its speed of heating and convenience (Heddleson and Doores, 1994).

Microwaves are electromagnetic waves generated by a magnetron, and they radiate outward from a source and can be absorbed, transmitted, and reflected (Giese, 1992). Microwaves generate microwave energy, which is a form of noninonizing electromagnetic radiation in the frequency range from 300 MHz to 300 GHz (Risman, 1991). According to the US-FDA, only 915 and 2450 MHz frequencies were allowed for use in commercial and home ovens (Chipley, 1980). Microwaves can penetrate food materials up to 30 cm at 915 MHz and at 2450 MHz the penetration level is 10 cm (IFT, 1989). The ability of microwaves to transfer heat depends on the dielectric properties of food, which are expressed as "dielectric constant" and "dielectric loss factor". The

"dielectric constant" represents the ability of a food to store electric energy, and the "dielectric loss factor" describes the ability of a food to dissipate electric energy as heat. Food with larger "dielectric loss factor" and smaller "dielectric constant" will generate more heat (Giese, 1992). The physical properties of the food, including shape, surface area and density, also determine the heat transferring ability of microwaves. For example, high density, sphere shape and great surface area help transfer heat to the microwaving food rapidly (Giese, 1992).

Fung and Cunningham (1980) and Valsechi et al. (2004) made a detailed review concerning the destruction effects of microwaves on microorganisms in meat and poultry products, eggs, dairy products, cereal products, fruits, vegetables, and miscellaneous foods, and microorganisms involved in their reviews including yeasts, *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium perfringens*, *Eschrichia coli*, *Enterococcus*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella* Typhimurium. They also concluded that the destruction effects of microwaves on microorganisms in foods are influenced by intrinsic (pH, moisture content, oxidation-reduction potential, nutrient content, antimicrobials, biological structures, chemical compositions, and shape and size of the food) and extrinsic (ambient temperature, humidity, frequency of the radiation, time of exposure, and position of the foods) factors. The mechanism of microbial destruction by microwaves is mainly attributed to the heat generated by the microwave energy.

The inactivation effect on *L. monocytogenes* by microwave heating has been well documented in a variety of foods. Choi et al. (1993) reported that microwave oven

heating (71.1°C) for 5 min of 20 ml milk reduced *L. monocytogenes* approximately by 5-7 log CFU/ml. Dasdag et al. (1995) found that microwave heating was more effective than conventional heating in eliminating *L. monocytogenes* in lamb and quail. A study conducted by Lund et al. (1989) showed that microwaving stuffed chicken (6-7 log CFU/g skin) to an internal temperature of 70°C resulted in a 6 log lethality of *L. monocytogenes*. Huang et al. (1993) reported that microwave cooked channel catfish fillets (6 log CFU/cm^2) to the internal temeparture of 60°C reduced *L. monocytogenes* by 4 logs in polyvinylidene chloride film covered and by 2 log in uncovered fillets. Gundavarapu et al. (1995) detected the microbiological safety of shrimp cooked in a microwave oven, and they observed that 5 log CFU/g of *L. monocytogenes* inoculated shrimp could be completely inactivated within 2 min of holding after microwaving for 168, 84, 62 and 48 s at 240, 400, 560, and 800 W, respectively. In a relatively recent study , Huang (2005) found that microwaving (600-W) beef frankfurters for 12-15 min achieved a 7-log reduction of *L. monocytogenes*, and the surface temperature of the samples reached 75-85°C.

Although microwave heating shows promising destruction effects against microorganisms, outbreaks attributed to consumption of microwave heated foods contaminated with *Salmonella* Typhimurium have still happened. An outbreak of illness occurred at a community picnic in Juneau, Alaska, in 1992. Of 30 persons who ate reheated pork, 10, who used a microwave oven, became sick, compared with none of 20 who used a conventional oven or skillet (Gessner and Beller, 1994). Failure to control foodborne pathogens by microwaving may be due to the non-uniform distribution of the

heat on the surface and in the food (Dealler and Lacey, 1990). Thus, the USDA-FSIS published a food safety fact sheet to guide consumers to cook safely in the microwave oven. They suggested use a food thermometer or an oven temperature probe to verify that the food has reached a safe temperature, which includes that the internal temperature of at least 160°F (71.1°C) for red meat and egg casseroles, and 180°F (82.2°C) for whole poultry, respectively. They also stated that whole, stuffed poultry is not recommended to be cooked in a microwave oven, and the reheated foods should reach the internal temperature of 165°F (73.9°C) (USDA-FSIS, 2000).

CHAPTER 3

INACTIVATION OF *ESCHERICHIA COLI* O157:H7 IN NONINACT BEEF STEAKS OF DIFFERENT THICKNESS COOKED BY PAN-BROILING, DOUBLE PAN-BROILING OR ROASTING USING FIVE TYPES OF COOKING APPLIANCES

ABSTRACT

This study compared thermal inactivation of *Escherichia coli* O157:H7 in nonintact beef steaks of different thicknesses cooked by different cooking methods and appliances. Coarse-ground (0.95 cm diameter plate) beef was inoculated with rifampicin-resistant *E. coli* O157:H7 (8-strain composite; 6-7 log CFU/g) and then mixed with sodium chloride (0.45%) plus sodium tripolyphosphate (0.23%), with a total of 10% water added. The meat was stuffed into bags (10 cm diameter), semi-frozen (-20°C, 6 h) and cut into 1.5, 2.5 and 4.0 cm thick steaks. Samples were then individually vacuum-packaged, frozen (-20°C, 42 h), and

tempered (4°C, 2.5 h) before cooking. Partially thawed (-2±1°C) steaks were pan-broiled (Presto® electric skillet and Sanyo® grill), double pan-broiled (George Foreman® grill), or roasted (Oster® toaster oven and Magic Chef® standard kitchen oven) to a geometric center temperature of 65°C. Extent of pathogen inactivation decreased in order of: roasting (2.0 to 4.2 log CFU/g) > pan-broiling (1.6 to 2.8 log CFU/g) ≥ double pan-broiling (1.1 to 2.3 log CFU/g). Cooking of 4.0 cm thick steaks required a longer time (19.8-65.0 min; variation due to different cooking appliances), and caused greater reductions in counts (2.3 to 4.2 log CFU/g) than in thinner samples (1.1 to 2.9 log CFU/g). The time to reach the target internal temperature increased in order of: George Foreman® grill (3.9 to 19.8 min) < Oster® toaster oven (11.3 to 45.0 min) < Presto® electric skillet (16.3 to 55.0 min) < Sanyo® grill (14.3 to 65.0 min) < standard kitchen oven (20.0 to 63.0 min); variation due to steak thickness. Results indicated that increased steak thickness allowed greater inactivation of *E. coli* O157:H7, as time to reach the target internal temperature increased. Roasting in a kitchen oven was most effective for pathogen inactivation.

3.1. INTRODUCTION

Escherichia coli O157:H7 is a highly virulent foodborne pathogen that causes approximately 62,000 cases of symptomatic infections, resulting in about 1,800 hospitalizations and 52 deaths annually in the U.S. (Mead et al., 1999). According to the U.S.

Department of Agriculture Food Safety and Inspection Service (USDA-FSIS), nonintact beef products include, in addition to ground beef, those muscle cuts that have been mechanically tenderized, restructured into formed entrees, or injected with marination or brining solutions for enhancement of flavor and/or tenderness (USDA-FSIS, 1999). *E. coli* O157:H7 translocated from the meat surface to internal tissue by mechanical tenderization or injection of solutions, or entrapped in the tissue during restructuring, could be protected from lethal heating effects, especially if the products are intentionally or unintentionally undercooked (Hajmeer et al., 2000; Ortega-Valenzuela et al., 2001; USDA-FSIS, 2000). The microbiological safety concern related to nonintact beef products other than ground beef is supported by relatively recent outbreaks of *E. coli* O157:H7 illness linked to consumption of such products (Laine et al., 2005; USDA-FSIS, 2005a; 2005b). These outbreaks were attributed to inadequate cooking or cooking products directly from the unthawed state (Laine et al., 2005; USDA-FSIS, 2005a; 2005b; 2007a; 2007b; 2007c, 2007d; 2007e; 2008a; Conference for Food Protection, 2008).

Information pertaining to the effectiveness of cooking on inactivation of *E. coli* O157:H7 internalized in nonintact beef products other than ground beef is limited. Sporing (1999) reported that cooking of blade-tenderized steaks to an internal temperature of 60°C or more inactivated *E. coli* O157:H7 by more than 5 log CFU/g, and furthermore, pathogen

inactivation by cooking decreased in order of: broiling > grilling > frying. Studies conducted in our laboratory (Mukherjee et al., 2007) also showed that broiling was more effective than grilling or frying when meat samples were cooked to internal temperatures of 60 or 65°C. In addition to the effectiveness of cooking methods, previous studies (Sporing, 1999; Thippareddi et al., 2000) also indicated that the thicker the meat samples, the more lethal the effect when cooking to the same internal temperature. However, there is no published information verifying thermal inactivation of *E. coli* O157:H7 in moisture-enhanced restructured nonintact beef steaks of different thicknesses by different cooking methods using different types of cooking appliances. Also, there is no information available on the temperature profiles of different areas (e.g., surface, edge and center) of steaks during cooking. A comparative risk assessment for nonintact (tenderized) and intact (non-tenderized) beef steaks, conducted by the USDA-FSIS (USDA-FSIS, 2002a; 2002b), indicated that there were insufficient data to determine whether traditional cooking methods are adequate to destroy *E. coli* O157:H7 translocated from the meat surface to internal tissue during the blade tenderization process. Because proper cooking renders nonintact products microbially safe, it is important to develop effective cooking protocols for such products. Thus, the objective of this study was to determine the effect of various cooking methods, using different appliances, on thermal inactivation of *E. coli* O157:H7 in nonintact, reconstructed beef steaks of different

thickness cooked to an internal temperature of 65°C.

3.2. MATERIALS AND METHODS

3.2.1. Bacterial strains and inoculum preparation

The inoculum was comprised of a mixture of rifampicin-resistant derivatives of *E. coli* O157:H7 strains ATCC 43888 (human isolate), ATCC 43895 (raw hamburger meat isolate), ATCC 43895/ISEHGFP (Noah et al., 2005), and C1-057, C1-072, C1-109, C1-154, and C1-158 (bovine fecal isolates, Carlson et al., 2009). Rifampicin-resistant strains were used to allow for the selective isolation of the inoculum from natural contaminating flora. The thermotolerance (60°C, 4 h) of the rifampicin-resistant strains and corresponding wild-type strains was evaluated in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD), and was found not ($P \geq 0.05$) to be different (unpublished data).

Each strain was activated and subcultured (35°C, 24 h) separately in 10 ml of TSB supplemented with rifampicin (200 µg/ml, Sigma-Aldrich, Inc., St. Louis, MO). The eight cultures were then combined and centrifuged (Eppendorf model 5810R, Brinkmann Instruments Inc., Westbury, NY) at 4,629×*g* for 15 min at 4°C. The harvested cells were washed with 10 ml of phosphate buffered saline (PBS, pH 7.4; 0.2 g of KH_2PO_4, 1.5 g of $Na_2HPO_4 \cdot 7H_2O$, 8.0 g of NaCl, and 0.2 g of KCl in 1 liter of distilled water), centrifuged as previously described, and resuspended in 80 ml of fresh PBS. The washed inoculum was

serially diluted in PBS to obtain an inoculation level of 6 to 7 log CFU/g when 50 ml of inoculum was added to 1 kg of coarse-ground beef.

3.2.2. Preparation of nonintact beef steaks

Fresh beef knuckles (97.5% lean) were coarse-ground (0.95 cm diameter plate, Hobart Mfg. Co., Troy, OH), and then 1 kg batches of ground meat were inoculated with 50 ml of the diluted rifampicin-resistant *E. coli* O157:H7 mixture to achieve an inoculation level of 6 to 7 log CFU/g. The meat and inoculum were thoroughly mixed for 2 min using a bowl-lift stand mixer (KitchenAid®, Professional 600, St. Joseph, MI) set at the speed setting "stir". The inoculated beef was then mixed for an additional 2 min with 50 ml of a sodium chloride plus sodium tripolyphosphate (BK Giulini Corporation, Simi Valley, CA) solution to yield concentrations (wt/wt) of 0.45% and 0.23%, respectively, in the final product. Thus, this nonintact product simulated a nonintact beef product that was moisture-enhanced to 110% of initial weight. The beef samples were then extruded into polyethylene bags (2.3 kg, 15.2 × 45.7 cm, Koch, Kansas City, MO), tied, and placed at -20°C for 6 h to facilitate cutting into steaks. Semi-frozen beef was cut into 1.5, 2.5 and 4.0 cm thick steaks, and individual steaks were placed into vacuum bags (15.2 × 21.6 cm, 3 mil std barrier, nylon/polyethylene vacuum pouch, water vapor and oxygen transmission rates of 9.3 $g/m^2/24$ h [97% relative humidity] and 54.3 $cm^3/m^2/24$ h [21°C, 0% relative humidity], respectively, Koch, Kansas City, MO),

vacuum-packaged (Hollymatic, Corp., Countryside, IL), and returned to the freezer. After 42 h, the steaks were cooked after tempering at 4°C for 2.5 h.

3.2.3. Cooking of nonintact beef steaks

The partially thawed (-2 ± 1°C) 1.5, 2.5 and 4.0 cm thick beef steaks were cooked to a geometric center temperature of 65°C by pan-broiling (referred to as "frying" by Mukherjee et al. [2007] and Sporing [1999]; Table 3.1), double pan-broiling (referred to as "grilling" by Mukherjee et al. [2007]; Table 3.1), or roasting (referred to as "modified broiling" by Kendall et al. [1974], and "broiling" by Mukherjee et al. [2007] and Sporing [1999]; Table 3.1). Cooking appliances used were a Presto® 16-inch electric skillet (National Presto Industries, Inc., Eau Claire, WI) and Sanyo® indoor barbecue grill (Sanyo Fisher Company, Chatsworth, CA) for pan-broiling; a George Foreman® grill (Salton, Inc., Lake Forest, IL) for double pan-broiling; and an Oster® stainless steel toaster oven (Sunbeam Products, Inc., Boca Raton, FL) and Magic Chef® standard kitchen oven (Maytag Corp., Newton, IA) for roasting (Table 3.1). An internal temperature of 65°C was selected as it simulates medium-rare doneness of beef steaks, which is preferred by a large proportion of U.S. consumers (Lorenzen et al., 1999). Terminology and methods for cooking used in this study were according to guidelines of the American Meat Science Association (American Meat Science Association, 1995), and were slightly revised according to experimental conditions in this

study. All cooking appliances with temperature settings (i.e., all but the George Foreman® grill) were set to 350°F (177°C), and all appliances were preheated before use. Temperatures of cooking appliances, and the geometric center, surface (5 mm below the surface of the steak), and edge (5 mm from the edge of the steak) of beef steaks were continuously monitored and recorded at 5 s intervals during cooking, using type-K thermocouples and PicoLog data acquisition software (Pico Technology Ltd, Cambridge, UK). In all cases, beef samples were placed in the center of each of the cooking appliances. Steak samples cooked by pan-broiling were flipped over when the internal (geometric center) temperature reached 42°C; whereas, samples cooked by double pan-broiling or roasting were not flipped over.

3.2.4. Microbiological, physical and chemical analyses

The weight of each steak sample was measured before cooking and immediately after cooking to determine cooking losses. Moisture and fat contents of uncooked and cooked samples also were measured using AOAC International official methods 950.46.B and 960.39, respectively (AOAC, 2000). The samples were analyzed for surviving pathogen and total bacterial populations before and immediately (within 2.5 min) after cooking. Individual samples were placed in a Whirl-Pak filter bag (1627 ml, 19 × 30 cm, Nasco, Modesto, CA) with 100 ml of maximum recovery diluent (0.85% NaCl and 0.1% peptone) and homogenized (Masticator, IUL Instruments, Barcelona, Spain) for 2 min. Serial tenfold

dilutions of each sample, in 0.1% buffered peptone water (Difco), were surface-plated onto tryptic soy agar (Acumedia, Lansing, MI) supplemented with 0.1% sodium pyruvate (Fisher Scientific, Fair Lawn, NY; TSAP) and TSAP plus rifampicin (100 μg/ml; TSAP+rif) for enumeration of total bacterial populations and inoculated *E. coli* O157:H7, respectively. Colonies were counted manually after incubation at 35°C for 48 h. The pH of the meat homogenate was measured after plating of the sample, using a digital pH meter with a glass electrode (Denver Instruments, Arvada, CO). The water activity of uncooked and cooked steak samples was measured using an AquaLab (model series 3, Decagon Devices Inc., Pullman, WA) water activity meter.

3.2.5. Statistical analysis

The experiment was performed twice, and each time included three individual samples per treatment. The pH, water activity, cooking losses, moisture and fat contents, and microbiological (converted to log CFU/g) data were analyzed using the Mixed Procedure of SAS (SAS Institute, 2002), with independent variables including steak thickness, cooking appliance, and the interaction of steak thickness × cooking appliance. Means and standard deviations for microbiological data were calculated, and the mean differences among interactions were separated with the least significant difference procedure at the significance level of α=0.05.

3.3. RESULTS AND DISCUSSION

3.3.1. Temperatures of cooking appliances and steaks

Temperatures of the cooking appliances during the duration of steak cooking were 176 ± 16°C and 177 ± 15°C for the Presto® electric skillet and Sanyo® grill used for pan-broiling, 176 ± 15°C for the George Foreman® grill used for double pan-broiling, and 176 ± 21°C and 178 ± 18°C for the Oster® toaster oven and Magic Chef® kitchen oven used for roasting, respectively. Part of the observed variation in appliance temperatures may be attributed to the heating element switching on/off whenever the temperature went below or above the set temperature of the thermostat (i.e., 177°C).

As indicated, in addition to the temperature of the geometric center of steaks, surface and edge temperatures of the samples were also monitored and recorded during the cooking period. For steaks that were cooked by pan-broiling, one thermocouple each was placed at the lower (closest to the heat source) and top (away from the heat source) surface of each steak. As expected, the temperature of the lower surface increased promptly before the samples were flipped over (when the center temperature reached 42°C) and reached temperatures as high as 83.7 to 107.5°C and 70.9 to 110.8°C when using the Presto® electric skillet (Figure 3.1; Appendix Table 1) and Sanyo® grill (Figure 3.2; Appendix Table 2), respectively. Immediately after flipping the samples over, temperatures of the same surface,

now on top, decreased to 49.5 to 65.0°C (Presto® electric skillet) and 54.6 to 62.9°C (Sanyo® grill). The temperature of the surface that was initially not in contact with the heat source increased slowly before the steaks were flipped over, and then increased quickly and reached approximately 80 to 90°C by the end of cooking (Figures 3.1 and 3.2; Appendix Tables 1 and 2). The temperature of the edge of samples cooked by pan-broiling also increased quickly, and final temperatures were 65.0 to 74.0°C and 51.8 to 76.5°C when using the Presto® electric skillet (Figure 3.1; Appendix Table 1) and Sanyo® grill (Figure 3.2; Appendix Table 2), respectively. In contrast to the results for the surface temperature, the final product edge temperature decreased as thickness of the samples increased. For example, the final edge temperature for 1.5, 2.5 and 4.0 cm thick steaks cooked on the Presto® electric skillet was 76.5, 66.1, and 51.8°C, respectively (Figure 3.1; Appendix Table 1). The product edge temperature was most likely influenced by the temperature of the air surrounding the steak and the temperature of the interior of the steak. In the case of thicker steaks, the temperature of the interior was most likely lower and transfer of heat from the outside to the inside was slower, compared to thinner samples. This, together with evaporative cooling occurring at the surface of the product was most likely responsible for the lower final edge temperature of the thicker steaks. For samples cooked by double pan-broiling using the George Foreman® grill, final sample surface and edge temperatures ranged from 91.4 to 117.7°C and 81.8 to 90.0°C,

respectively (Figures 3.3 and 3.4; Appendix Tables 3 and 4). Final sample surface and edge temperatures of steaks cooked by roasting were 77.8 to 93.2°C and 70.2 to 84.9°C (Oster® toaster oven, Figure 3.4; Appendix Table 3.4), and 82.2 to 92.6°C and 76.0 to 83.9°C (Magic Chef® kitchen oven, Figure 3.5; Appendix Table 5), respectively.

The temperature of the initial geometric center of uncooked steaks was -2 ± 1°C; thus, samples were at about the freezing point of meat when cooking was initiated. Cooking of beef steaks by double pan-broiling, pan-broiling, or roasting required 3.9 to 19.8 min (Table 3.2; Figure 3.3; Appendix Table 3), 14.3 to 65.0 min (Table 3.2; Figures 3.1 and 3.2; Appendix Tables 1 and 2) and 11.3 to 63.0 min (Table 3.2; Figures 3.4 and 3.5; Appendix Tables 4 and 5), respectively, to reach the internal center temperature of 65°C. The large variation in cooking times within each cooking method was due to the different thicknesses of the samples; the thicker the samples, the longer the time needed to reach the target temperature. For instance, roasting of samples using the Magic Chef® kitchen oven required 20.0, 36.3 and 63.0 min to reach the center temperature of 65°C for 1.5, 2.5 and 4.0 thick samples, respectively (Table 3.2; Figure 3.5; Appendix Table 5). Sporing (1999) reported that 1.3 cm thick steaks required the shortest time while 3.2 cm thick samples required the longest time to reach the desired internal temperatures when cooked by grilling on a propane

grill, frying on an electric skillet or broiling in a standard kitchen oven, which was in accordance with our findings.

3.3.2. Cooking losses, pH, water activity, and moisture and fat contents

Cooking resulted in weight losses ranging from 27.2 to 32.3%, 23.1 to 31.1% and 21.3 to 27.2% for steaks cooked by pan-broiling, double pan-broiling, and roasting, respectively (Table 3.3), which were similar to those reported by others (Trout, 1989; Mukherjee et al., 2007). In general, cooking losses increased as thickness of steaks increased, and this was attributed to thicker steaks needing a longer time to reach the target temperature of 65°C. Cooking losses, however, were not different ($P \geq 0.05$) among cooking appliances within the same cooking method (Table 3.3).

The pH of uncooked beef steaks ranged from 5.67 to 5.76 (Table 3.4). Pan-broiling, double pan-broiling, and roasting caused increases ($P < 0.05$) in the pH of samples, resulting in cooked product pH values ranging from 6.03 to 6.14, 6.02 to 6.04 and 5.98 to 6.04, respectively (Table 3.4). These results agree with those of previous studies (Trout, 1989; Berry, 1998; Mukherjee et al., 2007) which reported that the pH of beef products increased during cooking.

The water activity and moisture content of uncooked beef steak samples were 0.990 to 0.993 (Table 3.4) and 76.1%, respectively (Table 3.5). These values were higher than those

reported by Mukherjee et al. (2007) (0.979 and 70.6%, respectively); however, this was expected as the restructured product in our study was moisture-enhanced to 110% of initial weight, whereas the restructured product used by Mukherjee et al. (2007) was moisture-enhanced to 104% of initial weight. In the present study, water activities and moisture contents of cooked steaks, for all steak thickness, were 0.974 to 0.987 (Table 3.4) and 64.2 to 72.1% (Table 3.5), respectively, and were lower ($P < 0.05$) than those of uncooked samples. The variation in water activities and moisture contents of cooked steaks was due to different cooking methods and appliances. Troutt et al. (1992) also reported that the moisture content of ground beef patties was lower after cooking. The decrease in water activity and moisture contents of beef steak samples during cooking was likely the result of expulsion and loss of water from beef tissue caused by long cooking times required to reach the target internal temperature.

The fat content of uncooked steak samples was 2.4-2.5% (Table 3.5). Cooked samples had a fat content of 3.6-7.9%, which increased with thickness of steaks irrespective of cooking method or appliance (Table 3.5). Mukherjee et al. (2008) and Troutt et al. (1992) both reported that cooking of low-fat ground beef samples increased their fat content.

3.3.3. Cooking inactivation effects on bacterial populations

The overall mean *E. coli* O157:H7 inoculum level in control (uncooked) beef steak

samples was 6.4 ± 0.1 log CFU/g (Table 3.6). Total bacterial counts on TSAP were similar ($P \geq 0.05$) to those observed on TSAP+rif for all treatments, indicating that the majority of colonies found on TSAP were inoculated *E. coli* O157:H7. The interaction of cooking appliances and thickness of samples were significant ($P < 0.05$), indicating that the thermal inactivation effect of *E. coli* O157:H7 in moisture-enhanced reconstructed nonintact beef steaks depended on cooking appliances and samples thickness. Cooking of steak samples to 65°C (simulating medium-rare degree of doneness of beef) caused overall pathogen reductions of 1.1 (1.5 cm thick) to 2.3 (4.0 cm thick), 1.6 (1.5 cm thick) to 2.8 (4.0 cm thick), and 2.0 (1.5 cm thick) to 4.2 log CFU/g (4.0 cm thick) for samples cooked by double-pan broiling, pan-broiling, and roasting, respectively (Table 3.6). Therefore, overall inactivation of *E. coli* O157:H7 by cooking decreased in order of: roasting > pan-broiling ≥ double pan-broiling. These findings were in agreement with results of Mukherjee et al. (2007), who found that when cooking beef patties to 65°C, inactivation of *E. coli* O157:H7 decreased in the order of: broiling > frying ≥ grilling. A possible explanation for this is that roasting transmitted heat to the samples by convection (American Meat Science Association, 1995), which is regulated and maintained by a thermostat (Sporing, 1999), resulting in a more even distribution of heat into the samples during cooking compared to pan-broiling and double pan-broiling. Irrespective of steak thickness, the level of pathogen destruction achieved by

the different cooking methods did not appear to be related to the average time taken for the geometric center of the steaks to reach the target temperature of 65°C. Specifically, steaks cooked by double pan-broiling took a shorter time (3.9 [1.5 cm thick] to 19.8 min [4.0 cm thick]) than pan-broiling (14.3 [1.5 cm thick] to 65.0 min [4.0 cm thick]) to reach the target internal temperature; however, surviving pathogen counts in these samples were not different ($P \geq 0.05$; Tables 3.2 and 3.6). Rhee et al. (2003) reported that double sided grilling reduced *E. coli* O157:H7 counts in ground beef patties by 6.9 log CFU/g, which was higher than the reductions caused by single sided grilling (4.7 to 5.6 log CFU/g), although the cooking time to reach the target temperature of 71.1°C was shorter in double sided grill (2.7 min) than single sided grill (6.6 to 10.9 min). In contrast, surviving pathogen counts were numerically, and sometimes lower ($P < 0.05$) in roasted than in pan-broiled samples, even though times to reach the target internal temperature were similar for the two cooking methods (Tables 3.2 and 3.6). As indicated previously, differences in the way by which heat has transferred into the steaks by these cooking methods (i.e., broiling and roasting; Table 3.1) (American Meat Science Association, 1995) most likely caused the higher pathogen reductions in samples cooked by roasting. Furthermore, based on the temperature profiles shown in Figures 3.1 to 3.5, it was apparent that the level of pathogen destruction may have been affected, not only by the time needed to reach the target internal temperature and by how the heat was

transferred, but also by the rate at which the temperature inside various parts of the steaks increased, which varied among cooking methods, appliances, location of the thermocouples in the steak, and steak thickness. For example, a study of Mattick et al. (2002) found that barbecuing and frying sausages sometimes allowed *Salmonella* cells to survive, while cooking sausages by grilling killed all pathogens. In another study, Pittia et al. (2008) reported that minced meat roll cooked in a convention oven (71 to 72°C, internal target temperature) by medium brown cooking cycle caused 7 log CFU/g of total bacterial counts, which was higher than that of light brown cooking cycle (5 log CFU/g).

No differences ($P \geq 0.05$) in surviving pathogen counts were obtained between different cooking appliances within the same cooking method. For example, surviving pathogen counts were 3.6 (4.0 cm thick) to 4.7 (1.5 cm thick) and 3.6 (4.0 cm thick) to 4.8 log CFU/g (1.5 cm thick) for samples cooked by pan-broiling using the Presto® electric skillet or Sanyo® grill, respectively (Table 3.6). Similarly, counts of samples cooked by roasting in the Oster® toaster oven and Magic Chef® kitchen oven were 2.7 (4.0 cm thick) to 4.2 (1.5 cm thick) and 2.2 (4.0 cm thick) to 4.4 log CFU/g (1.5 cm thick), respectively (Table 3.6). Porto-Fett et al. (2009) also found that the reduction levels of *Yersinia pestis* in ground beef patties cooked (60 or 71.1 °C, internal target temperature) on an open-flame gas grill (5.5 log CFU/g) and a clam-shell type electric grill (5.3 log CFU/g) were similar.

Sporing (1999) reported reductions of approximately 3 to 5, 4 to 6 and 5 to 6 log CFU/g for beef steaks cooked to 65.6°C using electric skillet frying, gas grilling and oven broiling, respectively; these reductions were higher than those achieved in our study. This discrepancy could be due to a number of reasons, including the use of different types of beef products, inoculation methods, cooking methods and appliances, starting cooking surface temperatures, and starting sample temperatures. In the study by Sporing (1999), steaks were surface inoculated with *E. coli* O157:H7 and then blade-tenderized, which resulted in minimal translocation of the inoculum to the interior of the product (i.e., approximately 3% from the surface to the interior). Thus, the inoculum was primarily located on the surface of the steak, where the product was exposed to higher temperatures, for longer times, resulting in large microbial reductions (Sporing, 1999). In contrast, in the present study, the product was restructured and the inoculum was more evenly distributed throughout the steak. Thus, the extent of pathogen destruction was related to the temperatures and time taken to reach the target temperature at the "cold spot" (i.e., the geometric center of the sample), and other factors described above, with probably lower temperatures, for relatively shorter periods of time, being responsible for the lower reductions in pathogen numbers.

As expected, the 4.0 cm thick steaks had larger ($P<0.05$) reductions compared to the 1.5 and 2.5 cm thick samples by all cooking methods and appliances. Cooking of 4.0 cm thick

samples by the different methods resulted in 0.5 (George Foreman® grill) to 2.2 log CFU/g (Magic Chef® kitchen oven) higher reductions than those for 1.5 and 2.5 cm thick samples (Table 3.6). The highest reduction (4.2 log CFU/g) was achieved in the 4.0 cm thick samples roasted with the Magic Chef® kitchen oven. These results are in agreement with those observed by others (Sporing, 1999; Thippareddi, 2000). Sporing (1999) reported that thicker steaks (3.2 cm) compared to thinner ones (1.3 cm) resulted in greater reductions of *E. coli* O157:H7 when cooked to the same internal temperature by broiling in a standard kitchen oven. Thippareddi et al. (2000) also found greater reductions of *Salmonella* at 71.1°C in nonintact pork loin chops of 2.5 cm thickness compared to those of 1.25 cm thickness. The possible reason for this is that thicker products require a longer cooking time, as seen in the present study (Table 3.2), to reach the same internal temperature as thin samples, resulting in higher total lethality.

3.4. CONCLUSIONS

The results of this study indicated that extent of inactivation of *E. coli* O157:H7 in undercooked nonintact products depended on steak thickness and in the way by which the cooking method transferred heat into the steak, which affected rate of heat penetration and total time needed to reach the target internal temperature. Additionally, findings showed that roasting in a kitchen oven was the most effective cooking method in reducing *E. coli*

O157:H7 inoculated in nonintact beef steaks (4.0 cm thick), confirming that the thicker the steaks, the greater the inactivation of the pathogen present internally in the product when cooked in the same appliance, and elucidated the temperature variability occurring in different parts of the steaks cooked with different appliances during cooking. Thus, the findings of this study should be useful in risk assessments of nonintact, as well as intact beef products, and in developing cooking guidelines for food service and consumers.

Table 3.1. Terminologies used to describe various cooking methods, and appliances used for cooking in this study

Type of heat transfer	Terminologies of previous studies	Terminologies of this study	Appliances used in this study
Conduction	Frying (Mukherjee et al. [2007]; Sporing [1999])	Pan-broiling	Presto® electric skillet, and Sanyo® grill
	Grilling (Mukherjee et al. [2007])	Double pan-broiling	George Foreman® grill
Convection	Modified broiling (Kendall et al. [1974]) Broiling (Mukherjee et al. [2007]; Sporing [1999])	Roasting	Oster® toaster oven, and Magic Chef® kitchen oven

Table 3.2. Mean (± standard deviation) of time (min) to reach the internal (geometric center) temperature of 65°C during cooking of nonintact beef steaks of different thickness

Cooking		Time (min)		
		Steak thickness (cm)		
Procedure	Appliance	1.5	2.5	4.0
Pan-broiling	Presto® electric skillet	16.3±2.0	27.2±5.4	55.0±2.5
	Sanyo® grill	14.3±1.3	27.8±7.7	65.0±4.7
Double pan-broiling	George Foreman® grill	3.9±0.4	9.9±0.8	19.8±2.7
Roasting	Oster® toaster oven	11.3±0.2	29.7±3.0	45.0±2.9
	Magic Chef® kitchen oven	20.0±2.3	36.3±1.4	63.0±2.8

Table 3.3. Mean (± standard deviation) cooking losses (%) of nonintact beef steaks of different thickness cooked to the internal (geometric center) temperature of 65°C with different cooking appliances

Cooking		Cooking losses (%)		
		Steak thickness (cm)		
Procedure	Appliance	1.5	2.5	4.0
Pan-broiling	Presto® electric skillet	29.6±6.1aX	30.5±4.3aX	31.1±2.1aX
	Sanyo® grill	28.3±4.3aX	27.2±2.1acX	32.3±3.2aY
Double pan-broiling	George Foreman® grill	23.1±4.2bX	30.1±2.5aY	31.1±2.4aY
Roasting	Oster® toaster oven	21.3±4.1bX	22.4±4.3bX	27.2±5.4abY
	Magic Chef® kitchen oven	23.4±5.3bX	23.2±5.1bcX	25.3±5.2bY

a-c: Means with a common letter within a column are not different ($P \geq 0.05$).
X-Y: Means with a common letter within a row are not different ($P \geq 0.05$).

Table 3.4. Mean (± standard deviation) pH and water activity values of nonintact beef steaks of different thickness uncooked or cooked to the internal (geometric center) temperature of 65°C with different cooking appliances

Cooking		pH			Water activity		
		Steak thickness (cm)			Steak thickness (cm)		
Procedure	Appliance	1.5	2.5	4.0	1.5	2.5	4.0
Control	Uncooked	5.76±0.06aX	5.67±0.11aX	5.69±0.10aX	0.992±0.002aX	0.990±0.003aX	0.993±0.001aX
Pan-broiling	Presto® electric skillet	6.14±0.08bX	6.10±0.06bX	6.08±0.04bX	0.987±0.002bX	0.984±0.002bX	0.982±0.002bX
	Sanyo® grill	6.05±0.06bX	6.03±0.06bX	6.04±0.08bX	0.979±0.002bX	0.982±0.002bX	0.980±0.003bX
Double pan-broiling	George Foreman® grill	6.04±0.10bX	6.02±0.09bX	6.02±0.08bX	0.986±0.001bX	0.984±0.001bX	0.979±0.004bY
Roasting	Oster® toaster oven	6.00±0.05bX	6.04±0.02bX	6.01±0.03bX	0.981±0.004bX	0.979±0.001bX	0.974±0.002bY
	Magic Chef® kitchen oven	6.00±0.06bXY	5.98±0.06bX	6.04±0.04bY	0.983±0.001bX	0.979±0.002bXY	0.977±0.005bY

a-b: Means with a common letter within a column are not different ($P \geq 0.05$).
X-Y: Means with a common letter within a row are not different ($P \geq 0.05$).

Table 3.5. Mean (± standard deviation) moisture and fat content (%) of nonintact beef steaks of different thickness uncooked or cooked to the internal (geometric center) temperature of 65°C with different cooking appliances

Cooking		Moisture content (%)			Fat content (%)		
		Steak thickness (cm)			Steak thickness (cm)		
Procedure	Appliance	1.5	2.5	4.0	1.5	2.5	4.0
Control	Uncooked	76.1±2.1aX	76.1±2.2aX	76.1±2.3aX	2.5±0.6aX	2.4±0.5aX	2.5±0.6aX
Pan-broiling	Presto® electric skillet	64.2±2.2bX	66.2±5.2bX	66.3±3.1bX	5.6±2.3bX	5.6±2.1bX	7.9±3.0bX
	Sanyo® grill	68.2±2.1bXY	64.3±8.3bX	69.2±6.1bY	5.7±0.8bX	5.9±2.9bX	7.0±2.8bX
Double pan-broiling	George Foreman® grill	68.2±2.1bX	64.3±8.2bY	68.2±6.1bX	5.7±0.8bX	5.8±0.6bX	7.1±1.8bX
Roasting	Oster® toaster oven	72.1±3.2bX	69.2±2.3bXY	67.2±7.4bY	3.6±1.5bX	4.5±1.7bX	4.9±3.2bX
	Magic Chef® kitchen oven	70.1±3.1bX	69.5±2.1bX	68.5±4.2bX	5.3±2.5bXY	4.5±0.7bX	7.2±3.9bY

a-b: Means with a common letter within a column are not different ($P \geq 0.05$).
X-Y: Means with a common letter within a row are not different ($P \geq 0.05$).

Table 3.6. Mean (± standard deviation; log CFU/g) of total bacterial and rifampicin-resistant *Escherichia coli* O157:H7 populations, recovered with tryptic soy agar supplemented with sodium pyruvate (0.1%, TSAP) and TSAP plus rifampicin (100 μg/ml), respectively, from nonintact beef steaks of different thickness that were uncooked and cooked with different appliances to the internal (geometric center) temperature of 65°C

Cooking		TSAP			TSAP plus rifampicin		
		Steak thickness (cm)			Steak thickness (cm)		
Procedure	Appliance	1.5	2.5	4.0	1.5	2.5	4.0
Control	Uncooked	6.5±0.1aX	6.5±0.1aX	6.5±0.1aX	6.4±0.1aX	6.4±0.1aX	6.4±0.1aX
Pan-broiling	Presto® electric skillet	4.8±0.2bX	4.4±0.4bX	3.6±0.8bY	4.7±0.1bX	4.3±0.4bX	3.6±0.6bY
	Sanyo® grill	5.0±0.4bX	4.6±0.2bX	3.8±0.6bY	4.8±0.5bX	4.3±0.3bX	3.6±0.6bY
Double pan-broiling	George Foreman® grill	5.3±0.8bX	4.4±0.3bY	4.2±0.4bY	5.3±0.8bX	4.6±0.3bXY	4.1±0.5bY
Roasting	Oster® toaster oven	4.2±0.2cX	4.0±0.5bX	2.8±0.9cY	4.2±0.2cX	3.7±0.5cX	2.7±1.1cY
	Magic Chef® kitchen oven	4.5±0.4bcX	3.9±1.0bX	2.5±1.1cY	4.4±0.3bcX	3.5±1.0bcX	2.2±1.0cY

a-c: Means with a common letter within a column are not different ($P \geq 0.05$).
X-Y: Means with a common letter within a row are not different ($P \geq 0.05$).

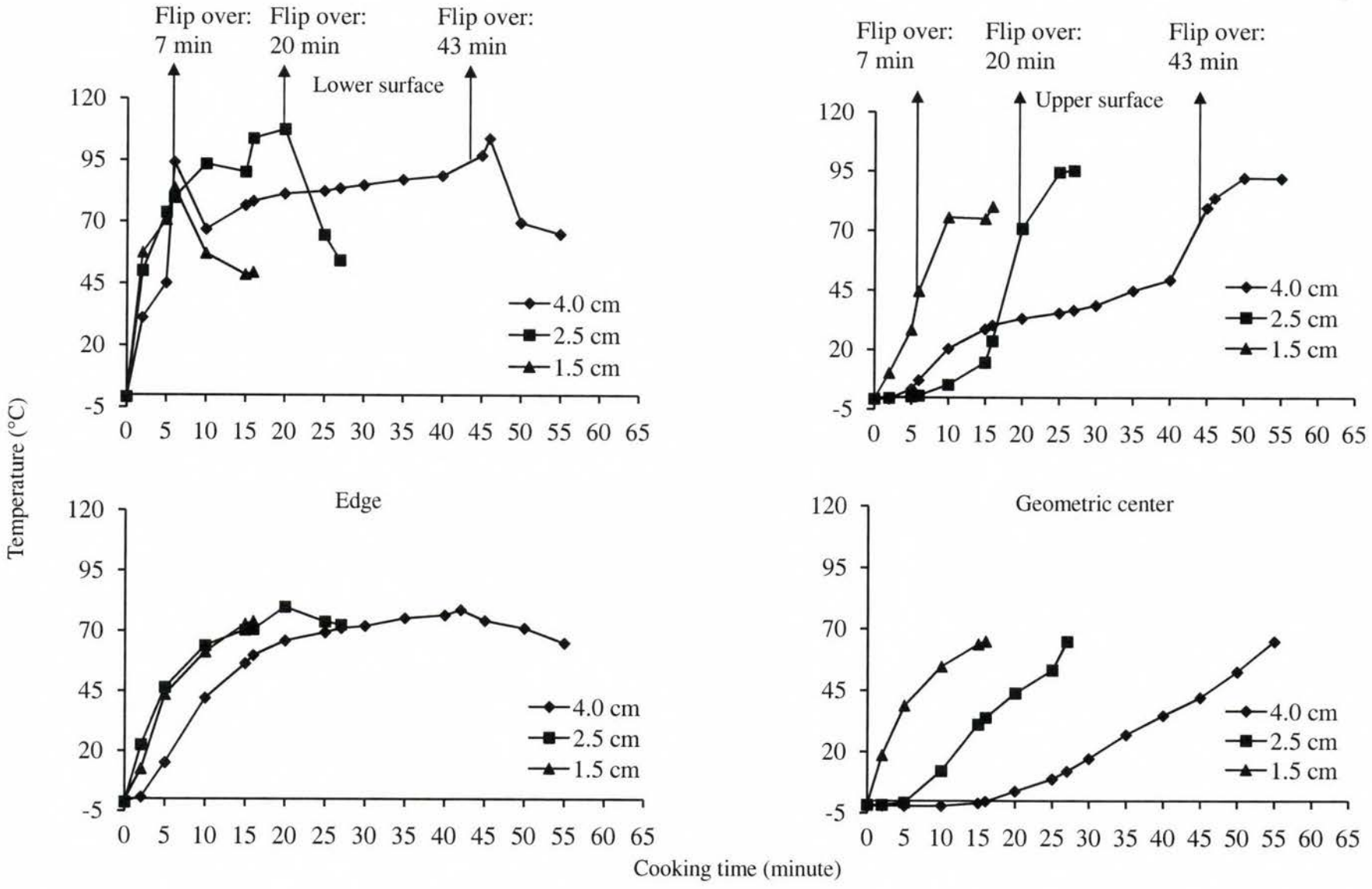


Figure 3.1 (Appendix Table 1). Cooking time and temperature curves for nonintact beef steaks of different thickenss cooked by pan-broiling using the Presto® electric skillet. Each point is the average of four determinants.

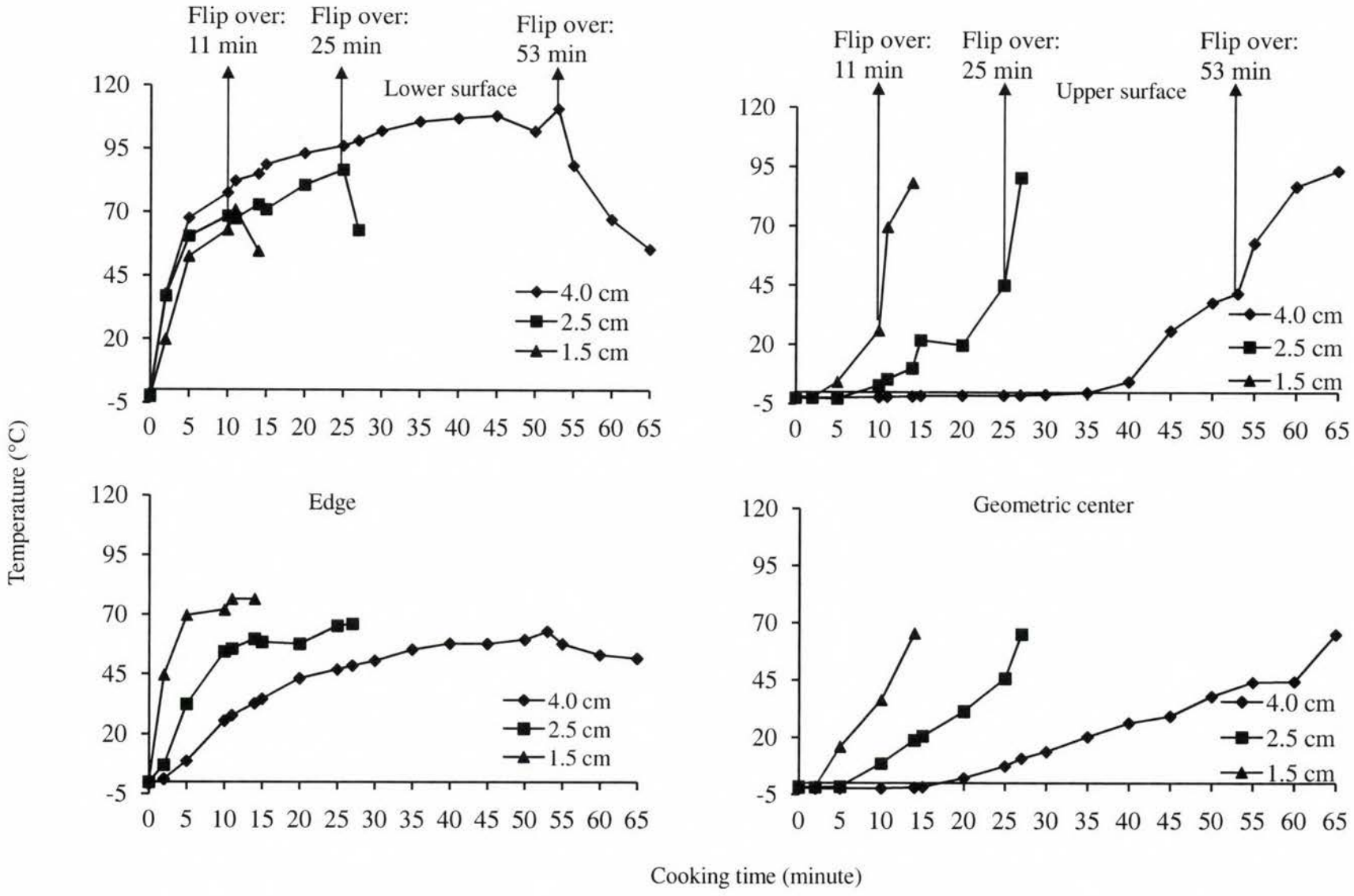


Figure 3.2 (Appendix Table 2). Cooking time and temperature curves for nonintact beef steaks of different thickness cooked by pan-broiling using the Sanyo® grill. Each point is the average of four determinants.

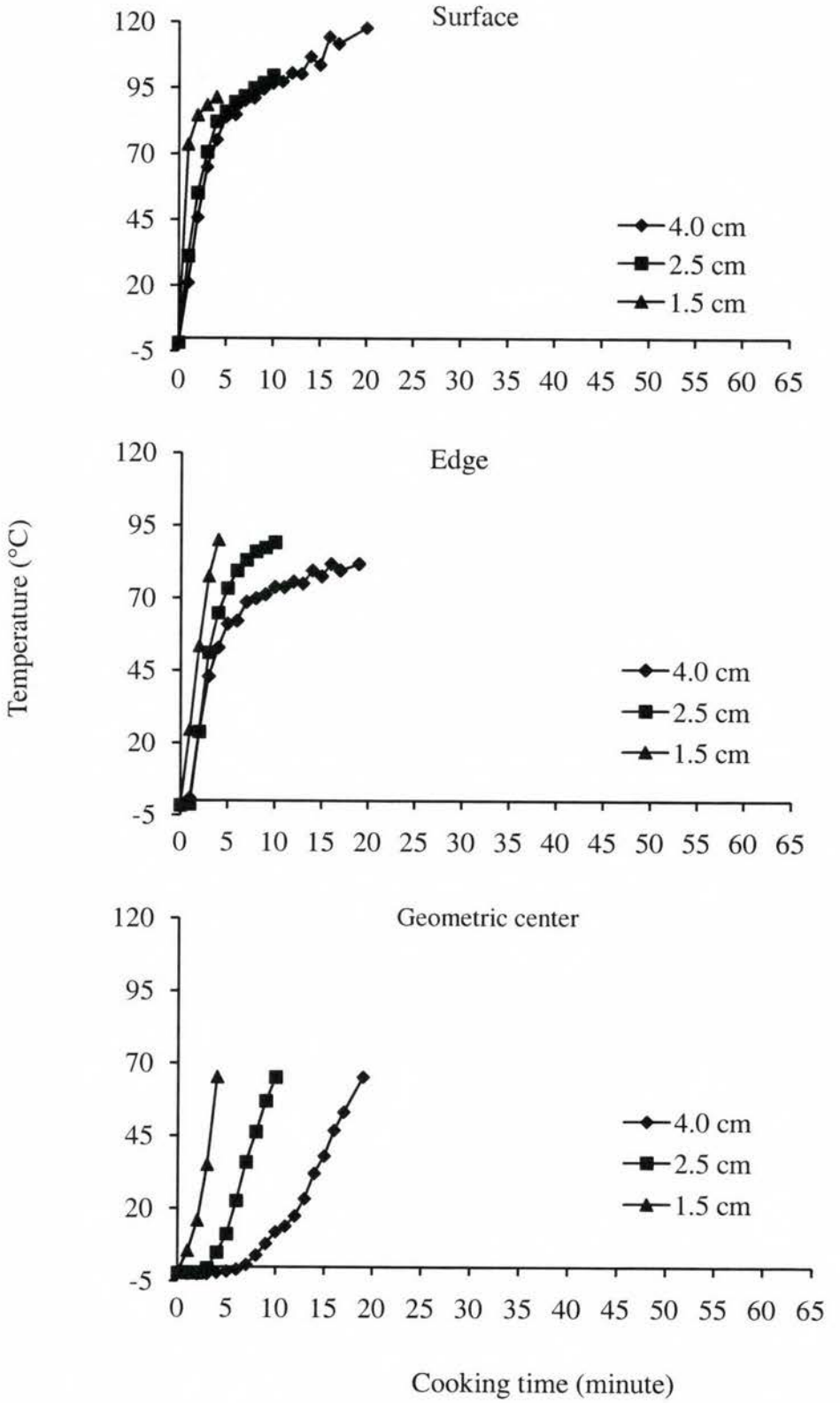


Figure 3.3 (Appendix Table 3). Cooking time and temperature curves for nonintact beef steaks of different thickness cooked by double pan-broiling using the George Foreman® grill. Each point is the average of four determinants.

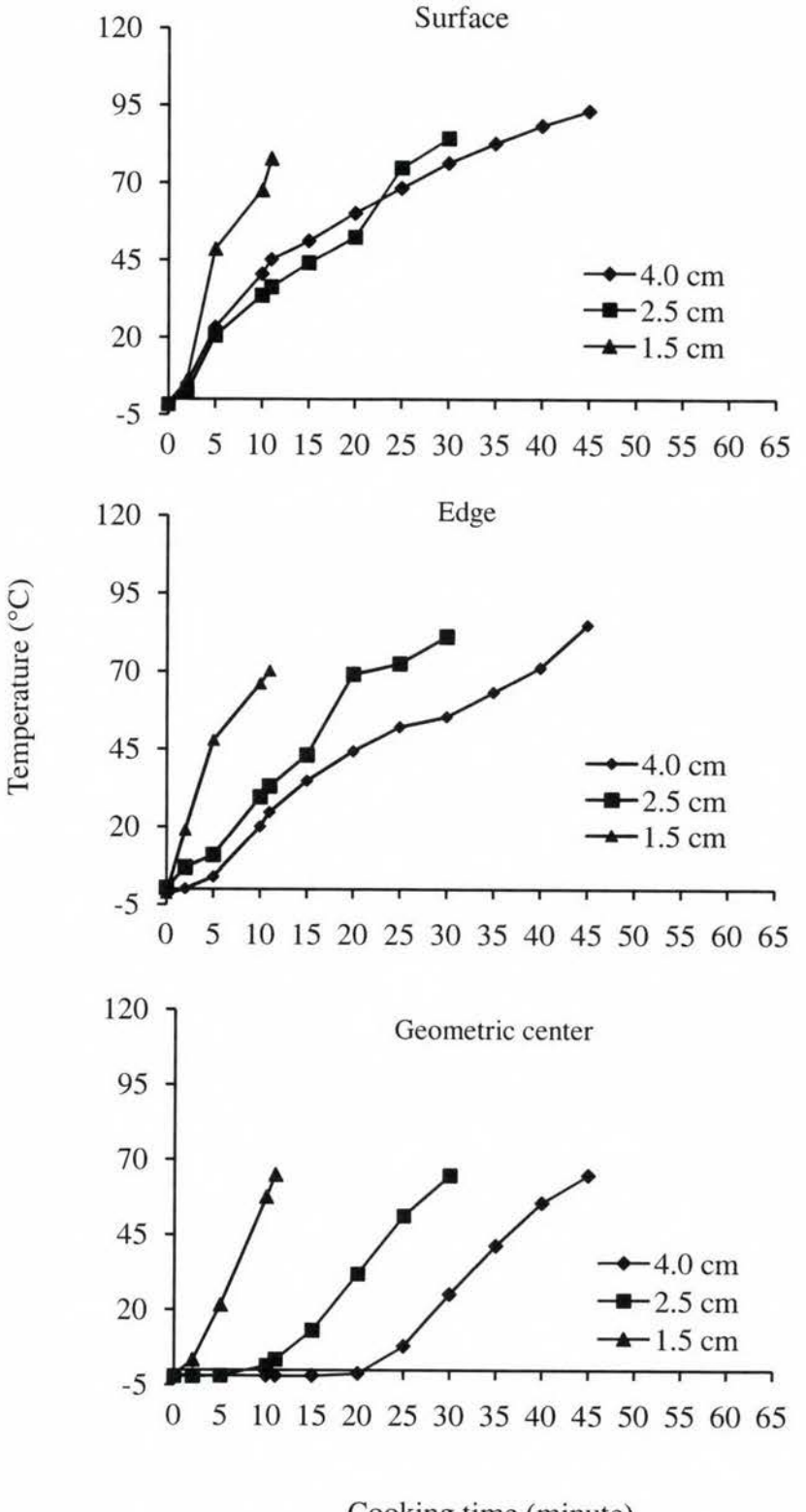


Figure 3.4 (Appendix Table 4). Cooking time and temperature curves for beef steaks of different thickness cooked by roasting using the Oster® toaster oven. Each point is the average of four determinants.

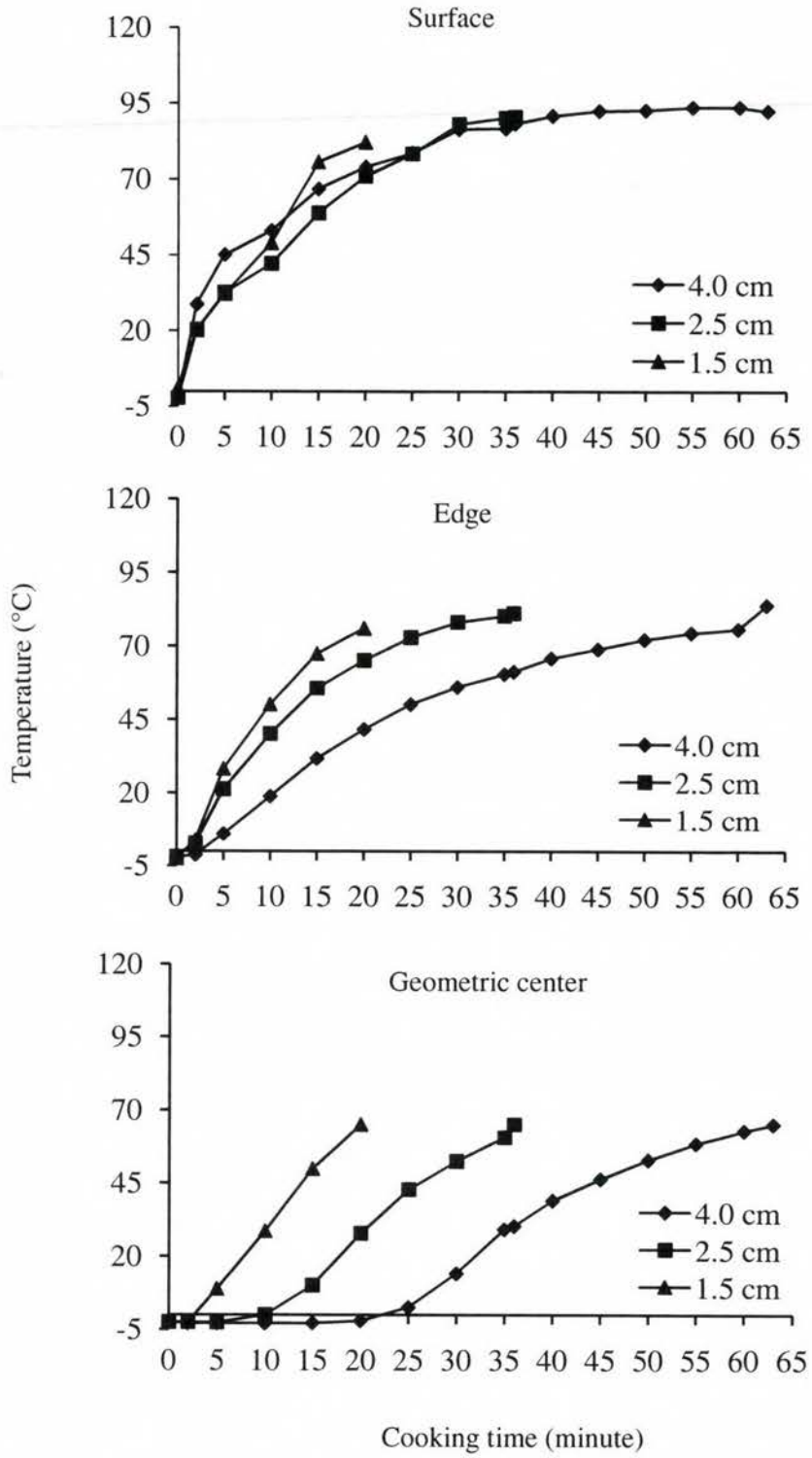


Figure 3.5 (Appendix Table 5). Cooking time and temperature curves for nonintact beef steaks of different thickness cooked by roasting using the Magic Chef® kitchen oven. Each point is the average of four determinants.

CHAPTER 4

THERMAL INACTIVATION OF *ESCHERICHIA COLI* O157:H7 IN MOISTURE ENHANCED NONINTACT BEEF BY PAN-BROILING OR ROASTING IN VARIOUS COOKING APPLIANCES SET AT DIFFERENT TEMPERATURES

ABSTRACT

This study compared inactivation of *Escherichia coli* O157:H7 in moisture-enhanced nonintact beef steaks cooked to 65°C using different cooking appliances set at different temperatures. Fresh beef (5% fat) was coarse-ground (0.95 cm diameter) and batches (2 kg) were mixed with an 8-strain composite (100 ml) of rifampicin-resistant *E. coli* O157:H7 (6.4 ± 0.1 log CFU/g) and sodium chloride (0.5%) plus sodium tripolyphosphate (0.25%) solution (100 ml), extruded into casings (10 cm diameter), and placed at -20°C for 6 h. Semi-frozen beef was cut into 2.54 cm thick steaks, vacuum-packaged, frozen (-20°C, 48 h), and

tempered (4°C, 2.5 h) before cooking. Steaks were pan-broiled (Presto® electric skillet or Sanyo® grill) or roasted (Oster® toaster or Magic Chef ® kitchen oven) to 65°C. Each of the four cooking appliances was pre-heated before and maintained during cooking as follows: 149 or 204°C (electric skillet), 149 or 218°C (grill), 149 or 232°C (toaster oven), and 149, 204, or 260°C (kitchen oven). Temperatures of cooking appliances and samples were monitored with thermocouples. Samples were analyzed for survivors on tryptic soy agar plus sodium pyruvate (0.1%, TSAP) and TSAP plus rifampicin (100 μg/ml). In general, the higher the starting temperature, the shorter the time needed to reach 65°C (204 to 260°C, 27 to 35 min; 149°C, 35 to 60 min), and the higher the edge and surface temperatures of the steaks. Starting temperatures of 204 to 260°C, regardless of appliance, resulted in greater ($P < 0.05$) pathogen reductions (3.3 to 5.5 log CFU/g) than those obtained at 149°C (1.5 to 2.4 log CFU/g). The highest ($P < 0.05$) reduction (5.5 log CFU/g) was obtained in steaks cooked in the kitchen oven set at 260°C. The results of this study should be useful in risk assessments for the food service industry as it strives to select effective nonintact beef cooking protocols, and for development of cooking instructions for consumers.

4.1. INTRODUCTION

As defined by the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS), nonintact beef products include ground beef, mechanically or chemically

tenderized meat cuts, restructured entrees, and those meat products that have been injected with marination or brining solutions to enhance flavor and/or tenderness (USDA-FSIS, 1999). Undercooked nonintact beef products have been involved in several outbreaks between 2000 and 2007 in the United States due to contamination with *Escherichia coli* O157:H7 (Laine et al., 2005; USDA-FSIS, 2005a; 2005b; 2007a; 2007b; 2007c; 2007d; 2007e; 2008a; Conference for Food Protection, 2008). During meat processing, such as mechanical tenderization, injection of solutions, or restructuring, *E. coli* O157:H7 or other pathogen contamination may be translocated or entrapped from the meat surface into the interior of the cut, and thus it could be protected from heating effects, especially if the products are intentionally or unintentionally undercooked (Hajmeer et al., 2009; Ortega-Valenzuela et al., 2001; USDA-FSIS, 2002a; 2002b).

Limited number of studies that have evaluated the inactivation efficacy of cooking on *E. coli* O157:H7 internalized in nonintact beef products, other than ground beef, have indicated that broiling was more effective than grilling or frying when meat samples were cooked to the internal temperature of 60 or 65°C (Sporing, 1999; Mukherjee et al., 2007; Chapter 3). The USDA-FSIS comparative risk assessment of nonintact and intact beef steaks indicated that oven broiling to an internal temperature of more than 60°C would result in safe blade-tenderized beef steaks (USDA-FSIS 2002a; 2002b). For corned beef products, it was

recommended that the temperature of the cooking oven should be set at 350°F (176°C) or no lower than 325°F (162.8°C) (USDA-FSIS, 2008c). However, there is no published information verifying thermal inactivation of *E. coli* O157:H7 in moisture-enhanced, restructured nonintact beef steaks cooked by pan-broiling or roasting in various cooking appliances set at different temperatures. Thus, the objective of this study was to determine the extent of *E. coli* O157:H7 thermal inactivation in nonintact meat cooked to 65°C (internal temperature) with various cooking appliances set at different temperatures.

4.2. MATERIALS AND METHODS

4.2.1. Bacterial strains and inoculum preparation

An eight-strain mixture of *E. coli* O157:H7 used in this study included rifampicin-resistant derivatives of strains ATCC 43888 (human isolate), ATCC 43895 (hamburger isolate), ATCC 43895/ISEHGFP (hamburger isolate, Noah et al., 2005), and C1-057, C1-072, C1-109, C1-154, C1-158 (cattle feces isolates, Carlson et al., 2009). Rifampicin resistant strains were used to allow selective detection and enumeration of the inoculum from natural contaminating flora, as well as recovery of injured cells. The strains were cultured, maintained and washed as described in a previous study (Chapter 3). On the day of setting up the experiment, the washed inoculum was serially diluted in phosphate buffered saline (PBS, pH 7.4; 0.2 g of KH_2PO_4, 1.5 g of $Na_2HPO_4 \cdot 7H_2O$, 8.0 g of NaCl, and

0.2 g of KCl in 1 liter of distilled water) to obtain a target inoculation level of 6 to 7 log CFU/g when 100 ml of inoculum was added to 2 kg of fresh coarse ground beef.

4.2.2. Preparation and inoculation of nonintact beef steaks

The beef steaks were prepared and inoculated as described previously (Chapter 3). Briefly, each 2 kg batch of course ground (0.95 cm diameter kidney plate, Hobart Mfg. Co., Troy, OH) fresh beef knuckles (approximately 5% fat) was placed in a bowl-lift stand mixer (Professional 600, KitchenAid, St. Joseph, MI), inoculated by adding 100 ml of the PBS prepared inoculum slowly for 2 min (speed setting at "stir"), and then mixing was continued for another 2 min at the same speed, with the addition of 100 ml of sterile distilled water containing sodium chloride (0.5%) plus sodium tripolyphosphate (0.25%) to yield 0.5% and 0.25% for moisture enhancement. The inoculated beef was extruded into casings (in-stock poly bag, 5-lb, 6 × 18 inch, Koch, Kansas City, MO), tied, and placed in a freezer (-20°C) for 6 h. Semi-frozen beef logs were cut into 2.54 cm thick steaks, and then each piece was placed into a vacuum bag (15 × 22 cm, 3 mil std barrier, nylon/polyethylene vacuum pouch, water vapor and oxygen transmission rates of 9.3 $g/m^2/24h$ [97% relative humidity] and 54.3 $cm^3/m^2/24h$ [21°C, 0% relative humidity], respectively, Koch, Kansas City, MO), vacuum packaged (Hollymatic, Corp., Countryside, IL), frozen (-20°C) for 42 h, and then tempered (4°C) for 2.5 h before cooking.

4.2.3. Cooking of beef steaks

Beef steaks were cooked by pan-broiling using a Presto® 16-inch electric skillet (National Presto Industries, Inc., Eau Claire, WI) or a Sanyo® indoor barbecue grill (SANYO Fisher, Inc., Chatsworth, CA), or by roasting using an Oster® stainless steel toaster oven (Sunbeam Products, Inc., Boca Raton, Florida) or a Magic Chef ® kitchen oven (Maytag Corp., Newton, IA), to the final internal temperature of 65°C, which corresponded to a medium rare degree of doneness of beef steaks. Temperatures of cooking appliances, and surface, edge, and center of the products were monitored and recorded as described previously (Chapter 3). For cooking, the appliances were set and the settings maintained constant at different temperatures: 149 or 204°C (Presto® electric skillet), 149 or 218°C (Sanyo® grill), 149 or 232°C (Oster® toaster oven), and 149, 204, or 260°C (Magic Chef® kitchen oven), respectively. The temperature setting of each appliance was kept constant during cooking. The samples were placed on the pan-broiling surface or in the center of the oven for roasting. For pan-broiling, the steak samples were flipped over when the center internal temperature reached 42°C, and then they were cooked to the target temperature of 65°C.

4.2.4. Physical and chemical analyses

Cooking losses were measured by weighing samples before cooking and reweighing them immediately after cooking. Moisture and fat contents of uncooked and cooked samples

were measured using AOAC International official methods No. 960.39 and No. 950.46.B, respectively (AOAC, 2000). The pH of the homogenate of uncooked and cooked samples was measured using a digital pH meter with a glass electrode (Denver Instruments, Arvada, CO). The water activity of raw and cooked steak samples was measured within 10 h after cooking using an AquaLab (model series 3, Decagon Devices Inc., Pullman, WA) water activity meter.

4.2.5. Microbiological analyses

Counts of the background microbial flora of fresh beef were determined immediately after coarse grinding. Surviving counts of pathogen cells were determined in samples before and immediately after cooking. Maximum recovery diluent (MRD; 0.85% NaCl [Fisher Scientific, Fair Lawn, NY] and 0.1% peptone [Difco, Becton Dickinson, Sparks, MD]) was added into a sterile filter bag (Whirl-Pak, Nasco, Modesto, CA) with an individual sample steak at a ratio of 1:1 (steak weight [g]: volume of MRD [ml]), and homogenized (Masticator, IUL Instruments, Barcelona, Spain) for 2 min. Each sample was serially diluted (10-fold) in 0.1% buffered peptone water, and then surface (0.1 ml) plated onto tryptic soy agar (Acumedia, Lansing, MI) plus pyruvate (Fisher Scientific, Fair Lawn, NY) (0.1%, TSAP) for enumerating total microbial populations, and onto TSAP plus rifampicin (100 μg/ml; Sigma-Aldrich Inc., St. Louis, MO) for enumeration of *E. coli* O157:H7 cell numbers,

respectively. Colonies were counted manually after the plates were incubated at 35°C for 48 h.

4.2.6. Statistical analysis

The experiment was replicated two times with three individual samples per treatment each time. The pH, water activity, cooking losses, moisture and fat contents, and microbiological counts (converted to log CFU/g) were analyzed using the One Way ANOVA in the GLM procedures of SAS (SAS Institute, 2002). Microbiological data included surviving counts after cooking treatments, and reductions of counts compared to uncooked samples (reduction level=log CFU/g uncooked/cooked). Means and standard deviations for all data were calculated, and the mean differences were separated with the least significant difference procedure at the significance level of α=0.05.

4.3. RESULTS AND DISCUSSION

4.3.1. Temperatures of cooking appliances

In pan-broiling appliances, the average cooking surface temperature of the Presto® electric skillet was 167 ± 13 and 218 ± 11°C (Figure 4.1; Appendix Table 6) when set at 149 and 204°C, respectively, while the surface temperature of the Sanyo® grill was 165 ± 16 and 238 ± 9°C (Figure 4.1; Appendix Table 6) when set at 149 and 218°C, respectively. For roasting appliances, the temperature of the cooking chamber was 139 ± 5 and 228 ± 12°C

(Figure 4.1; Appendix Table 6) in the Oster® toaster oven when set at 149 and 232°C, respectively, and 142 ± 6, 207 ± 5, and 264 ± 5°C (Figure 4.1; Appendix Table 6) in the Magic Chef ® kitchen oven when set at149, 204 and 260°C, respectively. The variation of 4 to 16°C observed in appliance surface or chamber temperatures might be explained by the heating element switching on/off to maintain the thermostat balance.

4.3.2. Temperatures of steaks

As indicated, the surface, edge and geometric center temperatures of the samples were monitored and recorded during the cooking period (Figures 4.2 to 4.5; Appendix Tables 7 to 10). For pan-broiling, thermocouples were inserted, approximately 5 mm inside the tissue at the lower (closest to the heat source) and top (away from the heat source) surface of steaks to monitor surface temperature. As expected, the lower surface temperature increased promptly before the samples were flipped over (when the center temperature reached 42°C) and reached final temperatures as high as 91.0 and 103.8°C (Figure 4.2; Appendix Table 7) when the Presto® electric skillet was set at 149 and 204°C, respectively. In the Sanyo® grill, set at 149 and 218°C, the lower surface reached 103.3 and 106.1°C (Figure 4.3; Appendix Table 8), respectively. Immediately after flipping the samples over, the original lower surface now became the upper surface, and its temperature decreased to 63.7 to 67.0°C (Figure 4.2; Appendix Table 7; Presto® electric skillet) and 58 to 62°C (Figure 4.3; Appendix Table 8;

Sanyo® grill). The temperature of the upper surface increased slowly before the steaks were flipped over, because it did not touch the hot surface. The upper surface temperature increased quickly when the steak was flipped over and reached approximately 80 to 90°C by the end of cooking. The edge temperature of samples cooked by pan-broiling also increased quickly, and the final temperatures were 61.7 to 66.5°C (Figure 4.2; Appendix Table 7) and 57 to 64°C (Figure 4.3; Appendix Table 8) when using the Presto® electric skillet and Sanyo® grill, respectively. At the higher appliance temperatures, the edge sample temperatures reached higher levels than the lower ones after the same length of cooking time. For example, the edge temperature of steaks was 79.7 and 61.2°C (Figure 4.3; Appendix Table 8) after 20 min cooking with the Presto® electric skillet set at 204 and 149°C, respectively. In the roasting appliances, the final sample surface and edge temperatures of steaks were 81.3 to 89.7°C (Figure 4.4; Appendix Table 9) and 73.3 to 78.1°C (Figure 4.4; Appendix Table 9; Oster® toaster oven), and 70.5 to 75.0°C and 73.1 to 85.5°C (Figure 4.5; Appendix Table 10; Magic Chef® kitchen oven). The variability in final surface and edge temperature was due to the different appliance temperature; the higher the appliances temperature, the higher the temperature surrounding the cooked meat, resulting in higher surface and edge temperatures.

The initial geometric center temperature of uncooked steaks was -2.5 ± 1.0°C. Cooking beef steaks by pan-broiling and roasting required 33.0 to 45.0 (Figures 4.2 and 4.3; Appendix

Tables 7 and 8) and 27-60.0 min (Figures 4.4 and 4.5; Appendix Tables 9 and 10), respectively, to reach the internal center temperature of 65°C. The large variation in cooking times within each cooking appliance was due to the different temperatures at which appliances were set and maintained during cooking; generally, the higher the appliance temperature, the shorter the time needed to reach the target temperature. For example, roasting of steaks in the Magic Chef® kitchen oven required 30, 35 and 60 min (Figure 4.5; Appendix Table 10) to reach the center temperature of 65°C with at the appliance temperature set at 260, 204 and 149°C, respectively, and pan-broiling of samples on the Sanyo® grill took 33 and 45 min (Figure 4.3; Appendix Table 8) to reach the target temperature of 65°C when the appliance set at 218 and 149°C, respectively. Bengtsson et al. (1976) found that roasting semimembranosus muscle (800 to 900 g) in oven set at 225°C required less time (60 min) compared to that of set at 175°C (80 min), which agreed with results of this study. It is possible that the appliances set at higher temperature increase apparent heat transfer coefficient which shortens the time to reach the target temperature (Sato et al., 1987). However, the samples cooked on the Presto® electric skillet took the same time (35 min) to reach the target temperature of 65°C when it was set at 149 and 204°C. The possible reason for this is the different shape and design of the appliances which may cause different heat transfer efficiency into the geometric center of the products, although they use

the same heat transfer principle. For example, the Presto® electric skillet has a raised edge structure around the cooking surface, while Sanyo® grill did not have such edge.

4.3.3. Cooking losses, pH, water activity, moisture and fat contents

Cooking resulted in weight losses in the ranges of 26.1 to 32.4% and 15.8 to 30.5% for pan-broiling and roasting (Table 4.1), respectively, with weight losses increasing as the appliance set temperature increased. These results agree with those of Bengtsson et al. (1976), who found that weight losses of semimembranosus muscle roasting in an oven set at 225°C was 30% compared to 24% at 175°C. The pH of fresh uncooked beef steaks was 5.72 ± 0.05. Pan-broiling and roasting caused increases ($P < 0.05$) in pH to 5.87 to 5.90 (Table 4.2). The water activity and moisture content of fresh uncooked beef steak samples were 0.993 ± 0.001 and 77.0 ± 0.7% (Table 4.2), respectively. These values represented a 10% pump rate in non-intact products. Cooking decreased ($P<0.05$) water activity and moisture content to 0.969 to 0.979 and 62.7 to 67.4% (Table 4.2), respectively, among all appliances setting at different temperatures. The lowest ($P < 0.05$) water activity value (0.969 ± 0.002) occurred in the samples cooked using the Sanyo® grill set at 218°C. These results were similar to previous studies (Mukherjee et al., 2008; Troutt et al., 1992). The fat content of control steak samples was 1.8 ± 0.2%. Cooking at different set temperatures in various appliances increased fat content in the range 3.1 to 7.5% (Table 4.2), due to decreased moisture content.

4.3.4. Cooking inactivation effects on the pathogen

The total counts of background microbial flora in uninoculated fresh beef were 3.2 ± 0.3 log CFU/g. The initial inoculum level of *E. coli* O157:H7 in uncooked beef steaks was 6.4 ± 0.1 log CFU/g (Figure 4.6; Appendix Table 11). Total microbial counts on TSAP were similar (6.5 ± 0.2 log CFU/g, $P > 0.05$) to those observed on TSAP + Rif agar for all treatments (Figure 4.7; Appendix Table 11), indicating that the majority of colonies found on TSAP were *E. coli* O157:H7. As expected, cooking of inoculated steaks to the target temperature of 65°C (medium rare) caused overall reductions of pathogen counts ranging from 1.5 to 3.3 and 1.9 to 5.5 log CFU/g (Figure 4.6; Appendix Table 11) when cooked at different set temperatures by pan-broiling and roasting, respectively. Thus, generally, the inactivation efficacy of roasting was higher than pan-broiling, which is in agreement with our previous study (Chapter 3). Previous studies found that broiling in a convection oven caused higher reductions of *E. coli* O157:H7 in beef products compared to gas grilling or frying (Sporing, 1999; Ortega-Valenzuela et al., 2001; Mukherjee et al., 2007). In all cooking appliances, the higher appliance set temperatures resulted in higher pathogen reductions compared to those achieved at the lower ones. When the pan-broiling appliances, Presto® electric skillet and Sanyo® grill, were set at 204 and 218°C, respectively, the reductions in counts were 3.3 log CFU/g compared to the uncooked controls. This reduction was higher ($P < 0.05$) than those achieved at 149°C (1.5 log CFU/g). For roasting appliances, steaks cooked in the Oster®

toaster oven set at 218°C had a 4.1 log CFU/g (Figure 4.6; Appendix Table 11) reduction compared to the uncooked controls, which was also higher ($P < 0.05$) than the reduction (1.9 log CFU/g) achieved by setting the oven at 149°C. The reduction levels in samples cooked in the Magic Chef® kitchen oven ranged from 2.4 to 5.5 log CFU/g (Figure 4.6; Appendix Table 11), and were higher at the higher appliance temperature (149 vs 260°C). Maximum reductions were found in samples cooked at the temperature of 260°C, with only 0.9 log CFU/g (Figure 4.6; Appendix Table 11) of pathogen counts surviving in the sample after cooking. A possible reason for this could be that the velocity of heated air in a convectional oven increased with increasing set temperatures (1116.7 m/sec at 220°C vs 903.7 m/sec at 180°C) (Sato et al., 1987), which caused the heat to penetrate into the cooking samples more efficiently than that of lower ones, resulting in higher pathogen reductions. There is limited information reported on the thermal inactivation effects on foodborne pathogens by various cooking appliances set at different temperatures. Murphy et al. (2001) found that a pilot-scale air convection oven set at a high web bulb temperature of 93°C resulted in less surviving counts of *Salmonella* (a mixture of six species) and *Listeria innocua* (less than 1 log CFU/g) in chicken breast patties, compared to those at a low temperature of 48°C (more than 2 log CFU/g).

4.4. CONCLUSIONS

The results of this study indicated that the cooking appliance type and set cooking temperature affected thermal inactivation of *E. coli* O157:H7 internalized in moisture enhanced beef. The higher the appliance set temperatures, the higher the level of inactivation of *E. coli* O157:H7 inoculated in nonintact beef steaks. In general, roasting in a toaster oven or standard kitchen oven killed greater numbers of the pathogen compared to pan-broiling in electric skillet or grill. The results should be useful for risk assessment and to the food service industry for selection of effective nonintact beef cooking protocols, and for development of cooking instructions for consumers.

Table 4.1. Mean (± SD) cooking losses (%) of nonintact beef steaks cooked with various cooking appliances set at different temperatures

Cooking method	Appliances	Set cooking temperature (°C)	Cooking losses (%)
Pan broiling	Presto® Electric skillet	149	26.1 ± 0.5b
		204	32.4 ± 1.8a
	Sanyo® grill	149	26.7 ± 3.6b
		218	32.4 ± 3.5a
Roasting	Oyster® toaster oven	149	20.1 ± 5.8cd
		232	27.2 ± 6.5b
	Magic Chef® Kitchen oven	149	15.8 ± 2.1d
		204	19.5 ± 2.1b
		260	30.5± 4.8a

a-d: Means with a common letter within a column are not different ($P \geq 0.05$).

Table 4.2. Mean (± SD) pH, water activity, moisture and fat contents (%) of uncooked and cooked nonintact beef steaks cooked with various cooking appliances set at different temperatures.

Cooking method	Appliance	Set cooking temperature (°C)	pH	Water activity	Moisture (%)	Fat (%)
Uncooked		----	5.72 ± 0.06a	0.993 ± 0.001a	77.0 ± 0.7a	1.8 ± 0.2d
Pan-broiling	Presto® Electric skillet	149	5.90 ± 0.07b	0.978 ± 0.002b	65.5 ± 3.3b	5.2 ± 1.2abc
		204	5.90 ± 0.06b	0.977 ± 0.004b	66.1 ± 0.8b	4.0 ± 0.8bc
	Sanyo® grill	149	5.90 ± 0.06b	0.979 ± 0.001b	62.9 ± 4.7b	3.8 ± 0.7bcd
		218	5.89 ± 0.06b	0.969 ± 0.002c	65.2 ± 9.0b	5.6 ± 3.1ab
Roasting	Oyster® toaster oven	149	5.89 ± 0.08b	0.979 ± 0.003b	66.8 ± 0.3b	4.9 ± 0.5bc
		232	5.87 ± 0.09b	0.975 ± 0.006b	63.0 ± 5.3b	7.5 ± 1.6a
	Magic Chef® Kitchen oven	149	5.90 ± 0.07b	0.974 ± 0.001b	67.4 ± 5.1b	3.1 ± 0.6cd
		204	5.89 ± 0.07b	0.979 ± 0.002b	67.1 ± 2.6b	4.4 ± 1.0bc
		260	5.90 ± 0.07b	0.976 ± 0.001b	62.7 ± 5.7b	5.6 ± 2.8bc

a-d: Means with a common letter within a column are not different ($P \geq 0.05$).

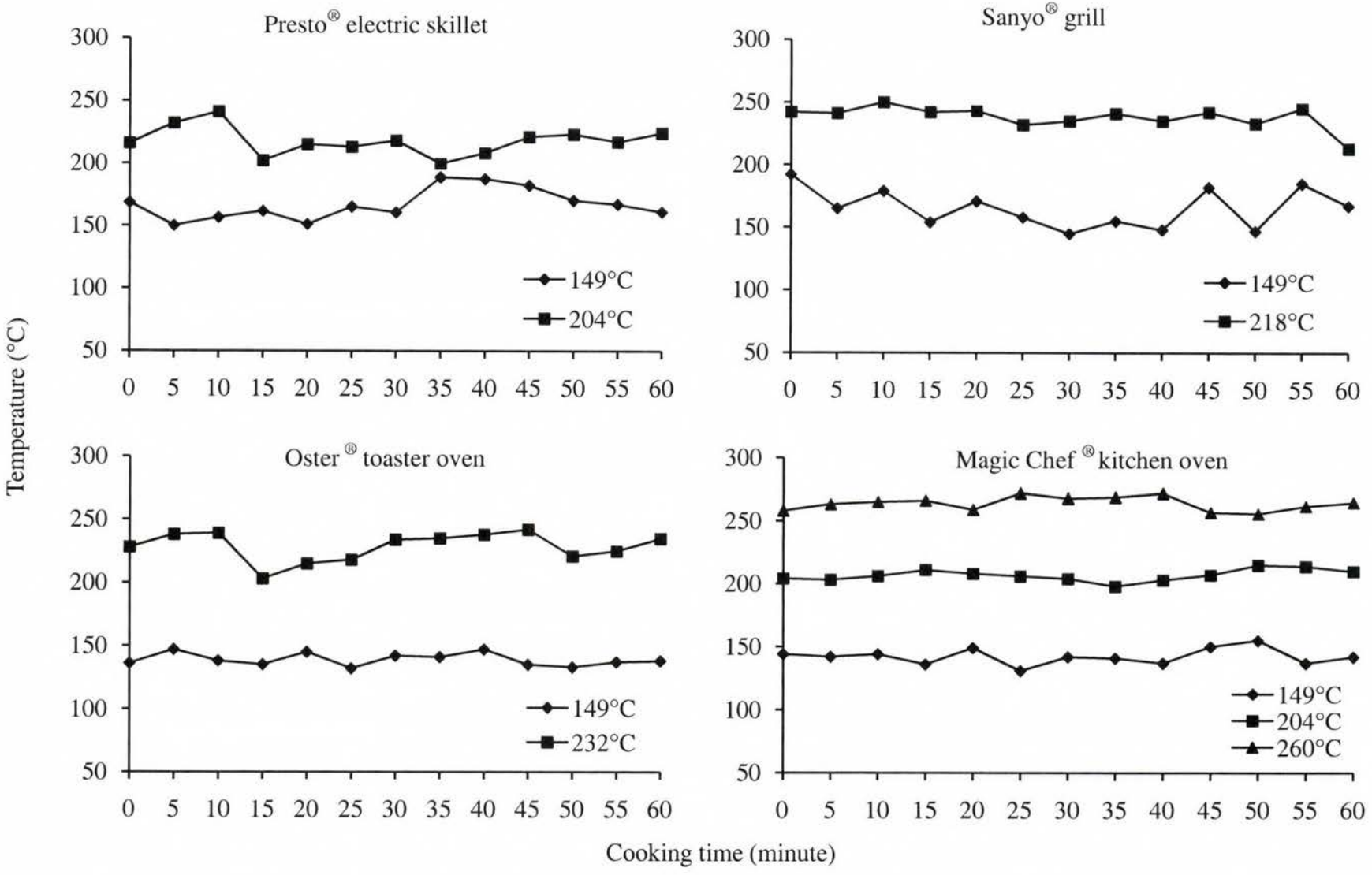


Figure 4.1 (Appendix Table 6). Temperature curves of pan-broiling and roasting appliances set and maintained at different temperatures settings during cooking of nonintact beef steaks.

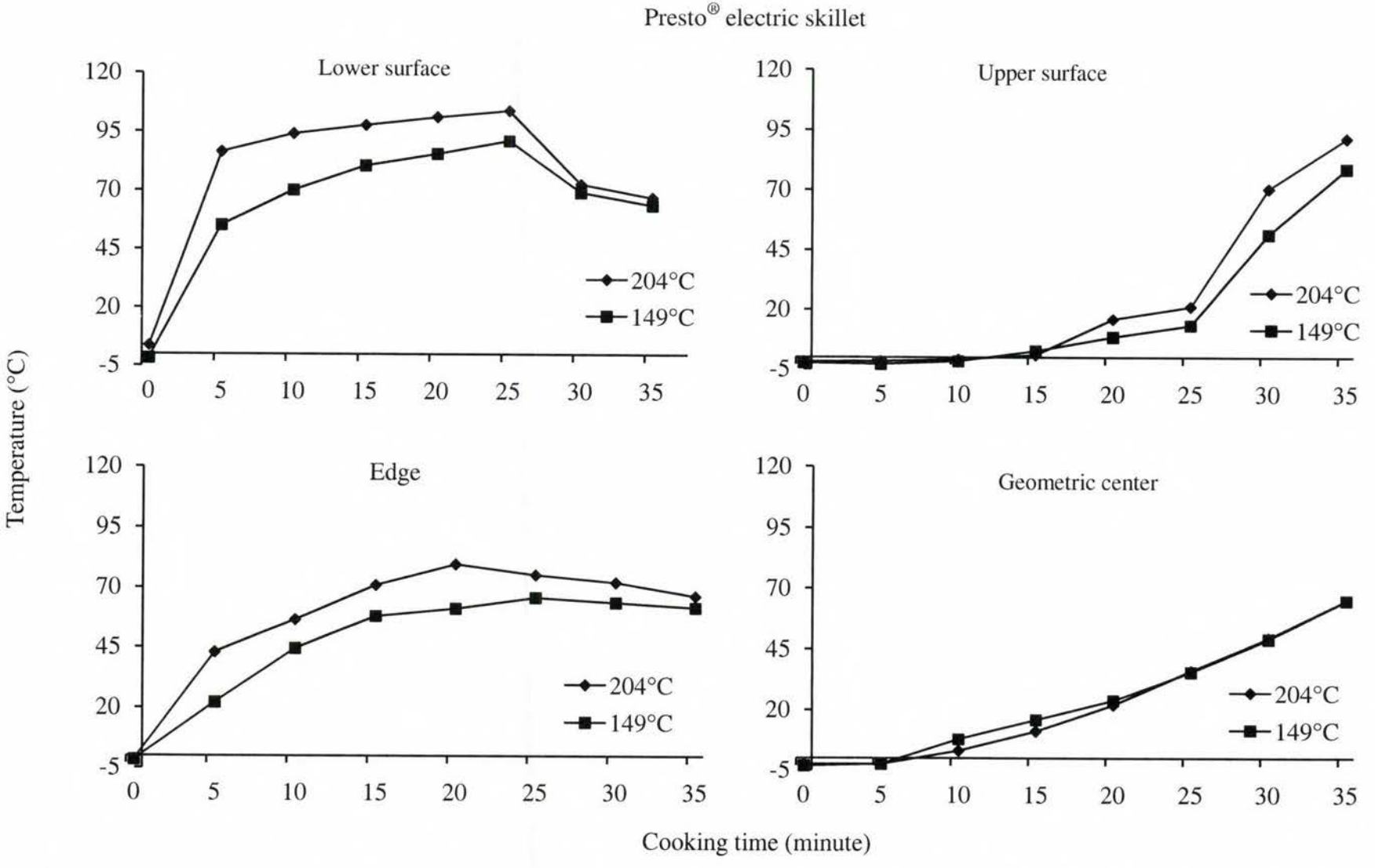


Figure 4.2 (Appendix Table 7). Cooking time (min) and temperature (°C) curves of nonintact beef steaks cooked by pan-broiling using the Presto® electric skillet setting at 149 or 204°C. Each point is the average of four determinants.

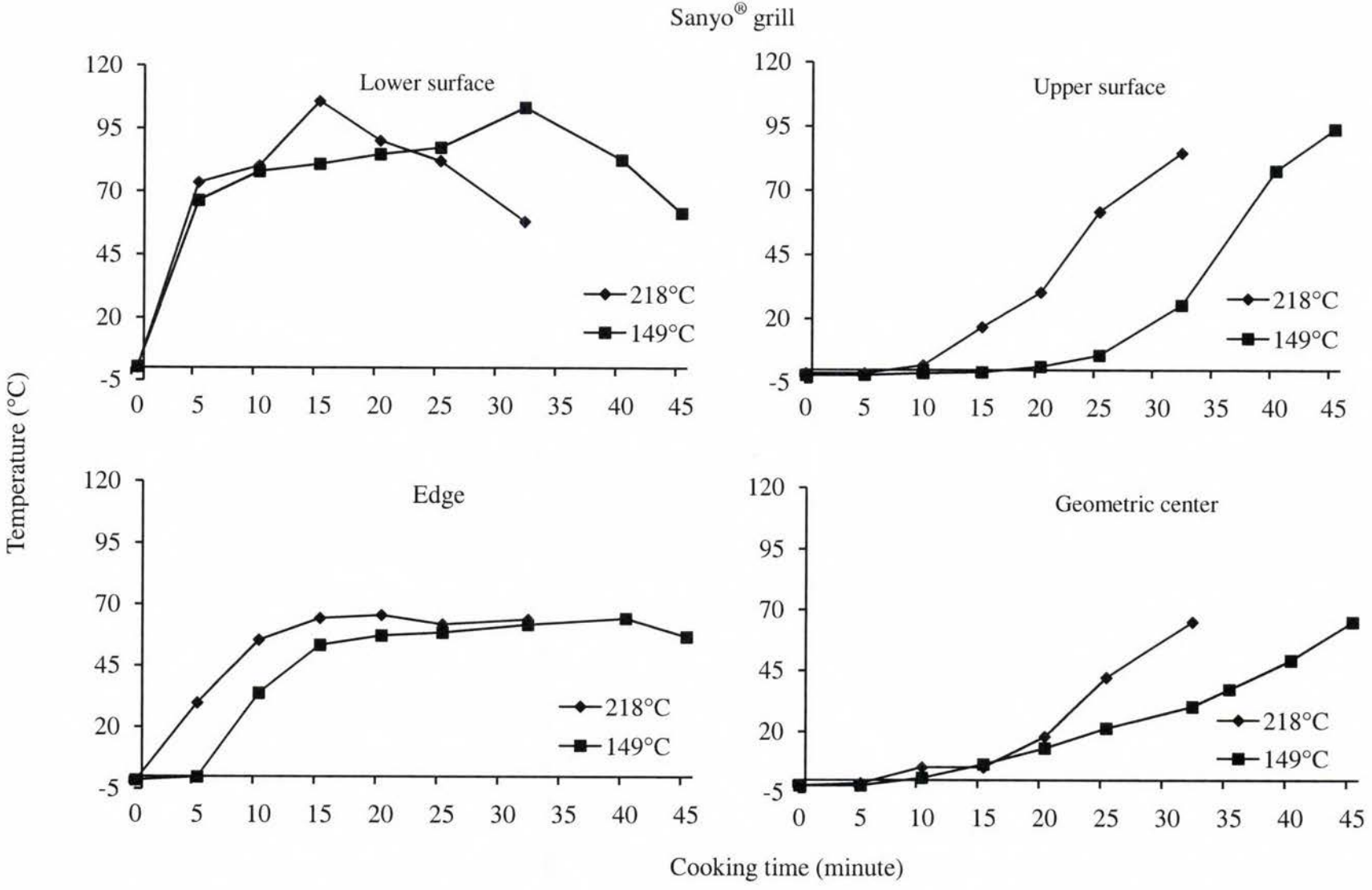


Figure 4.3 (Appendix Table 8). Cooking time (min) and temperature (°C) curves of nonintact beef steaks cooked by pan-broiling using the Sanyo® grill setting at 149 or 218°C. Each point is the average of four determinants.

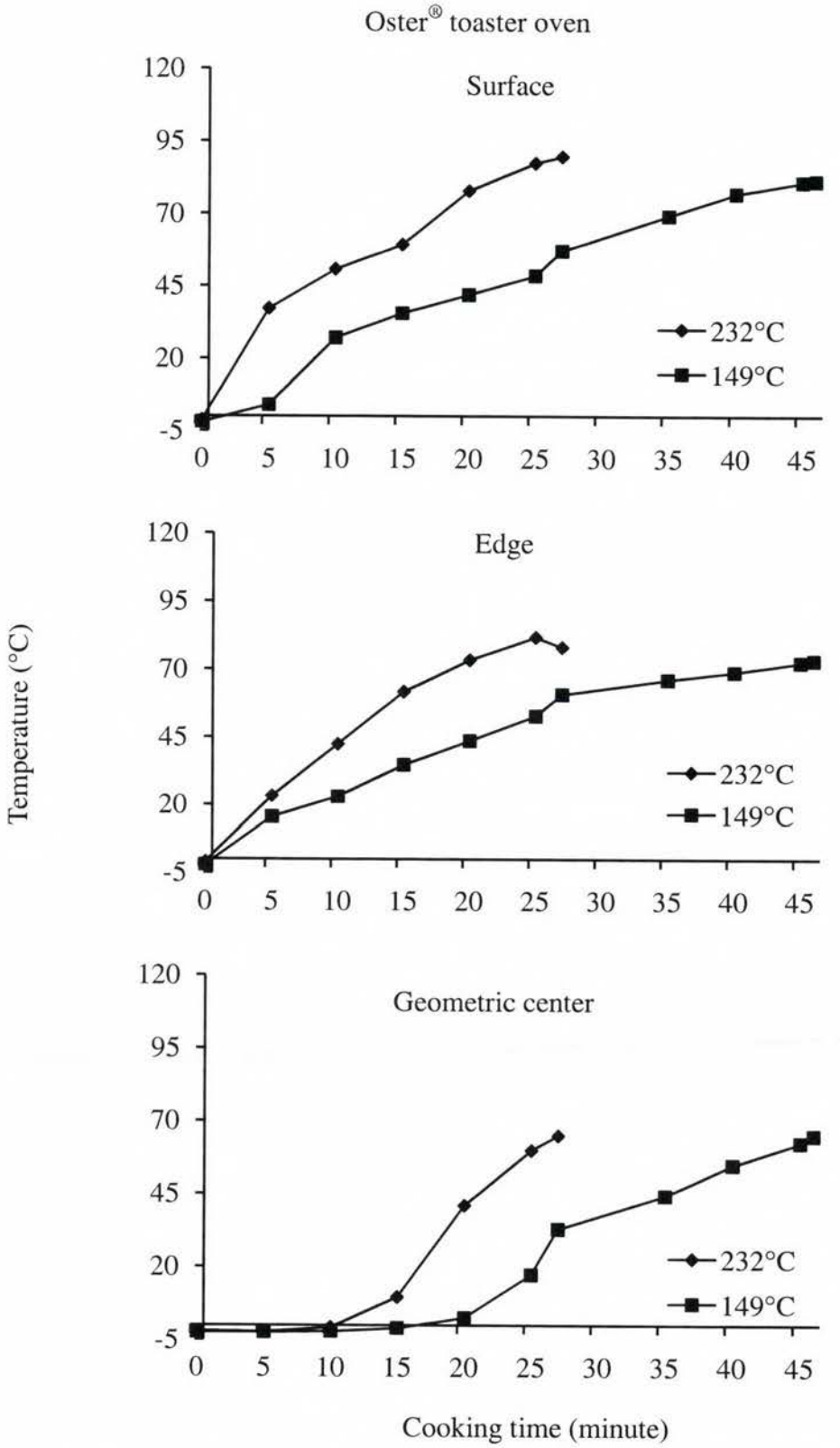


Figure 4.4 (Appendix Table 9). Cooking time (min) and temperature (°C) curves of nonintact beef steaks cooked by roasting using the Oster® toaster oven setting at 149 or 232°C. Each point is the average of four determinants.

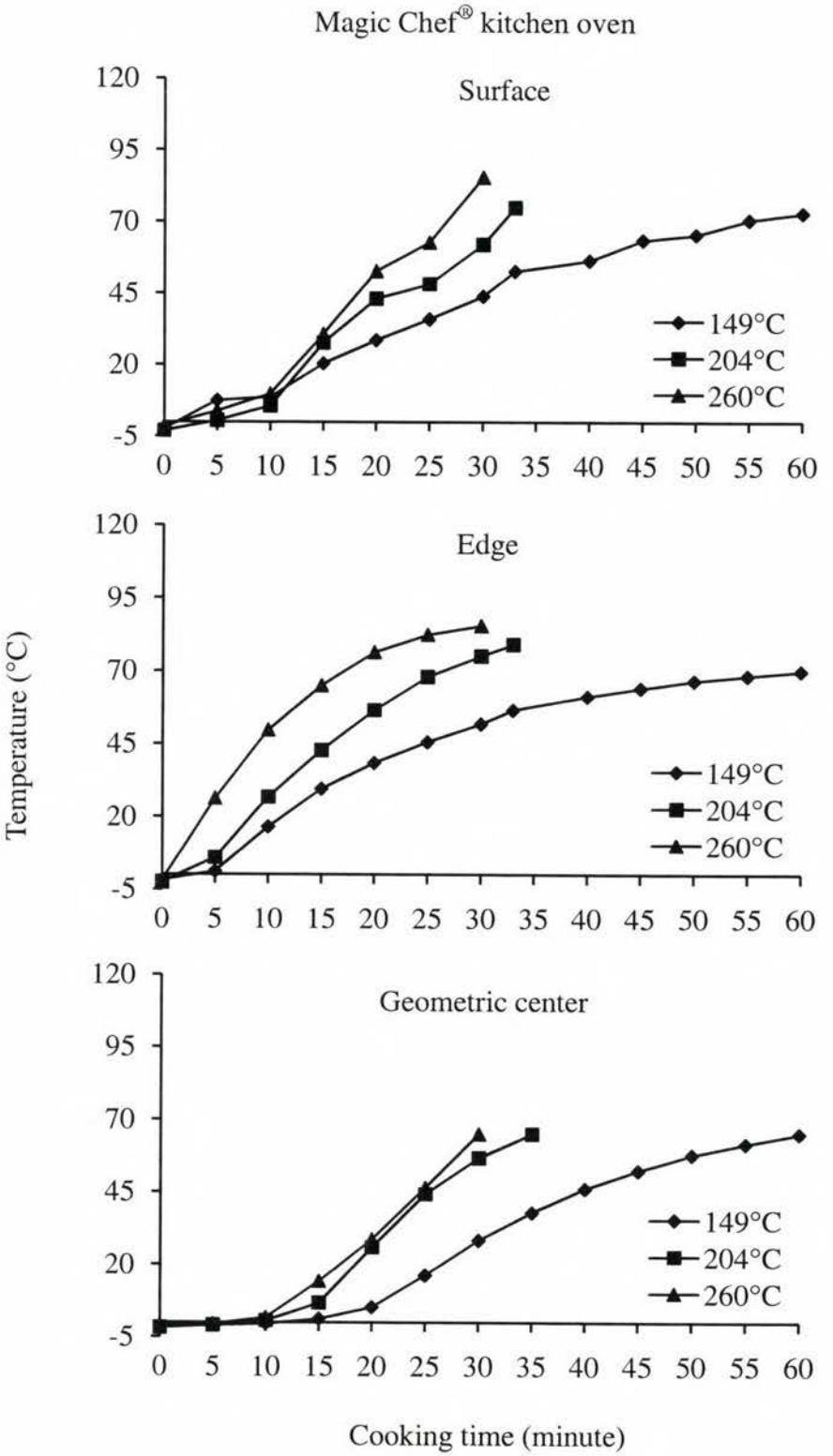


Figure 4.5 (Appendix Table 10). Cooking time (min) and temperature (°C) curves of beef steaks cooked by roasting using the Magic Chef® kitchen oven setting at 149, 204 or 260°C. Each point is the average of four determinants.

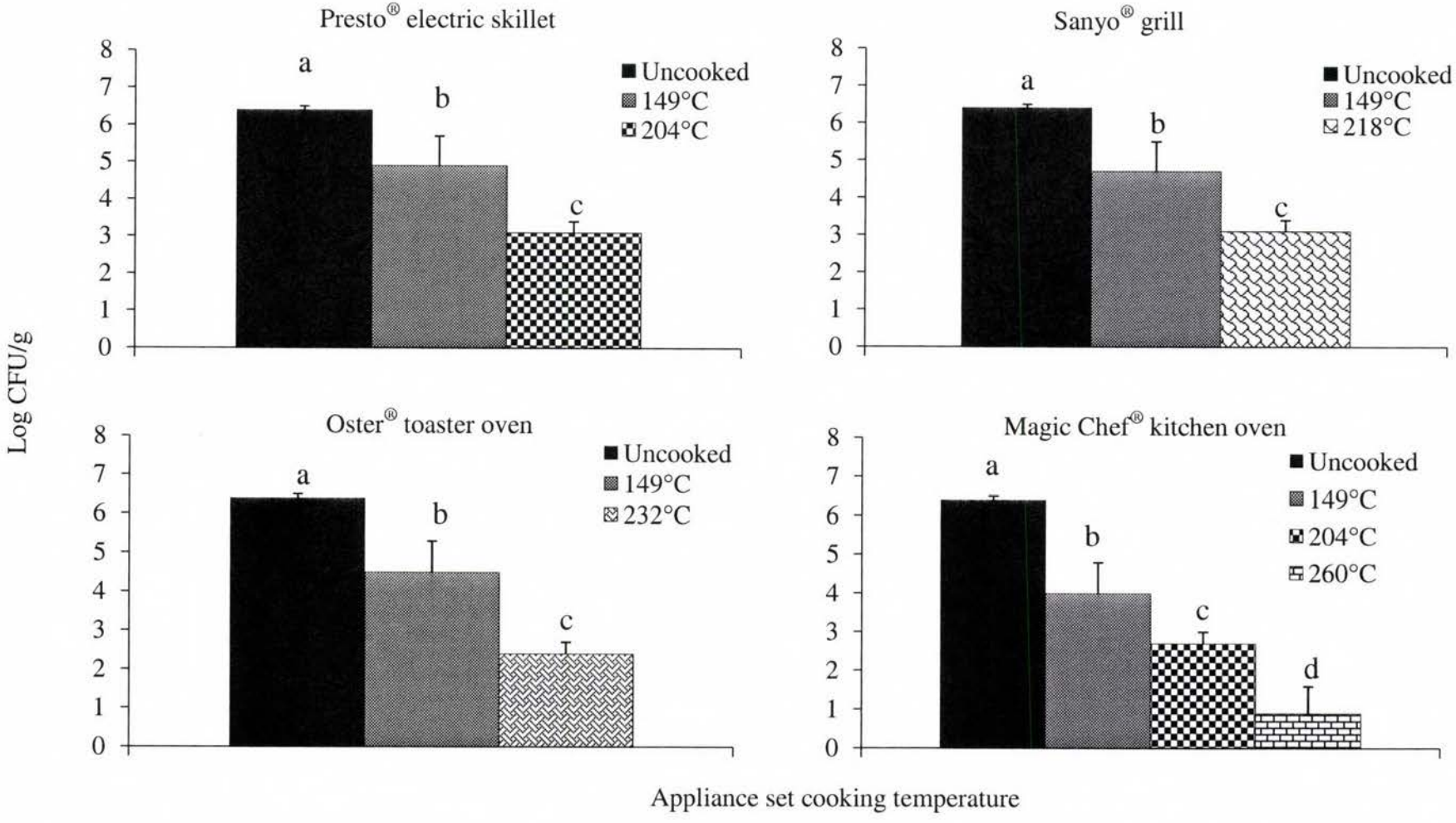


Figure 4.6 (Appendix Table 11). Mean (± SD, log CFU/g, n=9) of *Escherichia coli* O157:H7 counts recovered with tryptic soy agar plus pyruvate (0.1%, TSAP) plus rifampicin (100μg/ml) from uncooked and cooked nonintact beef steaks to the internal temperature of 65°C with a Presto® electric skillet, a Sanyo® grill, an Oster® toaster oven, and a Magic Chef® kitchen oven set at different temperatures. a-d: Means with a different letter within a figure are different ($P < 0.05$).

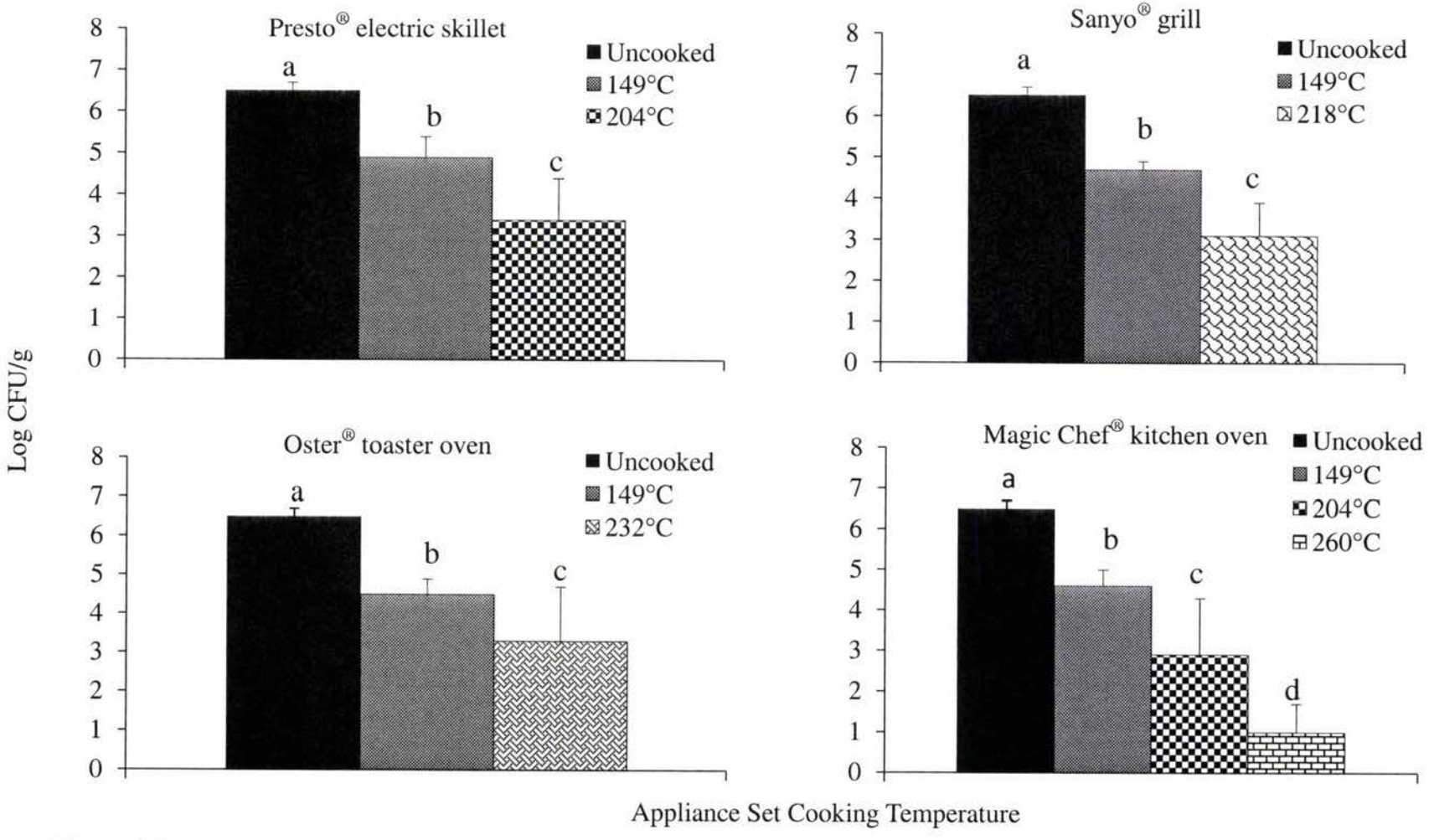


Figure 4.7 (Appendix Table 11). Mean (± SD, log CFU/g, n=9) of total bacterial counts recovered with tryptic soy agar plus pyruvate (0.1%, TSAP) from uncooked and cooked nonintact beef steaks to the internal temperature of 65°C with a Presto® electric skillet, a Sanyo® grill, an Oster® toaster oven, and a Magic Chef® kitchen oven set at different temperatures. a-d: Means with a different letter within a figure are different ($P < 0.05$).

CHAPTER 5

THERMAL INACTIVATION OF ACID, COLD, HEAT, STARVATION, AND DESICCATION STRESS-ADAPTED *ESCHERICHIA COLI* O157:H7 IN MOISTURE ENHANCED NONINTACT BEEF

ABSTRACT

This study compared thermal inactivation of stress-adapted and unstressed *Escherichia coli* O157:H7 in nonintact beef steaks containing different brining ingredients and cooked to an internal temperature of 65°C by pan-broiling. Fresh beef (5% fat) was coarse-ground (0.95 cm) and batches (1.5 kg) were mixed with an 8-strain composite (30 ml) of acid, cold, heat, starvation, or desiccation stress-adapted or unstressed rifampicin-resistant *E. coli* O157:H7 (5-6 log CFU/g) and different brining solutions (120 ml, to simulate a 10% pump rate), and were extruded into casings (10 cm diameter). Brine treatments included distilled water (DW-control), sodium chloride (0.5%, NaCl) plus sodium tripolyphosphate (0.25%, STP),

and NaCl (0.5%) + STP (0.25%) combined with cetylpyridinium chloride (CPC, Cecure®, 0.5%), lactic acid (LA, 0.3%) or sodium metasilicate (AvGard®XP, 0.2%). Semi-frozen (-20°C for 4.5 h) beef was cut into 2.54 cm thick steaks, vacuum-packaged, frozen (-20°C, 42 h), and tempered (4°C, 2.5 h) before cooking. Steaks were pan-broiled (Presto® electric skillet) to 65°C and analyzed for survivors immediately after 4.5 h of freezing, and before and after cooking. After 4.5 h of freezing, the stressed and unstressed pathogen counts in DW-control (5.5-5.9 log CFU/g) and NaCl+STP (5.5-5.8 log CFU/g) treated samples were similar; reductions of 0.4 to 1.0, 0.2 to 0.4, and 0.2 to 0.5 log CFU/g were found in samples treated with CPC, LA, and AvGard®XP, respectively, compared to DW samples. Further reductions (0.2 to 0.6 log CFU/g) were observed in CPC-treated samples following 42 h of frozen storage. Pan-broiling reduced counts of unstressed cells by 2.1 to 2.7 log CFU/g, while reductions of cold (3.0 to 4.5 log CFU/g) and desiccation (2.6 to 3.9 log CFU/g) stress-adapted cells were greater ($P < 0.05$); reductions of acid (1.3-1.9 log CFU/g) stress-adapted cells were lower ($P < 0.05$). Reductions of heat and starvation stress-adapted cells were similar ($P \geq 0.05$) to those of unstressed cells, regardless of brining treatment. After 42 h of freezing storage followed by pan-broiling, the lowest survivors (0.8 to 3.6 log CFU/g) were found in CPC or LA (0.8 to 3.5 log CFU/g) treated samples, among all stressed cultures. CPC or LA could be potential antimicrobials used in brining solutions of

reconstructed nonintact beef products to inactivate different types of stress-adapted pathogen cells.

5.1. INTRODUCTION

Noninact beef products have been involved in several *Escherichia coli* O157:H7 outbreaks between 2000 and 2007 in the United States (Laine et al., 2005; USDA-FSIS, 2005a; 2005b; 2007a; 2007b; 2007c; 2007d; 2007e; 2008a; Conference for Food Protection, 2008). A possible reason for this is that pathogen cells may be translocated or entrapped from the meat surface into internal tissue during processing of the products which were then inadequately cooked or cooked directly from the unthawed state (USDA-FSIS, 2002a; 2002b). Thorough cooking is effective in inactivating *E. coli* O157:H7 internalized in nonintact beef products. Recent studies have found that cooking of blade tenderized beef subprimals to the target temperature of 48 to 60°C in a commercial gas grill resulted a 2.6 to 4.2 log CFU/g reduction of *E. coli* O157:H7 (Luchansky et al., 2009), while cooking to the internal temperature of 65°C on a hot plate (170°C) reduced pathogen numbers by more than 7 log in brine injected nonintact beef steaks (Gill et al., 2009). However, rate and extent of cooking may depend on cooking method and appliances. Studies showed that broiling in an oven was more effective in reducing *E. coli* O157:H7 in meats cooked to the target temperatures of 60 or 65°C (Sporing, 1999; Mukherjee et al., 2007) compared to frying or

grilling; broiling resulted in more than a 5 log CFU/g reduction (Sporing, 1999). The comparative risk assessment of nonintact and intact beef published by United States Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) indicated that oven broiling to an internal temperature of more than 60°C would result in safe blade-tenderized beef steaks (USDA-FSIS 2002a; 2002b).

During meat processing and storage, microbial contamination in meat and meat product ingredients may be exposed to food related stresses such as acid, heat, salt and preservatives (Archer, 1996; Bower and Daeschel, 1999). Thus, stress-adapted or resistant cells may be developed, selected, or survive in the environment or products. For example, the acid washes applied on carcasses may lead to acid stress-adapted cells (Abee and Wouters, 1999; Dickson and Siragusa, 1994; Samelis and Sofos, 2003), while exposure of cells to low nutrient environments, including equipment surfaces, walls, and floors, may lead to selection of starvation stress-adapted cells (Dickson and Frank, 1993; Lou and Yousef, 1996; Matin, 1991). In addition, refrigeration of foods may lead to cold stress-resistant cells, while shifting foods from lower to higher temperatures, may result in heat shocked contaminants (Bunning et al., 1990; Farber and Brown, 1990). Evidence has indicated that if a microbial population is adapted to a particular stress it may exhibit a cross-protection resistance when subsequently it is exposed to a similar or unrelated stress (Blackburn and Davies 1994;

Samelis and Sofos, 2003). It has been reported that acid or starvation-stress adapted *E. coli* O157:H7 cells were resistant to further acid or heat treatments (Ryu and Beuchat, 1999; Berry and Cutter, 2000; Cheng et al. 2002; Rowe and Kirk, 2000). However, some stress-adapted cells may become more sensitive when exposed to subsequent stresses (Calicioglu et al., 2002a; 2002b). Leenanon and Drake (2001) reported that the D-values of three *E. coli* O157:H7 strains at 56°C in a broth system decreased by 2 to 3 min after exposure to cold stress. A recent study of Shaker et al. (2008) showed that desiccation, heat and cold stressed *Enterobacter* (Cronobacter) *sakazakii* cells showed increased thermal inactivation in rehydrated infant milk formula. Thus, it is necessary to evaluate the behavior of stress-adapted cells when exposed to heat treatments.

Cetylpyridinium chloride (CPC), a water soluble, colorless and odorless quaternary ammonium compound (Mastler, 1996), has been approved by USDA-FSIS as an antimicrobial to treat the surface of raw poultry carcasses prior to immersion in a chiller (USDA-FSIS, 2008b). Sodium metasilicate (AvGard®XP), a strong alkali with a pH of approximately 11.3 at 1% (wt/wt) solution (Weber et al., 2004), is currently accepted as a generally recognized as safe (GRAS) food ingredient to be used as an antimicrobial on fruits, vegetables, and nuts (US-FDA, 2003), as well as marinades for raw meat and poultry products, and on raw beef carcasses, subprimals and trimmings (USDA-FSIS, 2008b). Lactic

acid is the most commonly used organic acid in foods with an extensive application in decontamination of beef carcasses in the United States (Bosilevac et al., 2006; Heller et al., 2007). CPC, AvGard®XP, and lactic acid have been well documented as effective antimicrobials on beef and poultry carcasses (Kim and Slavik, 1995; Cutter et al., 2000; Ransom et al., 2003; Weber et al., 2004; Stopforth et al., 2005; Bosilevac et al., 2006). Thus, the objective of this study was to compare thermal inactivation of acid, cold, heat, starvation, and desiccation stress-adapted or unstressed *E. coli* O157:H7 cells in moisture-enhanced nonintact beef containing different brining ingredients when cooked to an internal temperature of 65°C by pan-broiling in an electric skillet.

5.2. MATERIALS AND METHODS

5.2.1. Bacterial strains and stress-adapted inoculum preparation

The eight strains making up the rifampicin-resistant *E. coli* O157:H7 cocktail used in this study included derivatives of strains ATCC 43888 (human isolate), ATCC 43895 (hamburger isolate), ATCC 43895/ISEHGFP (hamburger isolate, Noah et al., 2005), and C1-057, C1-072, C1-109, C1-154, C1-158 (cattle feces isolate, Carlson et al., 2009). The preparation of unstressed or stress-adapted cells was based on the methods reported by others (Leenanon and Drake, 2001; Calicioglu et al., 2002a; Shaker et al., 2008) and slightly revised according to the experimental conditions in this study. The unstressed cells (Calicioglu et al.,

2002a) were prepared by inoculating a single colony of each strain into 10 ml of glucose-free tryptic soy agar (TSB, Difo, Becton Dickinson, Sparks, MD) and incubating at 35°C for 24 h. Then, 0.1 ml of the 24-h culture was subcultured into 10-ml of glucose-free TSB for another 24 h. For preparation of inocula adapted to each of the other stresses, first each strain was prepared as for unstressed inocula and then the following procedure was followed for each type of stress. For preparion of acid stress adapted cells (Calicioglu et al., 2002a), 0.1 ml of each subcultured strain was added into 10-ml of glucose-free TSB with added 1% glucose and incubated at 35°C for another 24 h. Cold stress adapted cells (Leenanon and Drake, 2001) were prepared by centrifuging at 4,629×*g* for 15 min at 4°C (Eppendorf model 5810R, Brinkmann Instruments Inc., Westbury, NY), the unstressed culture of each strain, washing with 10 ml of phosphate buffered saline (PBS, pH 7.4; 0.2 g of KH_2PO_4 , 1.5 g of $Na_2HPO_4 \cdot 7H_2O$, 8.0 g of NaCl, and 0.2 g of KCl in 1 liter of distilled water), centrifuging again as previously described, resuspending in 10 ml of fresh glucose-free TSB, and incubating at 4°C for 7 days. For starvation stress adapted cells (Leenanon and Drake, 2001), the unstressed culture of each of the eight strains was centrifuged as above, the cells were washed with PBS, centrifuged again, resuspended in 10 ml of 0.85% NaCl (pH 6.6) solution and incubated at 35°C for 48 h. Heat stress adapted cells (Shaker et al., 2008) were prepared from the unstressed culture of each of the eight strains by centrifuging as above, washing

with PBS, centrifuging again, and resuspending in 10 ml of fresh PBS. A 3 ml portion of freshly washed cells was added to 27 ml of PBS in an 85-ml centrifuge tube (Polycarbonate Oakridge Centrifuge Tube, Nalgene, Nalge Nunc International, Rochester, NY) which had been warmed in a water bath (55°C, 45 min). After 5 min of holding, the tubes were removed and cooled immediately into ice for 5 min. For preparing desiccation stress adapted cells (Shaker et al., 2008), unstressed inocula of each strain were centrifuged as above, washed with PBS, centrifuged again and resuspended in 2 ml of fresh PBS. A 0.06 ml portion of each strain was then added into sterile petri dish as a single dot, with a total of 30 dots added separately into each petri dish. The petri dishes, without lid, were placed at 42°C to dry for 1.5 h, and then incubated at 21°C for 4 days. After 4 days, the desiccation stressed cells were rehydrated by adding 3 ml of fresh PBS. Finally, all eight strains prepared under each type of stress were combined for inoculation into coarse ground beef. The unstressed or stress-adapted cells were adjusted by dilution in PBS to obtain a target inoculation level of 5 to 6 log CFU/g when 30 ml of inoculum was added to 1.5 kg of fresh coarse ground beef. The pH of the suspensions of unstressed and stress-adapted cells was measured before inoculation of the meat. The unstressed, acid, cold, and starvation stress-adapted cells were centrifuged and washed before use as described above. The heat and desiccation stressed cells were directly used immediate after preparing.

5.2.2. Preparation and inoculation of nonintact beef steaks

The beef steaks were prepared and inoculated as described in Chapter 3. Briefly, each batch (1.5 kg) of course ground (0.95 cm diameter kidney plate, Hobart Mfg. Co., Troy, OH) fresh beef knuckles (approximately 5% fat) was placed in a bowl-lift stand mixer (Professional 600, KitchenAid, St. Joseph, MI), inoculated by adding 30 ml of the PBS prepared unstressed or stress-adapted inoculum, mixed slowly for 2 min at the speed of "stir", and then mixing was continued for another 2 min at the same speed after adding 120 ml of treatment solution to simulate a 10% pump rate. The treatment solutions tested were: (i) distilled water (DW-control), (ii) sodium chloride (NaCl, 0.5%, Fisher Scientific , Fair Lawn, NJ) plus sodium tripolyphosphate (STP, 0.25%, BK Giulini Corporation, Simi Valley, CA), (iii) NaCl (0.5%) + STP (0.25%) combined with cetylpyridinium chloride (CPC, 0.2%, Cecure®, Safe Foods Corporation, North Little Rock, AR), (iv) NaCl (0.5%)+STP(0.25%) combined with lactic acid (LA, 0.3%, Purac America Inc., Lincolnshire, IL), (v) NaCl (0.5%) + STP(0.25%) combined with AvGard®XP (0.2%, active ingredient: sodium metasilicate; Danisco USA Inc., New Century, KS). All the chemicals were well dissolved in sterile distilled water (25°C) before adding into the product. The inoculated beef was extruded into casings (In-stock poly bag, 5-lb, 6 × 18 inch, Koch, Kansas City, MO), tied, and placed in a freezer (-20°C) for 4.5 h. Semi-frozen beef was cut into 2.54 cm thick steaks, each of which

was placed into a vacuum bag (15 × 22 cm, 3 mil std barrier, nylon/polyethylene vacuum pouch, water vapor and oxygen transmission rates of 9.3 $g/m^2/24h$ [97% relative humidity] and 54.3 $cm^3/m^2/24h$ [21°C, 0% relative humidity], respectively, Koch, Kansas City, MO), vacuum packaged (Hollymatic, Corp., Countryside, IL), frozen (-20°C) for 42 h, and then tempered (4°C) for 2.5 h before cooking.

5.2.3. Cooking of beef steaks

The beef steaks were cooked by pan-broiling using a Presto® 16-inch electric skillet (National Presto Industries, Inc., Eau Claire, WI) to reach the internal temperature of 65°C, which corresponds to medium rare doneness (Lorenzen et al., 1999). Temperatures of cooking appliances, and surface, edge and center of the products were monitored and recorded as described in a previous study (Chapter 3). During cooking, the appliance was set and maintained at 176°C. The samples were placed on the pan-broiling surface and flipped over when the temperature of the internal geometric center reached 42°C, before they were cooked to the target temperature of 65°C.

5.2.4. Physical and chemical analyses

Cooking weight losses, moisture and fat, pH of the homogenate of uncooked or cooked samples, and water activity of steak samples within 10 h after cooking were measured as described in a previous study (Chapter 3).

5.2.5. Microbiological analyses

Background natural microbial contamination numbers of fresh beef were determined immediate after coarse grinding (2.9 ± 0.4 log CFU/g). Pathogen survival numbers were analyzed in samples after 4.5 h of freezing, and before and immediately (within 2.5 min) after cooking. Maximum recovery diluent (MRD; 0.85% NaCl [Fisher Scientific, Fair Lawn, NY] and 0.1% peptone [Difco, Becton Dickinson, Sparks, MD]) was added into a sterile filter bag (Whirl-Pak, Nasco, Modesto, CA) containing individual samples at a ratio of 1:1 (steak weight [g]: volume of MRD [ml]), and homogenized (Masticator, IUL Instruments, Barcelona, Spain) for 2 min. Each sample was serially diluted (10-fold) in 0.1% buffered peptone water (Difco, Becton Dickinson, Sparks, MD), and then surface (0.1 ml) plated onto tryptic soy agar plus pyruvate (0.1%, TSAP) for enumeration of total microbial populations, and onto TSAP plus rifampicin (100 μg/ml) for enumeration of *E. coli* O157:H7 cell numbers, respectively. Colonies were counted manually after the plates were incubated at 35°C for 48 h.

5.2.6. Statistical analysis

The experiment was performed twice, each time including three individual samples per treatment. At each sampling point, microbiological (survivors or reductions, converted to log CFU/g) data were analyzed using the Mixed Procedure of SAS (SAS Institute, 2002), with

independent variables including different stress-adapted cells, different brining ingredients and the interaction of stresses × brine ingredients. The pH, water activity, cooking weight losses, and fat and moisture content were analyzed using One Way ANOVA of SAS. Means and standard deviations for microbiological data were calculated, and the mean differences among interactions were separated with the least significant difference procedure at the significance level of α=0.05.

5.3. RESULTS AND DISCUSSION

5.3.1. Temperatures of electric skillet and steak samples

The average cooking surface temperature of the Presto® electric skillet was 177 ± 8°C (Figure 5.1; Appendix Table 12). As indicated, during the cooking period, thermocouples were inserted into steaks at 5 mm depth in the lower (closest to the heat source) and top (away from the heat source) surfaces, edge, and inside the geometric center to monitor surface, edge and center temperatures. As expected, the temperature of the lower surface of the product reached 82.2°C (Figure 5.1; Appendix Table 12) before the samples were flipped over (when the center temperature reached 42°C). Immediately after flipping the samples over, their original lower surface became the upper surface, and the original lower surface temperature decreased to 56.6°C (Figure 5.1; Appendix Table 12). The temperature of the original upper surface that initially was not in contact with the heat source, increased slowly

before the steaks were flipped over, and then increased quickly when it became the lower surface, and reached approximately 90°C by the end of cooking. The edge temperature of the samples also increased quickly before flipping over and reached 72°C, and then it decreased to 57°C by the end of cooking (Figure 5.1; Appendix Table 12), which might be explained by the evaporative cooling occurring at the surface of the product after being flipped over (Chapter 3). The initial geometric center temperature of uncooked steaks was 1.8 ± 0.5°C. Cooking beef steaks to the internal center temperature of 65°C required 30 min (Figure 5.1; Appendix Table 12). There was no difference in time to reach the target temperature of 65°C between samples that were moisture enhanced with different brining ingredients.

5.3.2. Cooking losses, pH, water activity, moisture and fat contents

The highest ($P < 0.05$) and lowest ($P < 0.05$) cooking losses were obtained in samples treated with LA (35.9 ± 2.0%) and AvGard®XP (22.0 ± 1.3%), respectively (Table 5.1). Brew and Novakofski (1999) found that cooking losses of ground beef were higher at pH of 5.3 to 5.5 than when pH was 5.7 to 6.0. The possible reason is that steaks treated with LA had the lowest pH (5.2), while samples moisture enhanced with AvGard®XP had a higher pH of 6.3. Lower pH values result in decreased water holding capacity which causes higher weight losses during cooking (Wismer-Pedersen, 1971). Cooking losses of steaks treated with DW, NaCl+STP, and NaCl+STP+CPC were similar ($P \geq 0.05$), and ranged from 29.1 to 32.3%.

The pH of unstressed and acid stress adapted cell suspensions used to inoculate the meat was 6.68 ± 0.05 and 4.86 ± 0.03, respectively, which agreed with previous results (Samelis et al., 2003); while that of cold, heat, starvation and desiccation stressed cells was 6.65 ± 0.02, 6.67 ± 0.06, 7.07 ± 0.01, 6.62 ± 0.05, respectively. The pH of steak samples treated with DW, NaCl+STP and NaCl+STP+CPC was 5.68 ± 0.09, 5.77 ± 0.04, and 5.74 ± 0.01, respectively (Table 5.2). As expected, the pH values of samples were reduced and increased due to the enhancement with LA (5.22 ± 0.02) and AvGard®XP (6.31 ± 0.06), respectively, which was attributed to the acidic nature of LA and the strong alkali component of sodium metasilicate as the active ingredient in AvGard®XP. No changes ($P \geq 0.05$) in pH values were observed after 42 h of frozen storage for any treated samples. In general, cooked steak samples had a higher ($P < 0.05$) pH value than uncooked samples of all treatments (Table 5.2) which was in agreement with results of previous studies (Trout et al., 1989; Mukherjee et al., 2008).

The water activity, moisture and fat content of fresh beef were 0.985 to 0.989 (Table 5.3), 76.2 to 77.1% and 2.5 to 2.7% (Table 5.4), respectively, regardless of brining ingredients. Cooking of beef steaks to 65°C decreased ($P < 0.05$) the water activity and moisture content to 0.973 to 0.980 (Table 5.3) and 56.7 to 66.2% (Table 5.4), respectively, and increased ($P < 0.05$) the fat content to 6.2-12.9% (Table 5.3) for all treatments. Trout et al. (1989) reported that the moisture content of ground beef patties decreased after cooking, while Trout et al.

(1989) and Mukherjee et al. (2008) indicated that the fat content of low-fat ground beef increased after cooking. The lowest water activity (0.973 ± 0.002) and moisture content (56.7 ± 0.8%) after cooking were observed in samples treated with LA, accompanied by the highest fat content (12.9 ± 2.4%), compared to those of other samples. This also could be explained by the low pH of the meat treated with LA, resulting in lower water holding capacity.

5.3.3. Microbial data on unstressed and stress-adapted cultures before cooking

Initial counts of total bacterial and unstressed, acid, cold, heat, starvation and desiccation stress-adapted *E. coli* O157:H7 in DW and NaCl+STP treated uncooked beef steaks, immediately after 4.5 of freezing, were 5.6 to 5.9 (Table 5.5) and 5.5 to 5.9 log CFU/g (Table 5.5), respectively. The microbial counts on TSAP (Table 5.5) were similar ($P \geq 0.05$) to those observed on TSAP + Rif agar for all types of stress and brining solution treatments (Figure 5.2; Appendix Table 13), indicating that the majority of colonies found on TSAP were *E. coli* O157:H7. Compared to the DW-control, surviving pathogen counts for samples treated with the brining solutions containing CPC, LA, and AvGard®XP were 0.4 to 0.5 (unstressed cultures), 0.3 to 0.4 (acid stress-adapted cultures), 0.2 to 1.0 (cold stress-adapted cultures), 0.4 to 0.7 (heat stress-adapted cultures), 0.2 to 0.4 (starvation stress-adapted cultures), and 0.2 to 0.6 log CFU/g (desiccation stress-adapted cultures) lower, respectively (Figure 5.2;

Appendix Table 13). The reduction of CPC treated samples reached 1.0 log CFU/g in cold stress-adapted cultures (Figure 5.2; Appendix Table 13) compared to that of the DW-control. After 42 h of frozen storage, the pathogen counts in most treatments were similar ($P \geq 0.05$), with only CPC treated samples showing further reductions of counts by 0.5, 0.5 and 0.6 log CFU/g in unstressed, acid and starvation stress samples, respectively (Figure 5.2; Appendix Table 13).

5.3.4. Cooking inactivation effects on unstressed and stress-adapted cultures

The interaction of different stress-adapted cells and brining ingredients was significant ($P < 0.05$), indicating that the cooking inactivation effects of *E. coli* O157:H7 depended on different stress-adapted cells and brining ingredients. As expected, the reduction level of unstressed *E. coli* O157:H7 cultures in samples pan-broiled to the internal temperature of 65°C was 2.1 to 2.7 log CFU/g in all brining treatments (Chapter 3). Among the different types of stressed inocula tested, cold and desiccation stress-adapted cells were more sensitive to heat as their reduction levels were 3.0 (NaCl+STP) to 4.5 (NaCl+STP+CPC) and 2.6 (NaCl+STP+AvGard®XP) to 3.9 log CFU/g (NaCl+STP+CPC) (Figure 5.2; Appendix Table 13), respectively, which were higher ($P < 0.05$) than that for unstressed cells (2.1 to 2.7 log CFU/g). Previous studies also indicated that cold (Leenanon and Drake, 2001) and desiccation (Shaker et al., 2008) stress-adapted cells were more sensitive to subsequent heat

treatment. The possible reason is that cold stress-adapted cells may incorporate unsaturated fatty acids and reduce their melting point within their cell membranes (Semanchek and Golden, 1998), while the exposure of cells to desiccation and heat stresses may require energy-consuming production of stress shock proteins, causing metabolic exhaustion of the cells (Beales, 2004). Acid stress adapted cells were more resistant to heat treatment, as their reduction level was 1.3 (NaCl+STP) to 1.9 log CFU/g (NaCl+STP+LA), which were lower ($P < 0.05$) than that of unstressed cells (2.1 to 2.7 log CFU/g) (Figure 5.2; Appendix Table 13). The strategies used by microorganisms to resist acid stress include pH homeostasis, changes in membrane structure by alteration of protein permeability, internal buffering ability, and the pH stability of essential proteins (Slonczewski and Foster, 1996). Previous studies (Behjamin and Datta, 1995; Rowe and Kirk, 1999; Ryu and Beuchat, 1999; Berry and Cutter, 2000; Cheng et al., 2002) have shown that acid stress-adapted *E. coli* O157:H7 cultures had increased heat resistance. Research indicated that acid adaptation provided *E. coli* O157:H7 with cross-protection against further heat treatment (Buchanan and Edelson, 1999), which may be due to heat shock proteins generated during acid adaptation providing cross-protection to subsequent heat treatment (Leyer and Johnson, 1993). However, the reduction levels of heat (1.6 [NaCl+STP] to 3.0 log CFU/g [NaCl+STP+CPC]) and starvation (2.1 [NaCl+STP+AvGard®XP] to 3.1 log CFU/g [NaCl+STP+LA]) stress-adapted

cultures were similar ($P \geq 0.05$) to that of the unstressed cells, among all brining treatments (Figure 5.2; Appendix Table 13). Although several investigators have reported that heat (Murano and Pierson, 1992) or starvation (Jenkins et al., 1988; Rowe and Kirk, 2000; Leenanon and Drake, 2001) stress increased thermotolerance of *E. coli* O157:H7, the present study found that these stresses applied to cells prior to cooking did not make *E. coli* O157:H7 more resistant to heat. Pan-broiling steaks to the internal temperature of 65°C was sufficient to overcome heat resistance effects caused by the heat or starvation shock proteins (Lindquist and Craig, 1988; Tolker-Nielsen and Molin, 1996).

Reductions in counts of the pathogen after pan-broiling differed ($P < 0.05$) among treatments with different brining solutions. Thermal inactivation was greatest ($P < 0.05$) in CPC treated steaks inoculated with unstressed, heat, and desiccation stress-adapted cultures, when reductions of 2.7, 3.0, and 3.9 log CFU/g, respectively, were generated. Treatments of samples with LA caused the greatest ($P < 0.05$) reductions in counts (1.9, 4.5, and 3.1 log CFU/g in acid, cold and starvation stress samples, respectively). A recent study (Mukherjee et al., 2009) found that lactic acid (0.27%), as a beef formulation ingredient, enhanced *E. coli* O157:H7 reduction to 4.9 log CFU/g during cooking of ground beef samples to internal temperature of 65°C, compared to the control samples (reduction of 3.2 log CFU/g).

Survivors of *E. coli* O157:H7 counts after 42 h of frozen storage followed by

pan-broiling differed ($P < 0.05$) among moisture enhancing treatments with different brining solutions. CPC resulted in the lowest ($P < 0.05$) survivor counts in samples inoculated with unstressed (2.2 ± 0.2 log CFU/g), cold (0.8 ± 0.4 log CFU/g), heat (1.8 ± 0.8 log CFU/g), starvation (1.9 ± 0.6 log CFU/g), and desiccation (0.8 ± 0.6 log CFU/g) stress-adapted pathogen cells (Figure 5.2; Appendix Table 13). The lowest pathogen counts of 3.5 ± 0.6 log CFU/g (Figure 5.2; Appendix Table 13) in acid stressed samples were found in steaks treated by LA. However, samples moisture enhanced by AvGard®XP resulted the similar ($P \geq 0.05$) survival pathogen numbers (2.1 [cold stress] to 3.9 log CFU/g [acid stress]) compared to those of DW-Control (2.2 [cold stress] to 4.2 log CFU/g [acid stress]) and NaCl+STP (2.3 [cold stress] to 4.4 log CFU/g [acid stress]), among different types of stress. The mechanisms by which CPC kills bacteria include the interaction of cetylpyridinium ions with acid groups of bacteria to form a weakly ionized compound that inhibits bacterial metabolism and its positively charged cetyl radials binding negative charges of the bacterial cell wall to destroy cell walls and membranes (Huyck, 1944; Kourai et al., 1985). CPC has been approved by USDA-FSIS as an antimicrobial for decontaminating raw poultry carcasses (USDA-FSIS, 2008b) and has been shown to have good antimicrobial activities in poultry or beef carcasses (Kim and Slavik, 1995; Cutter et al., 2000; Ransom et al., 2003) and cooked roast beef (Singh et al., 2005; Lim and Mustapha, 2007) as spraying or dipping solutions. Although

CPC has not been approved for use as an antimicrobial ingredient in the meat products, research has shown that daily oral intake 6.25 mg/kg body weight did not cause side effects in rats for up to 90 days (Gunner, 1991). Based on this criterion for an average adult (75 kg body weight), the daily consumption of 234 g of noninact beef products containing 0.2% CPC would not provide any adverse effect. Thus, it is possible that CPC could be a potential antimicrobial agent used in moisture-enhanced brining solutions to prepare noninact beef products, based on the further studies to determine minimal antimicrobial concentrations.

5.4. CONCLUSIONS

In conclusion, pathogen cells adapted to various stresses behaved differently when exposed to heating by pan-broiling of brine-enhanced beef. Cold and desiccation stress are more sensitive to heat inactivation effects, while acid stress is resistant to heat inactivation. Cetylpyridinium chloride and lactic acid may be potential antimicrobials to be used in the brining solutions of reconstructed noninact beef steaks, based on their reduction effects in stress-adapted or unstressed pathogen cells in this study. These findings will be useful in risk assessments of nonintact beef products, as well as for development of cooking protocols for consumers and the food service industry.

Table 5.1. Mean (± SD) weight losses (%) of nonintact beef steaks moisture enhanced with various brining solutions before and after cooking to 65°C

Treatments	After cooking 65°C (%)
Distilled water	32.3 ± 5.3b
NaCl+STP	29.1 ± 2.4b
NaCl+STP+CPC	30.1 ± 2.5b
NaCl+STP+LA	35.9 ± 2.0a
NaCl+STP+AvGard®XP	22.0 ± 1.3c

a-c: Means with a common letter within a column are not different ($P \geq 0.05$)
NaCl: sodium chloride (0.5%); STP: sodium tripolyphosphate (0.25%); CPC: cetylpyridinium chloride (0.2%); LA: lactic acid (0.2%).

Table 5.2. Mean (± SD) pH of nonintact beef steaks moisture enhanced with various brining solutions before and after cooking to 65°C

Treatments	After 4.5 h frozen	Before cooking	After cooking 65°C
Distilled water	5.68 ± 0.09aX	5.64 ± 0.09aX	5.83 ± 0.06aY
NaCl+STP	5.77 ± 0.04aX	5.77 ± 0.05aX	5.95 ± 0.02aY
NaCl+STP+CPC	5.74 ± 0.01aX	5.78 ± 0.08aX	5.89 ± 0.05aY
NaCl+STP+LA	5.22 ± 0.02bX	5.21 ± 0.05bX	5.40 ± 0.10bY
NaCl+STP+AvGard®XP	6.31 ± 0.06cX	6.29 ± 0.09cX	6.34± 0.04cX

a-c: Means with a common letter within a column are not different ($P \geq 0.05$)
X-Y: Means with a common letter within a row are not different ($P \geq 0.05$)
NaCl: sodium chloride (0.5%); STP: sodium tripolyphosphate (0.25%); CPC: cetylpyridinium chloride (0.2%); LA: lactic acid (0.2%).

Table 5.3. Mean (± SD) water activity of nonintact beef steaks moisture enhanced with various brining solutions before and after cooking to 65°C

Treatments	Before cooking	After cooking 65°C
Distilled water	0.988 ± 0.002aX	0.980 ± 0.002aY
NaCl+STP	0.989 ± 0.003aX	0.974 ± 0.004bY
NaCl+STP+CPC	0.985 ± 0.002aX	0.975 ± 0.003bY
NaCl+STP+LA	0.985 ± 0.003aX	0.973 ± 0.002bY
NaCl+STP+AvGard®XP	0.986 ± 0.001aX	0.976 ± 0.002abY

a-b: Means with a common letter within a column are not different ($P \geq 0.05$)
X-Y: Means with a common letter within a row are not different ($P \geq 0.05$)
NaCl: sodium chloride (0.5%); STP: sodium tripolyphosphate (0.25%); CPC: cetylpyridinium chloride (0.2%); LA: lactic acid (0.2%).

Table 5.4 Mean (± SD) moisture and fat content (%) of nonintact beef steaks moisture enhanced with various brining solutions before and after cooking to 65°C

Treatments	Moisture (%)		Fat (%)	
	Before cooking	After cooking	Before cooking	After cooking
Distilled water	76.4 ± 1.2aX	59.6 ± 6.3bY	2.5 ± 0.1aX	9.2 ± 4.8abY
NaCl+STP	76.2 ± 2.1aX	65.0 ± 1.9aY	2.5 ± 0.1aX	6.6 ± 2.2bY
NaCl+STP+CPC	76.8 ± 1.0aX	58.5 ± 2.9bY	2.6 ± 0.2aX	10.2 ± 4.1abY
NaCl+STP+LA	77.1 ± 0.9aX	56.7 ± 0.8bY	2.7 ± 0.5aX	12.9 ± 2.4aY
NaCl+STP+AvGard®XP	76.5 ± 1.1aX	66.2 ± 4.5aY	2.7 ± 0.4aX	6.2 ± 2.9bY

a-b: Means with a common letter within a column are not different ($P \geq 0.05$)
X-Y: Means with a common letter within a row are not different ($P \geq 0.05$)
NaCl: sodium chloride (0.5%); STP: sodium tripolyphosphate (0.25%); CPC: cetylpyridinium chloride (0.2%); LA: lactic acid (0.2%).

Table 5.5. Mean (± SD, log CFU/g, n=6) of total bacterial counts recovered with tryptic soy agar plus pyruvate (0.1%, TSAP) from uncooked and cooked nonintact beef steaks treated with brines containing various antimicrobials and cooked to the internal temperature of 65°C

Type of Stress	Treatment	After frozen 4.5 h	Before cooking	After cooking
Unstressed	Distilled water	5.9 ± 0.1aX	5.7 ± 0.1aX	3.3 ± 0.1adgY
	NaCl+STP	5.8 ± 0.1aX	5.7 ± 0.1aX	3.6 ± 0.6adgY
	NaCl+STP+CPC	5.4 ± 0.1bX	4.9 ± 0.3bY	2.4 ± 0.2bfZ
	NaCl+STP+LA	5.5 ± 0.1bX	5.5 ± 0.1acX	3.3 ± 0.3bgY
	NaCl+STP+AvGard®XP	5.4 ± 0.2bX	5.4 ± 0.1cX	3.3 ± 0.1agY
Acid	Distilled water	5.9 ± 0.1aX	5.7 ± 0.1aX	4.2 ± 0.1cdY
	NaCl+STP	5.8 ± 0.1aX	5.7 ± 0.1aX	4.4 ± 0.4cY
	NaCl+STP+CPC	5.5 ± 0.1bX	5.0 ± 0.3bY	3.7 ± 0.6adZ
	NaCl+STP+LA	5.6 ± 0.1bX	5.5 ± 0.1cX	3.5 ± 0.5adY
	NaCl+STP+AvGard®XP	5.5 ± 0.1bX	5.4 ± 0.1cX	4.2 ± 0.1dY
Cold	Distilled water	5.3 ± 0.1aX	5.5 ± 0.1aX	2.2 ± 0.2bfY
	NaCl+STP	5.5 ± 0.2aX	5.3 ± 0.1aX	2.3 ± 0.5bfY
	NaCl+STP+CPC	4.5 ± 0.4bX	4.2 ± 0.2bX	0.8 ± 0.4eY
	NaCl+STP+LA	5.2 ± 0.1aX	5.3 ± 0.1aX	0.8 ± 0.4eY
	NaCl+STP+AvGard®XP	5.3 ± 0.1aX	5.1 ± 0.1aX	2.1 ± 0.6bfY
Heat	Distilled water	5.8 ± 0.1aX	5.6 ± 0.1aX	3.2 ± 0.7adY
	NaCl+STP	5.9 ± 0.2aX	6.0 ± 0.1aX	4.4 ± 0.4cdY
	NaCl+STP+CPC	5.3 ± 0.3bX	5.0 ± 0.2bX	1.9 ± 0.9fhY
	NaCl+STP+LA	5.5 ± 0.1bX	5.4 ± 0.1cX	3.4 ± 0.6gY
	NaCl+STP+AvGard®XP	5.5 ± 0.2bX	5.5 ± 0.1cX	4.1 ± 0.3dY
Starvation	Distilled water	5.8 ± 0.1aX	5.4 ± 0.1aY	3.4 ± 0.8adgZ
	NaCl+STP	5.6 ± 0.2aX	5.7 ± 0.3acX	3.0 ± 0.4gY
	NaCl+STP+CPC	5.2 ± 0.1bX	5.0 ± 0.3bX	2.1 ± 0.4fhY
	NaCl+STP+LA	5.4 ± 0.2acX	5.2 ± 0.1cX	2.7 ± 0.7fhY
	NaCl+STP+AvGard®XP	5.3 ± 0.1acX	5.1 ± 0.1cX	3.2 ± 0.8gY
Desiccation	Distilled water	5.9 ± 0.1aX	5.8 ± 0.1aX	2.6 ± 0.3bgY
	NaCl+STP	5.8 ± 0.2aX	5.9 ± 0.2bX	2.7 ± 0.6bgY
	NaCl+STP+CPC	5.2 ± 0.1bX	4.8 ± 0.3cX	1.0 ± 0.6eY
	NaCl+STP+LA	5.4 ± 0.3aX	5.2 ± 0.3aX	2.1 ± 0.7hY
	NaCl+STP+AvGard®XP	5.4 ± 0.2aX	5.4 ± 0.2aX	2.6 ± 0.3bgY

a-h: Means with a common letter within a column are not significantly different ($P \geq 0.05$).

X-Y: Means with a common letter within a row are not significantly different ($P \geq 0.05$).

NaCl: sodium chloride (0.5%); STP: sodium tripolyphosphate (0.25%); CPC: cetylpyridinium chloride (0.2%); LA: lactic acid (0.2%).

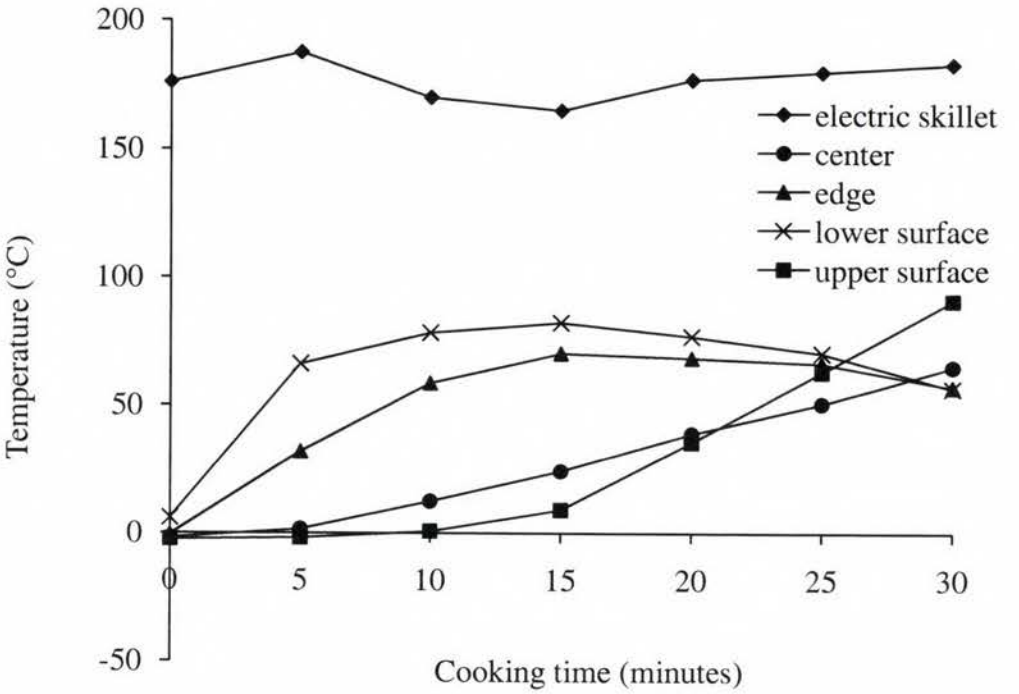
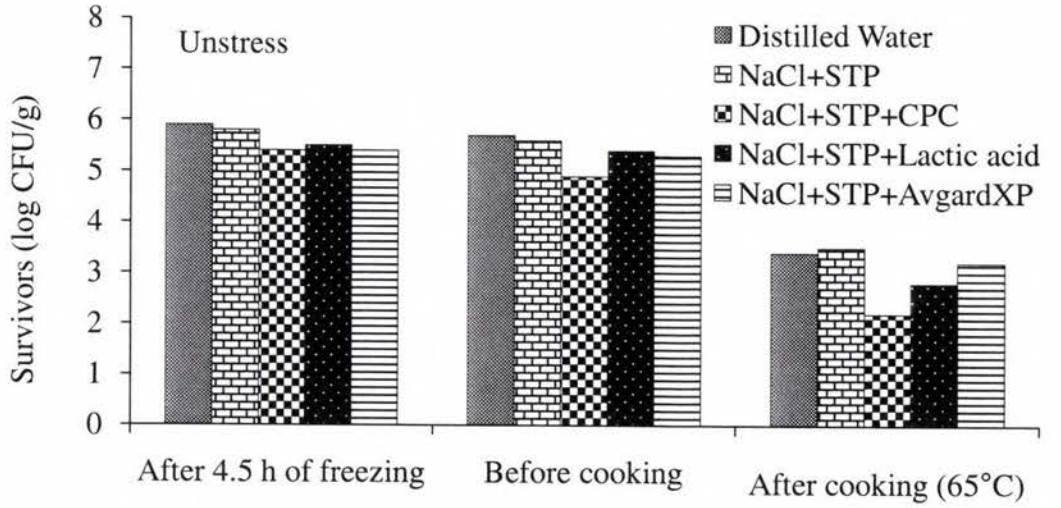
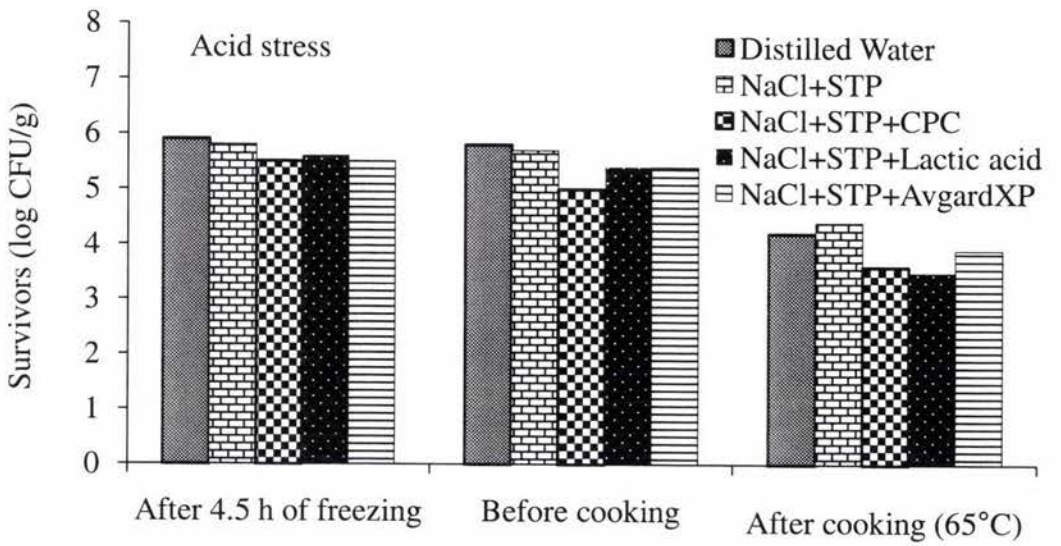
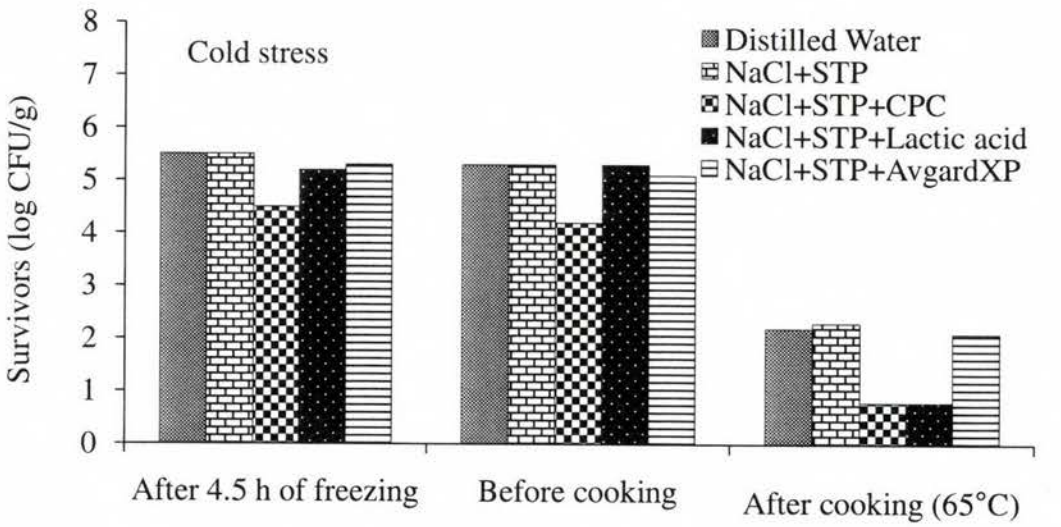


Figure 5.1 (Appendix Table 12). Temperature changes of the surface of Presto® electric skillet and the lower, and upper surface, edge, and geometric center of nonintact beef steaks during cooking to the internal geometric center temperature of 65°C. Each point is averaged across all brining treatments.


Unstress
Survivors (log CFU/g)
8
7
6
5
4
3
2
1
0
Distilled Water
NaCl+STP
NaCl+STP+CPC
NaCl+STP+Lactic acid
NaCl+STP+AvgardXP
After 4.5 h of freezing
Before cooking
After cooking (65°C)


Acid stress
Survivors (log CFU/g)
8
7
6
5
4
3
2
1
0
Distilled Water
NaCl+STP
NaCl+STP+CPC
NaCl+STP+Lactic acid
NaCl+STP+AvgardXP
After 4.5 h of freezing
Before cooking
After cooking (65°C)


Cold stress
Survivors (log CFU/g)
8
7
6
5
4
3
2
1
0
Distilled Water
NaCl+STP
NaCl+STP+CPC
NaCl+STP+Lactic acid
NaCl+STP+AvgardXP
After 4.5 h of freezing
Before cooking
After cooking (65°C)

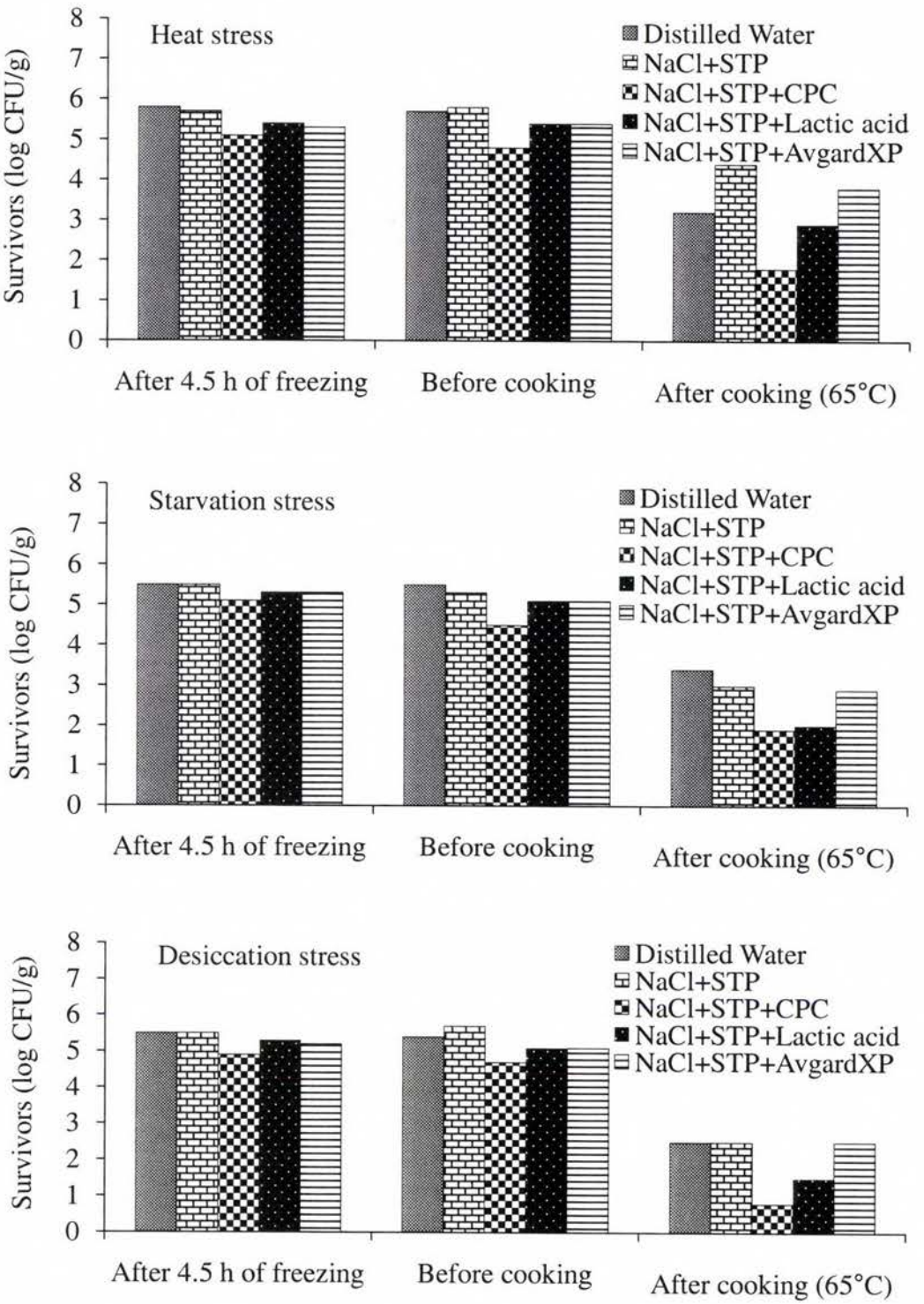


Figure 5.2 (Appendix Table 13). Survivor counts (mean, log CFU/g, n=6) of *Escherichia coli* O157:H7 recovered during cooking (65°C, internal) of non-intact beef steaks, moisture enhanced with variance brining solutions and inoculated with unstressed, acid, cold, heat, starvation and desiccation stress-adapted inocula with tryptic soy agar plus pyruvate plus rifampicin (100 µg/ml).

CHAPTER 6

ANTILISTERIAL ACTIVITY OF HOPS BETA ACIDS IN BROTH WITH OR WITHOUT OTHER ANTIMICROBIALS

ABSTRACT

Hops beta acids (HBA) are parts of hops flowers used to preserve wort and provide flavor in beer, and are reported as having antimicrobial properties. This study evaluated the antilisterial activity of HBA alone or in combination with other known antimicrobials in a culture broth medium. *Listeria monocytogenes* (10-strain mixture) was inoculated (2.6 to 2.8 log CFU/ml) into tryptic soy broth supplemented with 0.6% yeast extract (TSBYE) without (control) or with HBA (0.5-5.0 μg/ml), potassium lactate (1.0%), sodium diacetate (0.25%) or acetic acid (0.1%), alone or in combination with HBA (0.5-3.0 μg/ml). Survival/growth of the pathogen during storage at 4°C (35 days), 10°C (20 days) or 25°C (2 days) was periodically monitored by spiral plating onto tryptic soy agar plus 0.6% yeast extract. As

expected, TSBYE without antimicrobials (control) supported rapid pathogen growth with growth rates of 0.40, 2.88 and 9.58 log CFU/ml/day at 4, 10 and 25°C, respectively; corresponding Y_{end} values exceeded 9.0 log CFU/ml at 35, 20 and 2 days storage. HBA used alone (1.0-5.0 µg/ml), inhibited growth of *L. monocytogenes* at all three temperatures, with inhibition being more pronounced at higher concentrations and at the lower storage temperature (4°C). The antilisterial activity of HBA (0.5-3.0 µg/ml) was enhanced when combined with sodium diacetate, acetic acid or potassium lactate, achieving complete inhibition at 4°C when 3.0 µg/ml HBA were used in combination with each of the above antimicrobials. Overall, HBA exhibited promising antilisterial activity in a broth medium and further studies are needed to investigate its potential antilisterial effects in food products.

6.1. INTRODUCTION

Listeria monocytogenes, the causative agent of listeriosis, can survive and grow under adverse conditions including refrigeration temperatures, low pH, low water activity and high salt concentrations (Jay et al., 2005; Lado and Yousef, 2007). Although listeriosis outbreaks are not common, its fatality rate is high (20 to 40%) for high risk groups such as pregnant women, neonates, and immunocompromised adults (ILSI Research Foundation/Risk Science Institute, 2005). The U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) reported that 147,208 kg of ready-to-eat meat products were recalled for

possible *L. monocytogenes* contamination in 2005 (USDA-FSIS, 2006). Therefore, *L. monocytogenes* continues to be a major concern to the food industry and public health, and the investigation of new antilisterial agents is needed. Currently, there is a growing interest in using natural antimicrobials to control foodborne pathogens (Sofos et al., 1998; Sivarooban et al., 2007), including plant extracts, some of which have been approved by USDA-FSIS as a possible method for reducing pathogenic bacteria in meat and poultry products (USDA-FSIS, 2008b).

Among the many plants known to have antimicrobial properties is the hop plant (*Humulus lupulus*) of the family of *Cannabinaceae*. As early as the 12th century in Germany and the 15th century in England, hops were used to preserve wort and provide flavor in beer (Srinivasan et al., 2004). The antimicrobial properties of hop plants are mostly attributed to the hops acids, and they are mainly active against Gram-positive bacteria (Haas and Barsoumian 1994; Bhattacharya et al., 2003), but appear to have no activity against most Gram-negative microorganisms (Bhattacharya et al., 2003).

The hops beta acids (HBA) are constituents of hops acids, and consist of lupulone ($C_{27}H_{38}O_4$), colupulone ($C_{26}H_{37}O_4$) and adlupulone ($C_{27}H_{38}O_4$) (US-FDA, 2001). The U.S. Food and Drug Administration (FDA) and USDA-FSIS have approved HBA as generally recognized as safe for use as antimicrobial agents in the amount of 4.4 mg/kg of cooked meat

and 5.5 mg/kg of casings for meat products (US-FDA GRAS Notice No. 000063, 2001). To date, published studies on the antimicrobial activity of HBA are limited. Srinivasan et al. (2004) found that HBA had wide anti-protozoa effects, while Bhattacharya et al. (2003) showed that the minimum inhibitory concentrations of HBA against *Streptococcus mutans* were 2 to 50 μg/ml as determined by a turbidimetric assay. Larson et al. (1996) showed that hops extracts (containing 60% beta acids) exhibited 100% inhibition against *L. monocytogenes* in brain heart infusion broth when the concentration was higher than 10 μg/ml. The above studies, however, used the paper disc diffusion assay or optical density measurements to determine inhibition. They did not test the antimicrobial activity of HBA in broth media using an agar plating method, and did not compare it to other chemical antimicrobial agents used in the food industry. Potassium lactate, sodium diacetate and acetic acid are widely used as antimicrobial agents in the food industry to control foodborne pathogens in meat and poultry products, and their antilisterial activities have been well documented in broth media (Yoon et al., 2003; Vermeulen et al., 2007) and meat products (Samelis et al., 2001; Porto et al., 2002; Geornaras et al., 2006a; 2006b). Thus, the objective of the present study was to determine the antilisterial activity of HBA, alone or in combination with potassium lactate, sodium diacetate or acetic acid, in a culture broth medium stored at 4°C, 10°C or 25°C.

6.2. MATERIALS AND METHODS

6.2.1. Bacterial strains and inoculum preparation

A mixture of 10 *L. monocytogenes* strains was used in this study, and included NA-1 (serotype 3b, pork sausage isolate); N-7150 (serotype 3a, meat isolate); 558 (serotype ½, pork meat isolate); N1-227 (serotype 4b), R2-500 (serotype 4b), R2-501 (serotype 4b), and R2-764 (serotype 4b), all food isolates; N1-225 (serotype 4b) and R2-763 (serotype 4b), both clinical isolates; and R2-765 (serotype 4b, environmental isolate) (Lianou et al., 2007a; 2007b). Strains N1-225, N1-227, R2-500, R2-501, R2-763, R2-764 and R2-765 were kindly provided by Dr. Martin Wiedmann (Cornell University, Ithaca, NY, USA; Fugett et al., 2006). The strains were maintained (4°C) on slants of tryptic soy agar (Difco, Becton Dickinson, Sparks, MD, USA) supplemented with 0.6% yeast extract (Acumedia, Lansing, MI, USA) (TSAYE), and were subcultured monthly. Each strain was activated (30°C, 24 h) separately by transferring a single colony from PALCAM agar (Difco) plates into 10 ml of tryptic soy broth (Difco) supplemented with 0.6% yeast extract (TSBYE). Activated cultures were then subcultured (0.1 ml) into 10 ml of fresh TSBYE and incubated at 30°C for another 24 h.

Cultures of the 10 strains of *L. monocytogenes* were combined and centrifuged (Eppendorf model 5810R, Brinkmann Instruments Inc., Westbury, NY, USA) at 4,629×*g* for 15 min at 4°C. The harvested cells were washed with 10 ml of phosphate-buffered saline

(PBS, pH 7.4; 0.2 g of KH_2PO_4, 1.5 g of $Na_2HPO_4 \cdot 7H_2O$, 8.0 g of NaCl, and 0.2 g of KCl in 1 liter of distilled water), centrifuged as previously described, and resuspended in 100 ml of fresh PBS. The washed inoculum was serially diluted in PBS to obtain a target inoculation level of 2 to 3 log CFU/ml when 0.3 ml of inoculum was added to 75 ml of the control (no antimicrobials) or antimicrobial treatments.

6.2.2. Antimicrobial treatments and analyses

All the antimicrobial solutions were prepared in the culture broth medium, TSBYE. The treatments of the study included (i) control (TSBYE, no antimicrobials); HBA (preparation of 30% beta acids in 50% propylene glycol; S.S. Steiner Inc., NY, USA) at (ii) 0.5 µg/ml, (iii) 1.0 µg/ml, (iv) 3.0 µg/ml and (v) 5.0 µg/ml; (vi) potassium lactate (1.0%; Purac America, Inc., Lincolnshire, IL, USA); (vii) sodium diacetate (0.25%; Niacet Corp., Niagara Falls, NY, USA); (viii) acetic acid (0.1%; Mallinckrodt, Paris, KY, USA); (ix) HBA (0.5 µg/ml, 1.0 µg/ml, 3.0 µg/ml) + 1.0% potassium lactate; (x) HBA (0.5 µg/ml, 1.0 µg/ml, 3.0 µg/ml) + 0.25% sodium diacetate; and, (xi) HBA (0.5 µg/ml, 1.0 µg/ml, 3.0 µg/ml) + 0.1% acetic acid. All the solutions were autoclaved before the experiment, except for the HBA preparation, which was dissolved in distilled water, filter-sterilized (0.22 µm Steriflip, Millipore, Billerica, MA, USA) and added to the sterile TSBYE in appropriate amounts for the concentrations tested. As indicated above, the solutions (75 ml) were inoculated with 0.3 ml of the diluted

inoculum.

Microbiological testing to determine survival/growth of *L. monocytogenes* in the solutions was conducted on days 0, 7, 14, 21, 28 and 35 of storage at 4°C, on days 0, 3, 6, 10, 15 and 20 of storage at 10°C, and on days 0, 0.5, 1, 1.5 and 2 of storage at 25°C. At each analysis time, samples from each treatment were appropriately diluted in 0.1% buffered peptone water (Difco) and spiral plated (Spiral Plater Model D; Spiral Biotech, Bethesda, MD, USA) onto TSAYE. After incubation of the plates at 30°C for 48 h, colonies were counted using the Colony Image Analysis CASBA^{TM} 4 scanner and software system (Spiral Biotech). The pH of the solutions was measured, using a Denver Instruments (Arvada, CO, USA) pH meter and glass electrode, before and after inoculation, and during storage at each temperature.

6.2.3. Statistical analysis

The experiment was conducted twice, each time with three individual samples at each sampling time. The microbiological data were converted into log CFU/ml and analyzed using the DMFIT software (Institute of Food Research, Reading, UK), which was kindly provided by Dr. J. Baranyi and used to estimate the following parameters of *L. monocytogenes* growing in TSBYE without or with antimicrobials: length of lag phase period, growth rate of the pathogen, and the Y_{end} (upper asymptote), representing the maximum point of the

bacterial growth curve (Baranyi and Roberts, 1994). The pH data were analyzed using the mixed model procedure of SAS (SAS Institute, 2002), with independent variables including treatment, time and treatment × time interactions; mean differences were separated with the least significant difference procedure at the significance level of α=0.05.

6.3. RESULTS AND DISCUSSION

Initial pH values of TSBYE without (control) and with HBA (0.5 to 5.0 µg/ml) alone were 7.25 and 7.23 to 7.40 (Tables 6.1, 6.2 and 6.3), respectively, and no ($P \geq 0.05$) variation in pH was observed with increasing concentrations of HBA. The pH of the 1.0% potassium lactate, 0.25% sodium diacetate, and 0.1% acetic acid treatments on day-0 were 7.23, 6.41, and 6.10, respectively. Combinations of increasing concentrations of HBA (0.5 to 3.0 µg/ml) with the above antimicrobials resulted in slight ($P \geq 0.05$) increases in the pH of the solutions. Specifically, initial pH values for the combination treatments were 7.27 to 7.32 (HBA combined with potassium lactate), 6.43 to 6.46 (HBA combined with sodium diacetate), and 6.15 to 6.24 (HBA combined with acetic acid) (Tables 6.1, 6.2 and 6.3). During storage, especially after 1 day at 25°C, 6 days at 10°C and 21 days at 4°C, pH decreased ($P < 0.05$) in controls, the single antimicrobial treatments of 0.5 and 1.0 µg/ml HBA, potassium lactate, sodium diacetate, acetic acid, and the combination treatments containing 0.5 or 1.0 µg/ml HBA (Tables 6.1, 6.2 and 6.3). These pH reductions may have been attributed to *L.*

monocytogenes growth (>7.0 log CFU/ml). The pH of solutions that did not allow growth (3.0 and 5.0 µg/ml HBA) did not change ($P \geq 0.05$) during 35 days, 20 days and 2 days of storage at 4, 10 and 25°C, respectively (Tables 6.1, 6.2 and 6.3).

The initial (day-0) inoculum level of *L. monocytogenes* in all treatments was 2.6 to 2.8 log CFU/ml. Thus, exposure of *L. monocytogenes* to HBA alone or in combination with the other antimicrobials did not appear to have any immediate lethal effect. As expected, TSBYE without antimicrobials (control) allowed pathogen growth following a lag phase period of 2.64 days, with a growth rate of 0.40 log CFU/ml/day, and the Y_{end} exceeded 9.0 log CFU/ml at 35 days at 4°C (Table 6.4). At the same storage temperature, extent of pathogen growth in the potassium lactate (1.0%) treatment was similar to that of the control, with a lag phase duration of 2.61 days and a growth rate of 0.42 log CFU/ml/day (Table 6.5). Compared to the control, at 4°C, the single treatments of sodium diacetate (0.25%) or acetic acid (0.1%) inhibited *L. monocytogenes*, as they extended the lag phase duration by 0.32 to 0.86 days, and decreased growth rates by 0.11 log CFU/ml/day (Tables 6.6 and 6.7). HBA alone exhibited increasing inhibition of *L. monocytogenes* growth at 4°C as concentrations increased from 1.0 to 5.0 µg/ml with increasing lag phase durations and decreasing growth rates; complete inhibition (35 days) of the pathogen was obtained with 5.0 µg/ml HBA as indicated by the lag phase duration of at least 35 days, followed by a growth rate of -0.01 log

CFU/ml/day (Table 6.4). All HBA (0.5-3.0 µg/ml) treatments combined with potassium lactate, sodium diacetate or acetic acid inhibited growth of *L. monocytogenes* and were more effective than when used alone (Tables 6.4, 6.5, 6.6 and 6.7), except for the combination of 0.5 µg/ml HBA and potassium lactate (1.0%) which showed lag phase duration (3.06 days), growth rate (0.36 log CFU/ml/day) and Y_{end} (8.86 log CFU/ml) values similar to those of the control (Table 6.5). Particularly, the combination of 3.0 µg/ml HBA with potassium lactate (1.0%), sodium diacetate (0.25%) or acetic acid (0.1%) exhibited complete inhibitory effects against the pathogen (growth rate of 0 log CFU/ml/day) during the entire storage period at 4°C. In addition to 5.0 µg/ml HBA, the most effective antilisterial treatment was 3.0 µg/ml HBA combined with acetic acid showing a lag phase duration of at least 35 days and a growth rate of -0.01 log CFU/ml/day (Table 6.4). This result agreed with findings of Larson et al. (1996), who indicated that the inhibitory effects of hops extracts (containing 60% beta acids) were generally enhanced in a slightly acidic environment and at lower temperatures.

At 10 and 25°C, growth of the pathogen in controls (no antimicrobials) was obtained after a lag phase period of 1.41 and 0.19 days and levels increased to 9.0 log CFU/ml within 6 and 1 days, with growth rates of 2.88 and 9.58 log CFU/ml/day, respectively (Table 6.4). During storage at 10 or 25°C, pathogen growth in single treatments of potassium lactate was slower than in controls, with growth rates of 1.93 and 8.85 log CFU/ml/day, respectively

(Table 6.5). Inhibitory effects, compared to the controls, were observed in the single treatments of sodium diacetate or acetic acid stored at 10 and 25°C; however, in all cases, Y_{end} values for the pathogen reached about 9.0 log CFU/ml (Tables 6.3 and 6.4). Inhibition of *L. monocytogenes* growth was found at HBA concentrations of 1.0-5.0 μg/ml at the above two storage temperatures (Table 6.4). Lag phase durations of the pathogen in 5.0 μg/ml HBA stored at 10 and 25°C were 9.29 days and 1.12 days , respectively, and growth rates were 0.34 and 1.89 log CFU/ml/day, respectively, which were much less than those of the control (Table 6.4). The inhibitory effects of single treatments of HBA were enhanced when combined with potassium lactate, sodium diacetate or acetic acid. More specifically, *L. monocytogenes* exposed to combination treatments of 3.0 μg/ml HBA with sodium diacetate or acetic acid exhibited lag phase durations of 5.86 or 8.52 days and 1.12 or 1.25 days, with growth rates of 0.46 or 0.40 log CFU/ml/day and 2.82 or 2.58 log CFU/ml/day at 10 and 25°C, respectively, which was better than those of 3.0 μg/ml HBA tested alone (Tables 6.4, 6.6 and 6.7). At 25°C, the most effective treatment was the solution containing 3.0 μg/ml HBA combined with potassium lactate (1.0%), showing a lag phase duration of at least 2 days followed by a growth rate of 0.56 log CFU/ml/day (Table 6.5).

6.4. CONCLUSIONS

In conclusion, HBA (1.0-5.0 μg/ml) exhibited inhibitory activity against *L.*

monocytogenes in a broth medium during storage at 4, 10 and 25°C, and activity was enhanced as concentrations increased and when combined with other antimicrobials, and when stored at lower temperatures (4°C). However, in order for the HBA to be used in food systems, further studies need to be conducted to determine their antilisterial effects on food products, as well as their effects on product sensory characteristics. Also, the most effective method of their application in food products, such as inclusion as formulation ingredients or as post-processing surface dipping or spraying treatments need to be investigated.

Table 6.1. Mean (± standard deviation) pH values of *Listeria monocytogenes* cultures in tryptic soy broth supplemented with 0.6% yeast extract (TSBYE) with hops beta acid (HBA), potassium lactate (PL), sodium diacetate (SD) or acetic acid (Ac) alone or in combination stored at 4°C for 35 days.

Treatment		Storage time (day)					
Antimicrobial (%)	HBA(μg/ml)	0	7	14	21	28	35
None	0	7.21±0.01aX	7.33±0.10aX	7.25±0.01aX	5.29±0.07aY	5.18±0.06aY	5.19±0.02aY
	0.5	7.23±0.08aX	7.29±0.14aX	7.24±0.10aX	6.12±0.24bY	5.22±0.21aZ	5.20±0.21aZ
	1.0	7.28±0.08aX	7.34±0.11aX	7.18±0.04aX	7.00±0.28cY	5.66±0.21bZ	5.67±0.21bZ
	3.0	7.29±0.06aX	7.32±0.12aX	7.28±0.05aX	7.38±0.10dX	7.26±0.07cX	7.43±0.07cX
	5.0	7.32±0.02aX	7.20±0.02aX	7.26±0.01aX	7.24±0.03dX	7.30±0.01cX	7.34±0.01cX
PL (1.0)	0	7.23±0.05aX	7.37±0.07aX	7.21±0.07aX	5.83±0.37eY	5.29±0.12dZ	5.28±0.14dZ
	0.5	7.27±0.03aX	7.39±0.08aX	7.27±0.09aX	6.60±0.25fY	5.59±0.15eZ	5.54±0.24eZ
	1.0	7.28±0.06aX	7.36±0.10aX	7.19±0.05aX	7.30±0.03dX	6.98±0.10fY	6.58±0.05fZ
	3.0	7.32±0.14aX	7.40±0.13aX	7.22±0.05aX	7.35±0.07dX	7.31±0.08gX	7.27±0.05gX
SD (0.25)	0	6.41±0.03bX	6.53±0.22bX	6.48±0.09bX	6.53±0.13fX	5.97±0.13hY	5.48±0.13bdZ
	0.5	6.44±0.11bX	6.62±0.14bX	6.50±0.05bX	6.56±0.09fX	6.49±0.15iX	6.29±0.36iX
	1.0	6.43±0.07bX	6.63±0.13bX	6.45±0.06bX	6.63±0.10fX	6.49±0.10iX	6.49±0.16iX
	3.0	6.46±0.08bX	6.64±0.10bX	6.46±0.04bX	6.58±0.04fX	6.51±0.04iX	6.65±0.14iX
Ac (0.1)	0	6.10±0.05cX	6.29±0.10cX	6.13±0.02cX	6.17±0.02bX	5.80±0.15bX	5.47±0.15bY
	0.5	6.15±0.09cX	6.31±0.21cX	6.19±0.05cX	6.24±0.05bgX	6.27±0.11iX	6.22±0.18iX
	1.0	6.22±0.17cX	6.35±0.10cX	6.24±0.07cX	6.35±0.07gX	6.30±0.12iX	6.32±0.18iX
	3.0	6.24±0.02cX	6.30±0.17cX	6.22±0.07cX	6.29±0.08bgX	6.29±0.13iX	6.33±0.20iX

a- i: Means with a common letter within a column are not different ($P \geq 0.05$).

X-Z: Means with a common letter within a row are not different ($P \geq 0.05$).

Table 6.2. Mean (± standard deviation) pH values of *Listeria monocytogenes* cultures in tryptic soy broth supplemented with 0.6% yeast extract (TSBYE) with hops beta acid (HBA), potassium lactate (PL), sodium diacetate (SD) or acetic acid (Ac) alone or in combination stored at 10°C for 20 days.

Treatment		Storage time (day)					
Antimicrobial (%)	HBA(μg/ml)	0	3	6	10	15	20
None	0	7.25±0.11aX	7.25±0.05aX	5.11±0.02aY	4.89±0.03aY	5.00±0.10aY	5.05±0.08aY
	0.5	7.24±0.14aX	7.23±0.08aX	5.62±0.33bY	4.93±0.02aY	5.05±0.10aY	5.01±0.18aY
	1.0	7.23±0.10aX	7.28±0.12aX	7.11±0.06ceX	5.40±0.10bY	5.10±0.11aZ	5.04±0.03aZ
	3.0	7.28±0.15aX	7.28±0.12aX	7.21±0.07cX	7.26±0.17cX	7.33±0.03bX	7.25±0.10bX
	5.0	7.30±0.01aX	7.26±0.02aX	7.25±0.01cX	7.17±0.02cX	7.20±0.02bX	7.32±0.04bX
PL (1.0)	0	7.33±0.14aX	7.31±0.06aX	6.40±0.07dY	5.19±0.04bZ	5.11±0.11aZ	5.17±0.15acZ
	0.5	7.37±0.15aX	7.29±0.04aX	6.93±0.09eY	5.13±0.12abZ	5.10±0.10aZ	5.30±0.22acZ
	1.0	7.38±0.08aX	7.28±0.10aX	7.17±0.05ceX	7.10±0.12cX	5.61±0.17cY	5.40±0.20cY
	3.0	7.34±0.07aX	7.23±0.05aX	7.28±0.14cX	7.29±0.08cX	7.25±0.07bX	7.28±0.11bX
SD (0.25)	0	6.42±0.14bX	6.54±0.09bX	6.22±0.16dfX	4.84±0.06dY	5.03±0.09aY	4.96±0.22aY
	0.5	6.47±0.16bX	6.56±0.09bX	6.42±0.08dX	5.90±0.09eY	5.15±0.15aZ	5.20±0.27aZ
	1.0	6.48±0.14bX	6.55±0.10bX	6.40±0.08dX	6.43±0.03fX	6.59±0.19dX	6.44±0.26dX
	3.0	6.50±0.14bX	6.58±0.07bX	6.47±0.03dX	6.49±0.04fX	6.63±0.13dX	6.59±0.13dX
Ac (0.1)	0	6.17±0.05cX	6.27±0.03cX	6.23±0.06dfX	5.04±0.08aY	4.73±0.15eY	4.78±0.16aY
	0.5	6.23±0.15cX	6.25±0.04cX	6.22±0.11dfX	6.17±0.07fX	5.17±0.14aY	4.93±0.12aY
	1.0	6.25±0.09cX	6.25±0.06cX	6.12±0.03fX	6.17±0.04fX	6.26±0.10dX	5.47±0.24cY
	3.0	6.31±0.24cX	6.19±0.04cX	6.14±0.07fX	6.26±0.11fX	6.39±0.09dX	6.24±0.24dX

a- f: Means with a common letter within a column are not different ($P \geq 0.05$).

X-Z: Means with a common letter within a row are not different ($P \geq 0.05$).

Table 6.3. Mean (± standard deviation) pH values of *Listeria monocytogens* cultures in tryptic soy broth supplemented with 0.6% yeast extract (TSBYE) with hops beta acid (HBA), potassium lactate (PL), sodium diacetate (SD) or acetic acid (Ac) alone or in combination stored at 25°C for 2 days.

Treatment		Storage time (day)				
Antimicrobial (%)	HBA(μg/ml)	0	0.5	1.0	1.5	2.0
None	0	7.25±0.04aX	7.32±0.05aX	6.03±0.16aY	5.07±0.07aZ	5.10±0.14aZ
	0.5	7.32±0.03aX	7.41±0.04aX	7.11±0.09bY	5.34±0.10aZ	5.06±0.18aZ
	1.0	7.35±0.07aX	7.35±0.07aX	7.31±0.05bX	7.27±0.07bX	5.51±0.31bY
	3.0	7.36±0.06aX	7.35±0.06aX	7.28±0.05bX	7.30±0.04bX	7.47±0.09cX
	5.0	7.40±0.08aX	7.32±0.08aX	7.25±0.06bX	7.27±0.03bX	7.42±0.07cX
PL (1.0)	0	7.27±0.04aX	7.30±0.03aX	6.40±0.29cY	5.15±0.05aZ	5.31±0.09bZ
	0.5	7.28±0.05aX	7.29±0.02aX	7.22±0.20bX	5.49±0.06aY	5.33±0.06bY
	1.0	7.31±0.03aX	7.30±0.05aX	7.34±0.07bX	7.14±0.07bX	6.60±0.36dY
	3.0	7.35±0.02aX	7.25±0.03aX	7.33±0.10bX	7.23±0.08bX	7.35±0.06cX
SD (0.25)	0	6.49±0.07bX	6.53±0.07bX	6.19±0.03acY	5.08±0.07aZ	5.19±0.11bZ
	0.5	6.55±0.03bX	6.51±0.05bX	6.48±0.05cX	6.27±0.16cX	5.46±0.20bY
	1.0	6.57±0.03bX	6.48±0.06bX	6.51±0.10cX	6.43±0.10cX	6.67±0.15dX
	3.0	6.61±0.08bX	6.48±0.08bX	6.52±0.06cX	6.41±0.02cX	6.65±0.20dX
Ac (0.1)	0	6.17±0.06cX	6.25±0.05cX	5.93±0.09aY	4.79±0.10aZ	4.87±0.19aZ
	0.5	6.25±0.04cX	6.26±0.04cX	6.31±0.03cX	6.17±0.06cX	5.41±0.24bY
	1.0	6.35±0.09bcX	6.28±0.03cX	6.29±0.05cX	6.22±0.10cX	6.35±0.21dX
	3.0	6.28±0.09cX	6.30±0.03cX	6.34±0.08cX	6.22±0.04cX	6.32±0.07dX

a- d: Means with a common letter within a column are not different ($P \geq 0.05$).

X-Z: Means with a common letter within a row are not different ($P \geq 0.05$).

Table 6.4. Growth kinetics of *Listeria monocytogenes* in tryptic soy broth supplemented with 0.6% yeast extract containing hops beta acids (HBA) and stored at 4°C for 35 days, 10°C for 20 days, or 25°C for 2 days; initial inoculum was 2.6-2.8 log CFU/ml.

Temperature (°C)	HBA (μg/ml)	Lag phase duration (days)	Growth rate (log CFU/ml/day)	Y_{end}[a] (log CFU/ml)	R^{2d}
4	0	2.64	0.40	9.15	0.99
	0.5	2.67	0.37	8.86	0.99
	1.0	6.77	0.25	8.40	0.99
	3.0	24.70	0.24	---[c]	0.95
	5.0	---[b]	-0.01	---[c]	0.40
10	0	1.41	2.88	9.08	0.99
	0.5	1.44	2.40	8.97	0.99
	1.0	1.60	0.91	8.83	0.99
	3.0	3.53	0.89	7.90	0.99
	5.0	9.29	0.34	---[c]	0.97
25	0	0.19	9.58	9.17	0.99
	0.5	0.20	6.33	9.00	0.99
	1.0	0.23	3.90	8.96	0.99
	3.0	0.96	3.60	---[c]	0.94
	5.0	1.12	1.89	---[c]	0.82

[a] Upper asymptote estimated by the Baranyi model (Baranyi and Roberts, 1994).

[b] No lag phase observed due to no growth.

[c] Stationary phase was not reached.

[d] Coefficient of determination: how well the parameters fitted to the Baranyi model (Baranyi and Roberts, 1994).

Table 6.5. Growth kinetics of *Listeria monocytogenes* in tryptic soy broth supplemented with 0.6% yeast extract with hops beta acids (HBA) and potassium lactate (PL) in combination stored at 4°C for 35 days, 10°C for 20 days, or 25°C for 2 days; initial inoculum was 2.6-2.8 log CFU/ml.

Temperature (°C)	PL (%)	HBA (μg/ml)	Lag phase duration (days)	Growth rate (log CFU/ml/day)	Y_{end}[a] (log CFU/ml)	R^{2d}
4	0	0	2.64	0.40	9.15	0.99
	1.0	0	2.61	0.42	9.02	0.99
		0.5	3.06	0.36	8.86	0.99
		1.0	13.93	0.23	---[c]	0.98
		3.0	---[b]	0.00	---[c]	0.36
10	0	0	1.41	2.88	9.08	0.99
	1.0	0	1.56	1.93	8.97	0.99
		0.5	1.69	1.68	8.90	0.99
		1.0	1.99	0.72	8.81	0.99
		3.0	5.42	0.42	7.18	0.99
25	0	0	0.19	9.58	9.17	0.99
	1.0	0	0.19	8.85	8.94	0.99
		0.5	0.20	6.39	8.99	0.99
		1.0	0.34	3.71	---[c]	0.99
		3.0	---[b]	0.56	---[c]	0.47

[a] Upper asymptote estimated by the Baranyi model (Baranyi and Roberts, 1994).

[b] No lag phase observed due to little or no growth.

[c] Stationary phase was not reached.

[d] Coefficient of determination: how well the parameters fitted to the Baranyi model (Baranyi and Roberts, 1994).

Table 6.6. Growth kinetics of *Listeria monocytogenes* in tryptic soy broth supplemented with 0.6% yeast extract with hops beta acids (HBA) and sodium diacetate (SD) in combination stored at 4°C for 35 days, 10°C for 20 days, or 25°C for 2 days; initial inoculum was 2.6-2.8 log CFU/ml.

Temperature (°C)	SD (%)	HBA (µg/ml)	Lag phase duration (days)	Growth rate (log CFU/ml/day)	Y_{end}[a] (log CFU/ml)	R^{2d}
4	0	0	2.64	0.40	9.15	0.99
	0.25	0	2.96	0.29	8.73	0.99
		0.5	10.80	0.30	8.15	0.99
		1.0	24.27	0.07	---[c]	0.71
		3.0	---[b]	0.00	---[c]	0.43
10	0	0	1.41	2.88	9.08	0.99
	0.25	0	1.47	1.61	8.91	0.99
		0.5	2.27	0.81	8.89	0.99
		1.0	5.40	0.65	8.17	0.99
		3.0	5.86	0.46	6.75	0.99
25	0	0	0.19	9.58	9.17	0.99
	0.25	0	0.24	6.51	9.04	0.99
		0.5	0.35	4.19	9.18	0.99
		1.0	0.81	3.96	---[c]	0.99
		3.0	1.12	2.82	---[c]	0.86

[a] Upper asymptote estimated by the Baranyi model (Baranyi and Roberts, 1994).

[b] No lag phase observed due to no growth.

[c] Stationary phase was not reached.

[d] Coefficient of determination: how well the parameters fitted to the Baranyi model (Baranyi and Roberts, 1994).

Table 6.7. Growth kinetics of *Listeria monocytogenes* in tryptic soy broth supplemented with 0.6% yeast extract with hops beta acids (HBA) and acetic acid (Ac) in combination stored at 4°C for 35 days, 10°C for 20 days, or 25°C for 2 days; initial inoculum was 2.6-2.8 log CFU/ml.

Temperature (°C)	Ac (%)	HBA (µg/ml)	Lag phase duration (days)	Growth rate (log CFU/ml/day)	Y_{end}[a] (log CFU/ml)	R^{2d}
4	0	0	2.64	0.40	9.15	0.99
	0.10	0	3.50	0.29	8.63	0.99
		0.5	14.38	0.24	---[c]	0.98
		1.0	20.32	0.16	---[c]	0.97
		3.0	---[b]	-0.01	---[c]	0.79
10	0	0	1.41	2.88	9.08	0.99
	0.10	0	1.48	1.81	8.76	0.99
		0.5	3.85	0.60	8.83	0.99
		1.0	5.57	0.54	8.23	0.99
		3.0	8.52	0.40	---[c]	0.96
25	0	0	0.19	9.58	9.17	0.99
	0.10	0	0.21	7.63	8.96	0.99
		0.5	0.46	4.29	9.04	0.99
		1.0	0.86	4.34	---[c]	0.97
		3.0	1.25	2.58	---[c]	0.78

[a] Upper asymptote estimated by the Baranyi model (Baranyi and Roberts, 1994).

[b] No lag phase observed due to no growth.

[c] Stationary phase was not reached.

[d] Coefficient of determination: how well the parameters fitted to the Baranyi model (Baranyi and Roberts, 1994).

CHAPTER 7

CONTROL OF *LISTERIA MONOCYTOGENES* ON FRANKFURTERS BY DIPPING IN HOPS BETA ACIDS SOLUTIONS

ABSTRACT

Hops beta acids (HBA) are parts of hops flowers used in beer brewing and have shown antilisterial activity in bacteriological broth. The U.S. Department of Agriculture Food Safety and Inspection Service has approved HBA for use to control *Listeria monocytogenes* on ready-to-eat meat products. This study evaluated the effects of HBA as dipping solutions to control *L. monocytogenes* during storage of frankfurters. Frankfurters (two replicates/three samples each) were inoculated (1.9 ± 0.1 log CFU/cm^2) with *L. monocytogenes* (10-strain mixture), dipped (2 min, 25 ± 2°C) in HBA solutions (0.03%, 0.06% and 0.10%) or distilled water (DW), and then vacuum-packaged and stored at 4°C or 10°C for up to 90 and 48 days, respectively. Samples were periodically analyzed for microbial survival/growth on tryptic soy

agar plus 0.6% yeast extract and PALCAM agar. Dipping in HBA solutions caused immediate *L. monocytogenes* reductions ($P < 0.05$) of 1.3 to 1.6 log CFU/cm^2, whereas DW reduced counts by 1.0 log CFU/cm^2. Pathogen growth was completely suppressed ($P < 0.05$) for 30 to 50 (4°C) or 20 to 28 (10°C) days on frankfurters dipped in HBA solutions, with antilisterial effects increasing with higher concentrations (0.03 to 0.10%). Fitting the data with the Baranyi model confirmed that the lag-phase duration of the pathogen was extended, and the growth rate was decreased on samples dipped in HBA solutions. Therefore, HBA may be considered for use to improve the microbial safety of RTE meat products, provided that future studies show no adverse effects on sensory qualities and that their use is economically feasible.

7.1. INTRODUCTION

Listeria monocytogenes is a postprocessing contaminant of ready-to-eat (RTE) meat products and has been associated with multistate outbreaks of listeriosis in the United States through consumption of frankfurters and poultry deli meats (CDC, 1998; 1999; 2000; 2002). These outbreaks were the catalyst for food scientists to seek alternatives for control of *L. monocytogenes* on RTE meat products. In addition to physical and biological interventions, chemical antimicrobial agents (e.g., benzoate, lactate, diacetate, sorbate, acetic acid, lactic acid) have demonstrated valuable effects in controlling *L. monocytogenes* on RTE meats

(Barmpalia et al., 2004; 2005; Byelashov et al., 2008; Geornaras et al., 2005; Samelis et al., 2001; 2002a). Recently, there has been a growing interest by consumers to purchase meat products for which "natural" antimicrobials have been used against foodborne pathogens (Sofos et al., 1998). Various plant extracts, including cloves (Hao et al., 1998a; 1998b), rosemary (Pandit and Shelef, 1994), sage (Shelef et al., 1984), coriander (Stecchini et al., 1993) and grape seed (Sivarooban et al., 2007), have been shown to contain natural compounds with antimicrobial activities.

The hop plant (*Humulus lupulus*) is one of the plants known to have antimicrobial properties. Hops have been used in brewing to provide flavor in beer as early as the 12th century in Germany and the 15th century in England (Srinivasan et al., 2004), and their antimicrobial properties are mostly due to the hops acids, which are mainly active against Gram-positive bacteria (Bhattacharya et al., 2003; Haas and Barsoumian, 1994), while they have no activity against most Gram-negative microorganisms (Bhattacharya et al., 2003). The hops beta acids (HBA) are major constituents of hops acids, and their primary components are lupulone ($C_{27}H_{38}O_4$), colupulone ($C_{26}H_{37}O_4$), and adlupulone ($C_{27}H_{38}O_4$) (US-FDA, 2001). In 2001, the U.S. Food and Drug Administration (FDA) and the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) approved HBA for use as antimicrobial agents in the amount of 4.4 mg/kg of cooked meat and 5.5 mg/kg in casings for

meat products (USDA-FSIS, 2008b).

The numbers of published studies on the antimicrobial activity of HBA are limited. Srinivasan et al. (2004) found that HBA had wide anti-protozoa effects, while Bhattacharya et al. (2003) showed that the minimum inhibitory concentration of HBA against *Streptococcus mutans* ranged from 2 to 10 µg/ml. Larson et al. (1996) showed that hops extracts (containing 60% beta acids) exhibited 100% inhibition against *L. monocytogenes* in brain heart infusion broth, coleslaw and skim milk, when the concentration was 10 to 30 µg/ml, 100 to 1000 µg/ml, and 1000 µg/ml, respectively. A recent study from our laboratory (Chapter 6) showed that HBA had antilisterial activity in a broth medium stored at 4, 10 or 25°C, and the activity was enhanced with increasing concentrations (1.0 to 5.0 µg/ml), in combination with sodium diacetate, acetic acid or potassium lactate, and when stored at the lower temperature of 4°C. Since no published data are available on the antilisterial effects of HBA in meat products, this study was conducted to evaluate the activity of HBA as dipping solutions against *L. monocytogenes* on frankfurters stored in vacuum-packages at 4°C or 10°C.

7.2. MATERIALS AND METHODS

7.2.1. Preparation of frankfurters

The basic frankfurter formulation (no antimicrobials included) consisted (Samelis et al.,

2002a) of (%, wt/wt): fresh pork (49) and fresh beef (33), ice (10), sodium chloride (2), dextrose (2), dry mustard (0.9), corn syrup solids (2), polyphosphate (0.4; sodium tripolyphosphate and sodium hexametaphosphate, Heller Inc., Bedford Park, IL), 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). Spices and seasonings were purchased from AC Legg Co. (Birmingham, Ala). The ingredients were emulsified in a bowl chopper, extruded into 22-mm diameter peelable cellulose casings (Nojax, Viskase Co., Inc., Darien, IL) and linked at lengths of 6.3 ± 0.3 cm, before smoking and cooking as described by Byelashov et al. (2008). Frankfurters were weighed before and after cooking and cooling to determine the cooking yield. After overnight cooling at 4°C, the casings were manually removed.

7.2.2. Bacterial strains and inoculum preparation

The 10-strain mixture of *L. monocytogenes* used in this study included strains NA-1 (serotype 3b, pork sausage isolate); N-7150 (serotype 3a, meat isolate); 558 (serotype ½, pork meat isolate); N1-227 (serotype 4b), R2-500 (serotype 4b), R2-501 (serotype 4b), and R2-764 (serotype 4b), all isolated from food; N1-225 (serotype 4b) and R2-763 (serotype 4b), both clinical isolates; R2-765 (serotype 4b, environmental isolate). Strains N1-225, N1-227, R2-500, R2-501, R2-763, R2-764 and R2-765 were kindly provided by Dr. Martin

Wiedmann (Cornell University, Ithaca, NY) (Fugett et al., 2006). The strains were activated and subcultured according to procedures described by Lianou et al. (2007a; 2007b). To prepare the inoculum, the culture of each strain was centrifuged (Eppendorf model 5810 R, Brinkmann Instruments Inc., Westbury, NY) at 4,629 × *g* for 15 min at 4°C. The harvested cells were washed with 10 ml of phosphate-buffered saline (PBS, pH 7.4; 0.2 g of KH_2PO_4, 1.5 g of $Na_2HPO_4 \cdot 7H_2O$, 8.0 g of NaCl, and 0.2 g of KCl in 1 liter of distilled water), centrifuged as previously described, and resuspended in 10 ml of frankfurter homogenate. The frankfurter homogenate (10%, wt/wt, in distilled water) was prepared as described in previous studies (Byelashov et al., 2008; Lianou et al., 2007a; 2007b). The culture of each strain suspended in frankfurter homogenate was stored at 7°C for 3 days to acclimate the cells to a low temperature food environment. On the day of the experiment, the habituated pathogen cells of each strain were combined and serially diluted in freshly prepared frankfurter homogenate to yield an inoculum level of approximately 2 log CFU/cm^2 when 0.25 ml of inoculum was applied to a frankfurter link.

7.2.3. Product inoculation and treatment

Frankfurters (surface area of 56 cm^2) were inoculated (0.25 ml) with the 10-strain mixture of *L. monocytogenes* under a biosafety cabinet. The inoculum was spread uniformly onto the surface of each link with a sterile bent glass rod, and inoculated frankfurters were

left to stand for 15 min at 5°C for attachment. Inoculated frankfurters were left undipped (control) or were dipped (25 ± 2°C) in 0.03%, 0.06% or 0.10% HBA (30% product, water soluble, density: 1.07 ± 0.01 g/ml; S.S. Steiner Inc., New York, NY) solutions or distilled water (DW). The pH values of the 0.03%, 0.06% or 0.10% HBA solutions were 7.44 ± 0.47, 7.61 ± 0.46 and 8.05 ± 0.38, respectively. All dipping treatments were applied by immersing 20 frankfurters into 250 ml of solution for 2 min, followed by draining for 1 min. After draining, pairs of frankfurters from each treatment were placed into vacuum bags (15 × 22 cm, 3 mil std barrier, nylon/polyethylene vacuum pouch, water vapor and oxygen transmission rates of 9.3 $g/m^2/24h$ [97% relative humidity] and 54.3 $cm^3/m^2/24h$ [21°C, 0% relative humidity], respectively, Koch, Kansas City, MO), vacuum sealed (Hollymatic, Corp., Countryside, IL), and stored at 4 or 10°C for 90 or 48 days, respectively. To verify absorption of HBA on the surface of each frankfurter (mg/kg), preliminary experiments were conducted to determine the weight gained by each frankfurter after 2-min of dipping in DW followed by draining for 1 min.

7.2.4. Microbiological, chemical and physical analyses

On days 0, 5, 10, 20, 30, 50, 70 and 90 (4°C) and 0, 4, 8, 12, 20, 28, 36 and 48 (10°C), individual samples were placed in a sterile bag (Whirl-Pak, Nasco, Modesto, CA) with 50 ml of maximum recovery diluent (MRD; 0.85% NaCl and 0.1% peptone) and shaken vertically

30 times (Barmpalia et al., 2005). Serial 10-fold dilutions of each sample, in 0.1% buffered peptone water (Difco, Becton Dickinson, Sparks, MD), were surface (0.1 ml) plated onto tryptic soy agar (Difco, Becton Dickinson) supplemented with 0.6% yeast extract (Acumedia, Lansing, MI; TSAYE) and PALCAM agar (Difco, Becton Dickinson) for enumeration of total microbial populations and *L. monocytogenes*, respectively. Colonies were counted manually after incubation at 25°C for 72 h (TSAYE) or 30°C for 48 h (PALCAM). On each plating day, frankfurter samples that had been microbiologically analyzed were homogenized (Masticator, IUL Instruments, Barcelona, Spain) for 2 min, and the pH of the homogenate was measured using a digital pH meter with a glass electrode (Denver Instruments, Arvada, CO). The water activity of frankfurter samples was measured only on day-0 using an AquaLab (model series 3, Decagon Devices Inc., Pullman, WA) water activity meter.

7.2.5. Data analysis

The experiment was performed twice and each time included three individual samples that were analyzed at each sampling time (n=6). The water activity (day-0), pH and microbiological data (converted to log CFU/cm^2) were analyzed using the Mixed Procedure of SAS (SAS Institute, 2002), with independent variables including treatment, time and treatment × time interactions. Means and standard deviations for microbiological data were calculated, and the mean differences among interactions were separated with the least

significant difference procedure at the significance level of α=0.05. DMFIT software (Institute of Food Research, Reading, UK), kindly provided by Dr. J. Baranyi, was used to estimate the following parameters: Y_0 and Y_{end} (lower and upper asymptotes), representing initial and end points of the bacterial growth curve, respectively; length of lag phase period; and growth rate of the pathogen (Baranyi and Roberts, 1994).

7.3. RESULTS AND DISCUSSION

7.3.1. Physical and chemical properties

The average cooking yield of two replicate frankfurter batches (day-0) was 89.5 ± 3.5%. The average weight of each frankfurter was 24.7 ± 0.8 g, and 0.2 ± 0.0 g extra weight was obtained after 2-min of dipping in DW followed by 1-min draining. Thus, it may be assumed that after dipping in 0.03%, 0.06% and 0.10% HBA solutions, the estimated concentrations of HBA on the surface of each frankfurter link were 2.5, 4.4 (USDA-FSIS recommended concentration) and 8.0 mg/kg, respectively.

The pH and water activity of untreated frankfurters on day-0 were 6.04 ± 0.01 (Figure 7.1; Appendix Table 14) and 0.974 ± 0.002, respectively, while after dipping in the HBA solutions (0.03 to 0.10%), pH and water activity values were 6.01 to 6.08 and 0.972 to 0.978, respectively. Thus, dipping in HBA solutions for 2 min did not change ($P \geq 0.05$) the product pH and water activity. During storage (especially after 50 days at 4°C and 20 days at 10°C),

pH decreases ($P < 0.05$) were observed in inoculated controls, and samples dipped in DW and 0.03% HBA solutions (Figure 7.1; Appendix Table 14), reflecting microbial growth. The pH of samples that were treated with 0.06 and 0.10% HBA solutions did not change ($P \geq 0.05$) during 90 and 48 days of storage at 4 and 10°C (Figure 7.1; Appendix Table 14), respectively.

7.3.2. Reduction and growth *L. monocytogenes* on frankfurters

Throughout storage at 4 and 10°C, total microbial counts on TSAYE were similar ($P \geq 0.05$) to those observed on PALCAM agar for all treatments (Figure 7.2; Appendix Tables 15 and 16), indicating that the majority of colonies found on TSAYE were *L. monocytogenes*. Total microbial populations on control samples reached 7.4 and 7.7 log CFU/cm^2 by the end of storage at 4 and 10°C (Figure 7.2; Appendix Tables 15 and 16), respectively. Untreated frankfurters showed increased *L. monocytogenes* counts after 10 days at 4°C or 4 days at 10°C, while counts increased from 1.9 to 7.0 log CFU/cm^2 after 50 days (4°C) or 28 days (10°C) (Figure 7.2; Appendix Tables 15 and 16). These results are in agreement with those of previous studies, indicating the ability of *L. monocytogenes* to grow on RTE meat products at refrigeration temperatures (Barmpalia et al., 2004, Samelis et al., 2002a). As expected, dipping frankfurters in DW caused immediate removal of 1.0 log CFU/cm^2 (day-0); after that, however, pathogen growth exceeded 7.0 log CFU/cm^2 by 28 days (10°C) or 70 days (4°C).

7.3.3. Survival and growth of *L. monocytogenes* on frankfurters dipped in HBA solutions

Dipping frankfurters in HBA solutions caused immediate reductions ($P < 0.05$) in pathogen numbers by 1.3 log CFU/cm^2 for 0.03% HBA, and 1.6 log CFU/cm^2 for both 0.06% and 0.10% HBA (Figure 7.2; Appendix Tables 15 and 16), as compared with undipped samples; these reductions were greater ($P < 0.05$) than those obtained by dipping in DW. This indicates that antimicrobial activity of HBA included inactivation as well as physical removal of contamination. During storage, *L. monocytogenes* growth was inhibited on HBA-dipped frankfurters, and inhibition increased with increasing concentration of HBA and lower temperature. During storage at 4°C, complete inhibition was found for up to 30 days for the 0.03% HBA treatment, and 50 days for the 0.06 and 0.10% HBA treatments, resulting in 1.0 to 1.8 log CFU/cm^2 lower pathogen counts compared to the control by the end of storage (Figure 7.2; Appendix Table 15). This finding agreed with that of Larson et al. (1996) who indicated that the inhibitory effect of hops extract was generally increased at low temperature. During storage at 10°C, complete inhibitory effects were shown in 0.03 and 0.06% HBA treated samples for up to 20 days, and in the 0.10% HBA treatment until 28 days, although final pathogen numbers at 48 days of 10°C storage ranged between 5.7 and 7.5 log CFU/cm^2 (Figure 7.2; Appendix Table 16).

Modeling of *L. monocytogenes* counts performed by DMFIT showed that the average lag phase duration and average calculated growth rates, from the Baranyi and Roberts mathematical model, of the pathogen on untreated control samples were 17.36 and 9.17 days and 0.31 and 0.50 log CFU/cm^2/day at 4 and 10°C, respectively (Table 7.1). *L. monocytogenes* on samples dipped in DW had lag phase durations of 22.54 and 8.48 days at 4 or 10°C, respectively, and showed a faster growth rate than on untreated control samples at the two storage temperatures (Table 7.1). One possible explanation for this phenomenon may be that dipping in DW may allow moisture to remain on the frankfurter surface and this moisture may encourage pathogen growth at the frankfurter-package interface (Byelashov et al., 2008; Samelis et al., 2002a). The duration of lag phase on samples dipped in HBA solutions (0.03 to 0.10%) were in the range 31.34 to 41.92 and 18.97 to 22.57 days at 4 or 10°C, respectively (Table 7.1), indicating that pathogen growth initiation was delayed for about 30 days at 4°C and 20 days at 10°C on samples treated with HBA solutions. The Y_0 values were lower in all HBA-treated samples compared to that of the control or DW. The average growth rate of *L. monocytogenes* decreased with increasing concentrations of HBA solutions (0.03 to 0.10%) during storage at 4 and 10°C, and the lowest average pathogen growth rate was observed on samples dipped in 0.10% HBA (Table 7.1). Counts on frankfurters dipped in 0.06 or 0.10% HBA and stored at 4°C and 0.10% HBA at 10°C did not

reach the stationary phase by the end of the storage.

Hops beta acids are reported to affect bacterial cells by causing leakage in the cytoplasmic membrane, which in turn inhibits the active transport of sugar and amino acids (Teuber and Schmalreck, 1973). The possible reason for the resistance of Gram-negative bacteria to hops beta acids may be the lipopolysaccharide-containing outer membrane, because lupulone and humulone of hops beta acids are inactivated by serum phosphatides (Sacks and Humphreys, 1951). Only limited numbers of studies have evaluated the antilisterial activity of HBA in food. As far as we know, the only study, reported by Larson et al. (1996), showed that hop extracts were ineffective against *L. monocytogenes* in full-fat Camembert cheese and whole fat milk, and indicated that HBA could be used against *L. monocytogenes* in low fat foods. In contrast to Larson et al. (1996), the results of this study suggested that HBA could be good antilisterial agents for use on the skin surface of frankfurters even with a high fat content. The observed discrepancy regarding the effectiveness of HBA could be explained by the differences in tested foods and methods used in the two studies. Specifically, in the present study, HBA was used as a post-processing dipping treatment on the skin surface of frankfurters, instead of as ingredients in cheese or whole milk.

7.4. CONCLUSIONS

Results of the present study indicated that HBA exhibited antilisterial effects on vacuum packaged frankfurters stored at 4 and 10°C, using post-processing surface dipping treatments. The results also confirmed that 0.06% of HBA (4.4 mg/kg, which is allowed by USDA-FSIS) used on RTE meats may be beneficial for *L. monocytogenes* control. Future studies are needed to determine whether sensory qualities of products are adversely affected by such treatments and whether their application is economically feasible.

Table 7.1. Growth kinetics of *L. monocytogenes* on the surface of untreated (control) frankfurters, and frankfurters dipped (2 min) in distilled water or hops beta acids (HBA), then vacuum packaged and stored for 90 days at 4°C or 48 days at 10°C

Storage temperature (°C)	Treatment	Lag phase duration (days)	Growth rate (log CFU/cm^2/day)	Y_0 (log CFU/cm^2)[a]	Y_{end} (log CFU/cm^2)[b]	R^2
4	Control	17.36	0.31	1.81	7.74	0.99
	Distilled Water	22.54	0.46	1.10	7.57	0.99
	HBA 0.03%	31.34	0.14	0.31	6.41	0.96
	HBA 0.06%	40.69	0.13	0.25	--[c]	0.97
	HBA 0.10%	41.92	0.12	0.26	--[c]	0.96
10	Control	9.17	0.50	1.89	7.85	0.99
	Distilled Water	8.48	0.58	1.03	7.95	0.99
	HBA 0.03%	18.97	0.40	0.42	7.60	0.97
	HBA 0.06%	21.52	0.39	0.21	6.11	0.91
	HBA 0.10%	22.57	0.23	0.24	--[c]	0.94

[a] Lower asymptote estimated by the Baranyi model (Baranyi and Roberts, 1994).

[b] Upper asymptote estimated by the Baranyi model (Baranyi and Roberts, 1994).

[c] Stationary phase was not reached.

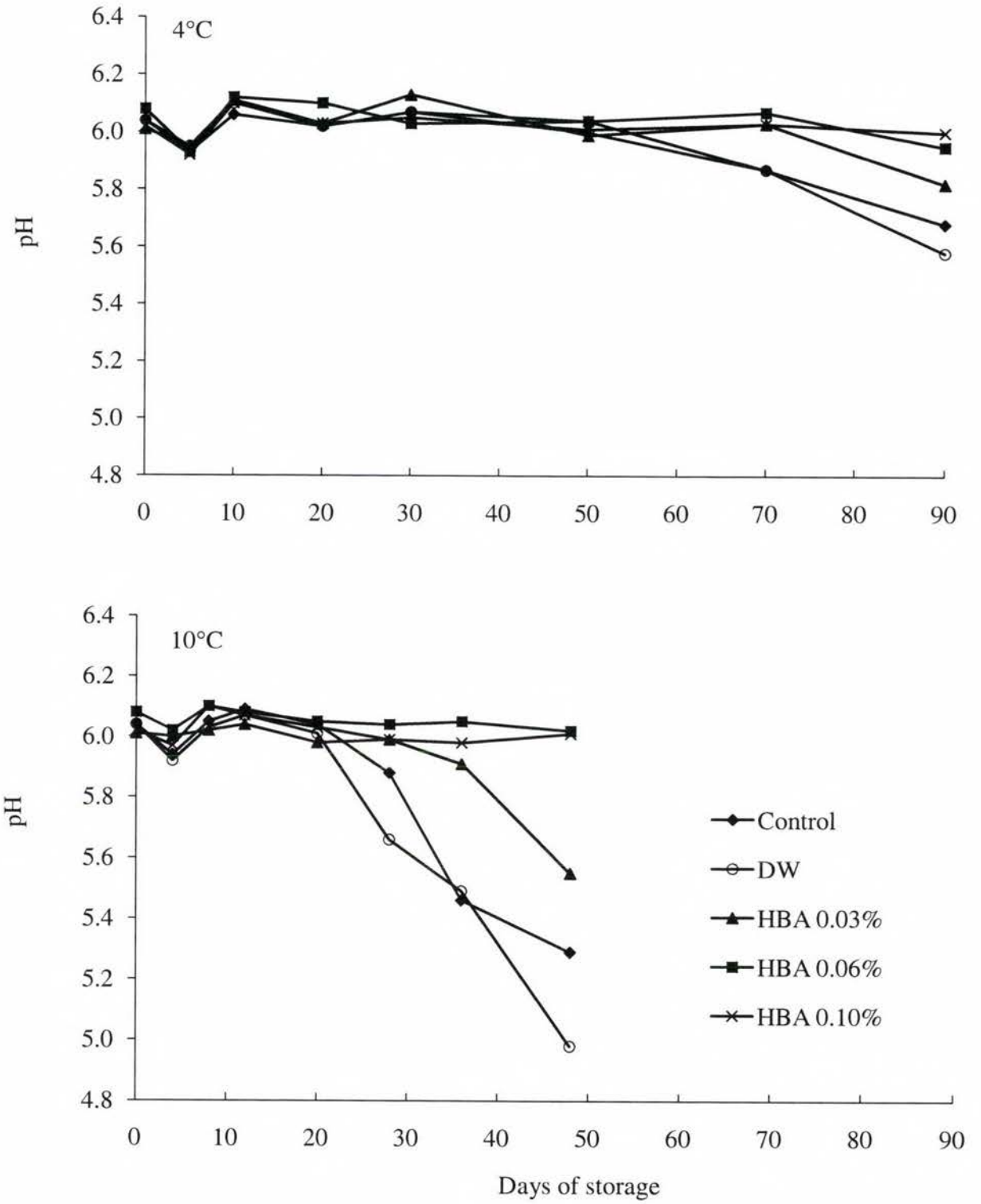


Figure 7.1 (Appendix Table 14). Mean pH values of untreated (control) frankfurters, and frankfurters dipped (2 min) in distilled water (DW) or hops beta acids (HBA) and stored for 90 or 48 days under vacuum at 4 or 10°C, respectively.

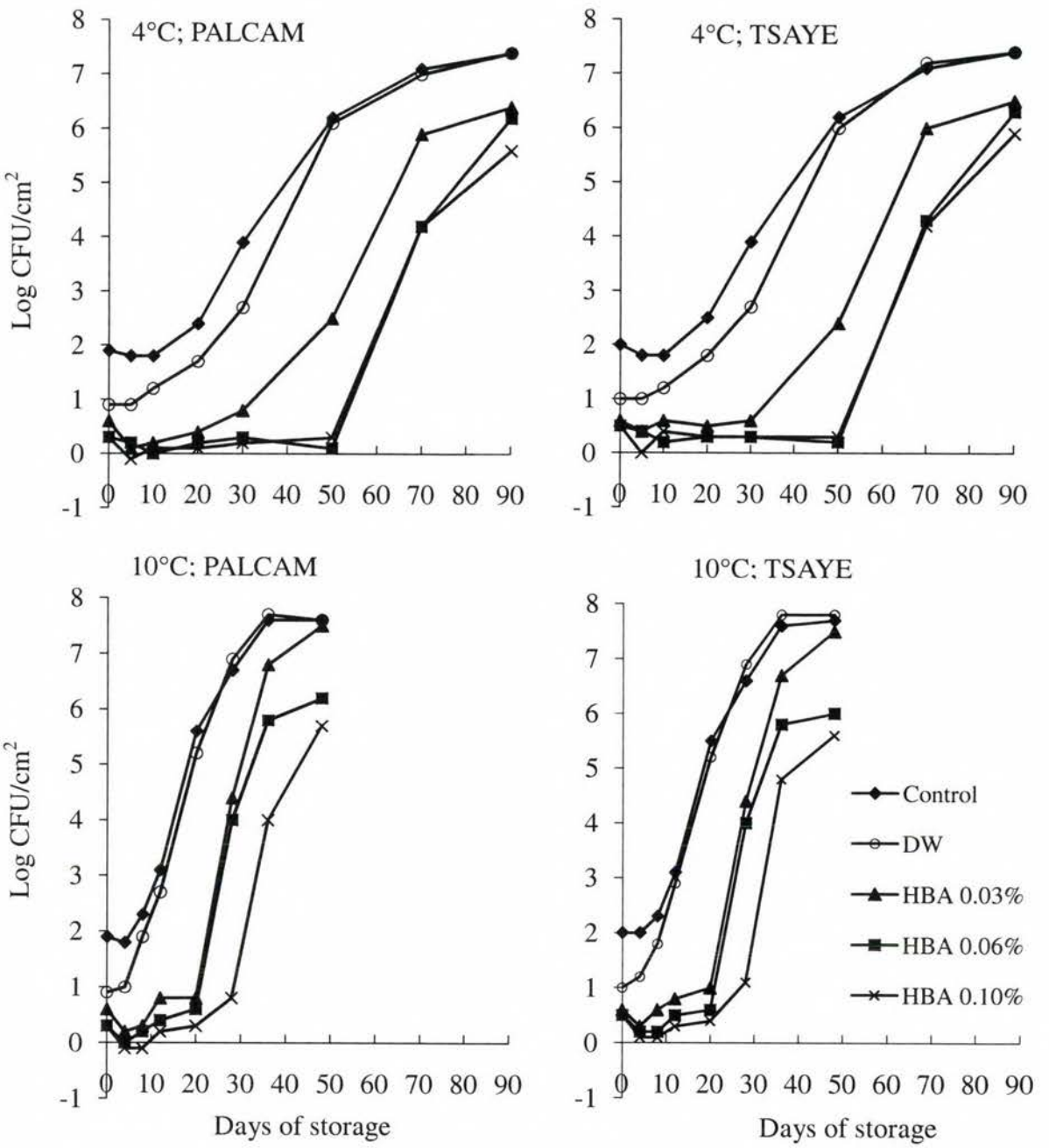


Figure 7.2 (Appendix Table 15 and 16). Mean (log CFU/cm^2) *L. monocytogenes* (PALCAM agar) and total microbial (TSAYE) populations on the surface of untreated (control) frankfurters, and frankfurters dipped (2 min) in distilled water (DW) or hops beta acids (HBA) during storage in vacuum packages at 4°C for 90 days or 10°C for 48 days.

CHAPTER 8

ANTILISTERIAL ACTIVITIES OF SALAD DRESSINGS, WITHOUT OR WITH PRIOR MICROWAVE OVEN HEATING, ON FRANKFURTERS DURING SIMULATED HOME STORAGE

ABSTRACT

This study evaluated the antilisterial effects of salad dressings, as well as oils mixed with lemon juice or vinegar, on frankfurters during simulated home storage, without or with prior microwave oven heating. Frankfurters were inoculated (2.4 ± 0.1 log CFU/cm^2) with *Listeria monocytogenes* (10-strain mixture) and stored aerobically in bags at 7°C. At 0, 7 and 14 days of storage, frankfurters were immersed (5 or 20 min, 25 ± 2°C) in sunflower oil plus lemon juice or vinegar, extra virgin olive oil plus lemon juice or vinegar, or salad dressings (i.e., Vinaigrette, Ranch, Thousand island, and Caesar), or distilled water (DW), without or with prior microwave oven heating (1100 Watts, 2450 MHz, high power) for 30 s. Samples were

analyzed for microbial growth during storage, and survivors following application of treatments, on tryptic soy agar plus 0.6% yeast extract and PALCAM agar. Immersion in salad dressings and in the combinations of oils with lemon juice or vinegar caused reductions ($P < 0.05$) in *L. monocytogenes* compared to dipping in DW. Reductions increased with previous product storage, from 0.5 to 0.9 (day-0) to 1.2 to 2.1 (day-14) log CFU/cm^2, as levels of contamination also increased. Reductions of pathogen counts by each treatment increased ($P < 0.05$) when applied following exposure to microwave oven heating; ranging from 1.2 to 1.9 (day-0) to 2.2 to 3.3 (day-14) log CFU/cm^2. Reductions were not ($P \geq 0.05$) different between 5 and 20 min of immersion in most treatments. In general, the reduction effects of salad dressings decreased in the order of sunflower or extra virgin olive oil plus vinegar ≥ sunflower or extra virgin olive oil plus lemon juice > Caesar ≥ Thousand island ≥ Ranch ≥ Vinaigrette. Results of this study indicated that salad dressings and oils with lemon juice or vinegar may contribute to control of *L. monocytogenes* on ready-to-eat meat products in the home environment, especially when these products are treated and used in salads.

8.1. INTRODUCTION

Listeria monocytogenes is a psychrotrophic Gram-positive bacterium, which exists in a wide variety of foods such as dairy products, meat and poultry products, vegetables and seafood, and is difficult to control because of its ability to grow at refrigerated temperatures

(Ryser and March, 2007). Foodborne illness originating in private home environments have been reported three times more frequently than in commercial cafeterias (Borneff et al., 1988). Improper temperature control, poor cleanliness and inappropriate refrigerator management have been identified as critical factors in foodborne listeriosis (Kennedy et al., 2005; Yang et al., 2006; Kilonzo-Nthenge et al., 2008). Thus, it is important to develop appropriate risk management strategies to control *L. monocytogenes* in the home environment.

Frankfurters, a ready-to-eat (RTE) meat product popular among consumers, were implicated in a multistate outbreak of listeriosis during 1998-1999 in the United States (CDC 1998; 1999). The addition of antimicrobial compounds to product formulations (Samelis et al., 2002a; Barmpalia et al., 2005) and post-processing dipping (Samelis et al., 2001; Barmpalia et al., 2004; Geornaras et al., 2005) or spraying (Byelashov et al., 2008) treatments with antimicrobial agents have been demonstrated to be effective for control of *L. monocytogenes* on frankfurters and other RTE meat products. However, most antimicrobial agents are synthetic chemical compounds and are not readily accepted by consumers (Sofos et al., 1998; Solomakos et al., 2008). Thus, there is increasing interest by consumers in using natural antimicrobial systems to maintain food safety in their home environment.

In the United States, commercially produced salad dressings are widely used and

maintain a good safety record (Smittle, 2000). Vinegar or lemon juice may be used alone, or as a mixture, as natural flavoring and acidifying liquids in some vegetable salads (Sengun and Karapinar, 2005) or in home-made egg mayonnaise (Lock and Board, 1995). Food oils of plant origin are used widely in the processing of foods and in homemade dishes (Medina et al., 2007). Mixtures of oil with vinegar are used as ingredients in prepared foods (Rodrigo et al., 1999). In Greece, olive oil with oregano and lemon juice is used as a sauce on cooked fish (Tassou et al., 1995) and vegetable salads. These salad dressings may provide a harsh environment for foodborne pathogens such as *Salmonella*, *Escherichia coli* O157:H7, *L. monocytogenes*, or *Staphylococcus aureus* to survive due to the presence of acetic or citric acids as low pH ingredients, salt, and preservatives such as sorbic and/or benzoic acid (Beuchat et al., 2006). Antimicrobial effects of salad dressings, including Ranch, Thousand Island, olive oil, lemon juice and vinegar has been well documented (Weagant et al., 1994; Entani et al., 1998; Castillo et al., 2000; Ciafardini and Zullo, 2002; Vijayakumar and Wolf-Hall, 2002a; 2002b; Beuchat et al., 2006). This leads to the hypothesis that exposing foods to salad dressings prior to consumption may control transmission of foodborne pathogens.

According to Sengun and Karapinar (2004; 2005) and Chang and Fang (2007), lemon juice or vinegar alone or in a mixture caused a significant reduction of *Salmonella*

Typhimurium on rocket leaves, carrots and spring onions, and removed *E. coli* O157:H7 on iceberg lettuce. Karapinar and Gonul (1992) reported a 5 log CFU/g reduction of *Yersinia enterocolitica* on parsley leaves dipped in 40% vinegar, while Kisla (2007) showed elimination of *Salmonella* Typhimurium in stuffed mussels immersed in fresh lemon juice or lemon dressing. Egg or milk mayonnaise prepared with virgin olive oil and lemon juice decreased *Salmonella* Enteritidis and *L. monocytogenes* populations, respectively, to below the detection limit after 30 min of exposure (Medina et al., 2007). The results of the above studies suggested that salad dressings such as Ranch, Thousand island, lemon juice, vinegar, and olive oil, used alone or as mixtures, may be considered as potential antimicrobial agents for use by consumers to reduce the risk of foodborne illness in the home environment. However, the above studies were limited to vegetables or seafood and there is no study investigating their antimicrobial effects on RTE meat products.

Currently, use of microwave heating to cook and reheat foods has become remarkably popular in food preparation at home (Fung and Cunningham, 1980; Giese, 1992). The U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) reported that over 90% of homes in America have at least one microwave oven (USDA-FSIS, 2008d) due to its speed of heating and convenience (Heddleson and Doores, 1994). The antilisterial activity of microwave oven heating (Fung and Cunningham, 1980) has been described in

milk (Choi et al., 1993), catfish fillets (Huang et al., 1993), lamb and quail meat (Dasdag et al., 1995), and beef frankfurters (Huang and Sites, 2007).

The "multiple hurdle" concept is commonly applied for control of foodborne pathogens on RTE meat products, including the combination of heat and acid (Luchansky et al., 2006). However, there is no information verifying the antilisterial effects of microwave heating followed by salad dressing treatments. Such information would be useful to consumers to potentially reduce the risk of foodborne illness due to *L. monocytogenes* in the home environment. Thus, the objective of the present study was to evaluate the antilisterial effects of salad dressings and oils with lemon juice or vinegar, without or with prior microwave oven heating, on frankfurters during simulated home storage at 7°C for 14 days.

8.2. MATERIALS AND METHODS

8.2.1. Preparation of frankfurters

Frankfurters were prepared with 60% fresh pork (pork shoulder, 70 to 72% lean) and 40% fresh beef (beef chuck, 76 to 78% lean). The formulation (Samelis et al., 2001) included (%, wt/wt): pork (49), beef (33), ice (10), sodium chloride (2), dextrose (2), dry mustard (0.9), corn syrup solids (2), polyphosphate (0.4; sodium tripolyphosphate and sodium hexametaphosphate, Heller Inc., Bedford Park, IL), 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). Spices and seasonings were purchased from AC Legg Co. (Birmingham, AL). The ingredients of each batch were emulsified in a vacuum (0.5 par) bowl chopper, extruded into 22-mm peelable cellulose casings (Nojax, Viskase Co., Inc., Darien, IL), smoked, cooked and peeled as described by Byelashov et al. (2008). Frankfurters were weighed before and after cooking to determine the cooking yield.

8.2.2. Preparation of inocula and inoculation of frankfurters

The 10-strain mixture of *L. monocytogenes* used in this study included strains NA-1 (serotype 3b, pork sausage isolate); N-7150 (serotype 3a, meat isolate); 558 (serotype ½, pork meat isolate); N1-227 (serotype 4b), R2-500 (serotype 4b), R2-501 (serotype 4b), and R2-764 (serotype 4b), food isolates; N1-225 (serotype 4b) and R2-763 (serotype 4b), both clinical isolates; R2-765 (serotype 4b, environmental isolate). Strains N1-225, N1-227, R2-500, R2-501, R2-763, R2-764 and R2-765 were kindly provided by Dr. Martin Wiedmann (Cornell University, Ithaca, NY; Fugett et al., 2006). The strains were activated and subcultured according to procedures described by Lianou et al. (2007a; 2007b). The cultures of each strain were centrifuged, washed and resuspended in 10 ml of frankfurter extract as described by Byelashov et al. (2008). Cultures of individual strains suspended in frankfurter extract (pH 6.02 ± 0.04) were habituated at 7°C for 3 days (Lianou et al., 2007a; 2007b) and then combined and serially diluted in freshly prepared frankfurter extract to yield

a target inoculum level of 2 to 3 log CFU/cm^2 on each frankfurter.

Frankfurters (surface area of 56 cm^2) were inoculated with the 10-strain mixture of *L. monocytogenes* by spreading 0.25 ml of the inoculum over the entire surface with a sterile bent glass rod (Geornaras et al., 2005). After 15 min (5°C), the inoculated samples were placed into zip-top type bags (6 per bag, Zip Vak 15.2 × 20.3 cm, nylon/EVA copolymer, Winpak Winnipeg, MB, Canada), vacuum packaged (Hollymatic Corp., Countryside, IL), and then opened to remove two frankfurters for day-0 testing. The bags with the four remaining frankfurters were reclosed (zip-top) and stored at 7°C for 14 days. This process simulated opening of a vacuum packaged product by a consumer, removing some of the frankfurters for consumption, reclosing the bag and storing the rest in a home refrigerator for later consumption.

8.2.3. Frankfurters treatments

At 0, 7 and 14 days of storage, two frankfurters from each bag were immersed for 5 or 20 min (25 ± 2°C) in Whirl-Pak bags (Nasco, Modesto, CA) containing one of the following treatments: (i) no treatment (control); (ii) sterile distilled water (DW, 20 ml); (iii) sunflower oil (18 ml, Kroger®, Kroger Co., Cincinnati, OH) mixed with lemon juice (2 ml, Kroger®) (pH 3.50); (iv) sunflower oil (18 ml) mixed with vinegar (2 ml, Private Selection®, Inter-American products, Inc. Cincinnati, OH) (pH 2.50); (v) extra virgin olive oil (18 ml,

Star®, Star Fine Foods, Fresno, CA) mixed with lemon juice (2 ml) (pH 3.50); (vi) extra virgin olive oil (18 ml) mixed with vinegar (2 ml) (pH 2.50), and each of four commercial salad dressings: (vii) Vinaigrette (20 ml, pH 3.30), (viii) Ranch (20 ml, pH 3.34), (ix) Thousand island (20 ml, pH 3.44) and (x) Caesar (20 ml, pH 3.41, Kraft®, Kraft Foods Global Inc., Northfield, IL), without or with 30 s prior microwave oven (Amana, model Radarange AMC5143AAW, Newton, IA) heating (1100 Watts, 2450 MHz, high power). For the microwave oven treatments, two frankfurters were immersed in 250 ml DW in sterile bowls (346 cm^3). All immersion treatments were conducted within 5 to10 s following the 30 s microwave treatment.

8.2.4. Microbiological, chemical and physical analyses of samples

After treatment with salad dressings, the two frankfurters were transferred into a Whirl-Pak bag containing 50 ml of maximum recovery diluent (MRD; 0.85% NaCl and 0.1% peptone), shaken for 30 s, and plated on tryptic soy agar (Difco, Becton Dickinson, Sparks, MD) supplemented with 0.6% yeast extract (Acumedia, Lansing, MI; TSAYE) and PALCAM agar (Difco, Becton Dickinson) for enumeration of total microbial populations and *L. monocytogenes*, respectively (Byelashov et al., 2008). Colonies were counted manually after incubation of plates at 25°C for 72 h (TSAYE) and 30°C for 48 h (PALCAM). The detection limit of the plating method was -0.4 log CFU/cm^2. The pH and water activity

(day-0) of each sample was measured using a digital pH meter with a glass electrode (Denver Instruments, Arvada, CO) and an AquaLab (model series 3, Decagon Devices Inc., Pullman, WA) water activity meter, respectively (Lianou et al., 2007a; 2007b).

8.2.5. Statistical analysis

The study was repeated twice, and each time three samples for each treatment were analyzed at each storage period. The pH and microbiological data (log CFU/cm^2) were analyzed using the Mixed Procedure of SAS® version 9.1 (SAS Institute, Cary, NC). For each analysis day, independent variables included immersion treatments, immersion time, and microwave treatment (without or with 30 s prior microwave heating) and interactions of immersion treatments × immersion time, immersion treatments × microwave treatment, and immersion treatments × immersion time × microwave treatment. The interaction of immersion treatments × immersion time and immersion treatments × immersion time × microwave treatment were not significant ($P \geq 0.05$), while the interaction of immersion treatments × microwave treatment were significant ($P < 0.05$), which indicated that the antilisterial effects on frankfurters depended on different salad dressing treatments and without or with prior microwave heating. Means and standard deviations for pH and microbiological data were calculated, and the mean differences were separated with the least significant difference procedure at the significance level of α=0.05.

8.3. RESULTS AND DISCUSSION

8.3.1. Chemical and physical properties of frankfurters

The cooking yield of the two replicates of frankfurters was 91.5 ± 1.2%. The water activity and pH of the frankfurters after inoculation was 0.980 ± 0.002 and 6.06 ± 0.12, respectively. The pH values of frankfurters were affected ($P < 0.05$) by immersing in salad dressings due to the acid ingredients. On day-0, without prior microwave heating, immersion in oils with lemon juice or vinegar for 5 min reduced the pH of samples, compared to the control (pH 6.06) by 0.17 to 0.18 ($P \geq 0.05$) and 0.25 to 0.27 ($P < 0.05$), respectively, while greater reductions ($P < 0.05$) of 0.29 to 0.43 were obtained for samples dipped in the four commercial salad dressings (Table 8.1). Similarly, reduced pH values were found in samples treated by most salad dressings for 20 min, and the lowest ($P < 0.05$) pH (5.46) was observed in frankfurters treated with Vinaigrette (Table 8.1). The pH values of the samples did not show large variation ($P \geq 0.05$) when exposed to microwave heating and between storage times. Beuchat et al. (2006) reported that the main factor in salad dressings causing death to pathogenic bacteria is the low pH.

8.3.2. Growth of *L. monocytogenes* on frankfurters during aerobic storage at 7°C

Initial counts of *L. monocytogenes* and total microbial populations on inoculated control frankfurters were 2.4 ± 0.1 and 2.5 ± 0.1 log CFU/cm^2, respectively. *L. monocytogenes*

counts reached approximately 6.0 log CFU/cm^2 after 14 days of aerobic storage at 7°C (Figure 8.1 and 8.2; Appendix Tables 17 and 18). Counts on TSAYE (Table 8.2) were similar ($P \geq 0.05$) to those on PALCAM agar for all treatments, indicating that the majority of colonies found on TSAYE were *L. monocytogenes* and that most microbial reductions caused by salad dressings or DW immersion treatments was due to physical removal or death rather than sublethal cell injury (Byelashov et al., 2008).

8.3.3. Antilisterial activities of salad dressings without prior microwave heating

Treatment of inoculated frankfurters with all salad dressings caused immediate reductions ($P < 0.05$) of pathogen populations. On day-0, immersion in oils with lemon juice or vinegar for 5 or 20 min reduced pathogen numbers by 0.7 to 0.9 log CFU/cm^2, which was higher ($P < 0.05$) than that caused by immersion in DW (0.5 to 0.6 log CFU/cm^2 reduction) (Figure 8.1; Appendix Table 17). Sengun et al. (2005) reported that lemon juice or vinegar immediately reduced *Salmonella* Typhimurium on whole rocket leaves or shredded spring onions by 1-3 log CFU/g. In the present study, immersion in Vinaigrette, Ranch, Thousand Island or Caesar salad dressings for 5 or 20 min reduced pathogen numbers by 0.5 to 0.8 log CFU/cm^2, and only Thousand Island caused a reduction ($P < 0.05$, 0.7 CFU/cm^2) compared to DW (Figure 8.1; Appendix Table 17), indicating that the decrease in pathogen counts caused by the commercial salad dressings could be attributed to physical removal of microbial cells.

Immersion of inoculated frankfurters in salad dressings for 20 min did not increase ($P \geq 0.05$) the magnitude of reductions in pathogen counts compared to 5 min of immersion for most treatments; only Ranch salad dressing resulted in a greater ($P < 0.05$) reduction (0.3 log CFU/cm^2) after 20 min of dipping compared to 5 min.

As storage time increased from 0 to 14 days, the contamination level of *L. monocytogenes* on frankfurters increased from 2.4 to 6.0 log CFU/cm^2 (Figures 8.1 and 8.2; Appendix Table 17). Reductions of pathogen numbers due to treatment with each of the salad dressings increased from 0.5 to 0.9 (day-0) to 1.2 to 2.1 (day-14) log CFU/cm^2, which was higher than the DW treatments (0.5 to 1.1 log CFU/cm^2 reduction for frankfurters stored for 0, 7 or 14 days). A possible reason for these results might be that *L. monocytogenes* was in the exponential growth phase (day-7 and day-14) which would make cells more susceptible to acid ingredients of salad dressings compared to when they were in the lag phase of growth (day-0). Specifically, the reduction effect of sunflower oil with lemon juice and of Ranch dressing increased from 0.9 (day-0) to 2.1 (day-14) and 0.5 (day-0) to 1.6 (day-14) log CFU/cm^2, respectively. The maximum lethal effect on products stored for 7 and 14 days was observed in samples treated for 20 min with extra virgin olive oil plus vinegar and sunflower oil plus lemon juice. For these treatments, pathogen numbers were reduced by 1.8 (day-7) and 2.1 (day-14) log CFU/cm^2 compared to control samples, respectively (Figure 8.1;

Appendix Table 17). Reductions in pathogen levels on samples stored for 7 or 14 days were not different ($P \geq 0.05$) among salad dressings exposed for 5 or 20 min.

An increasing selection of types of pourable salad dressings for use in home settings is available for purchase in large containers in recent years (Beuchat et al., 2006). This presents an increased possibility that consumers use more salad dressings to prepare foods. The above results indicate that use of salad dressings may contribute to the safety of foods before consumption in the home environment.

8.3.4. Antilisterial activities of salad dressings with prior microwave heating

As expected, microwave heating for 30 s reduced pathogen numbers by 0.8 to 1.3 log CFU/cm^2 compared to the untreated control (Figure 8.2; Appendix Table 17). The average surface temperature of frankfurters immediately after microwaving was 52°C. Immersion of frankfurters in DW for 5 or 20 min after microwave heating for 30 s caused a further reduction of 0.1 to 0.8 log CFU/cm^2 at all storage times (Figure 8.2; Appendix Table 17). Immersing frankfurters in salad dressings after 30 s of microwave heating caused a reduction ($P < 0.05$) in pathogen counts for most treatments, compared to reductions achieved by treating with DW alone; reduction levels increased from 1.2 to 1.9 on day-0 to 2.2 to 3.3 on day-14 log CFU/cm^2 as the contamination level increased (Figure 8.2; Appendix Table 17). The reductions were higher ($P < 0.05$) than those achieved by salad dressings or microwave

heating (30 s) alone. On day-0, treatment of frankfurters with oils plus lemon juice or vinegar, along with the four commercial salad dressings, reduced pathogen numbers by 1.5 to 1.9 and 1.2 to 1.5 log CFU/cm^2 from 2.4 log CFU/cm^2 (control), respectively. Application of extra virgin olive oil with vinegar for 20 min resulted in the lowest pathogen number (0.5 log CFU/cm^2) surviving on frankfurters (Figure 8.2; Appendix Table 17). On day-7 and day-14, the salad dressing treatments caused reductions of 2.3 to 2.9 and 2.2 to 3.3 log CFU/cm^2, respectively. Maximum reduction was observed on samples immersed in sunflower oil with lemon juice (day-7) and sunflower oil with vinegar (day-14) for 20 min, which reduced counts by 2.9 (day-7) and 3.3 (day-14) log CFU/cm^2 (Figure 8.2; Appendix Table 17). Reductions were not different ($P \geq 0.05$) between 5 and 20 min of exposure for most treatments. Results of this study suggested that the multiple hurdle concept enhances pathogen destruction compared to using a single method. Immersion in salad dressings combined with microwave oven heating increased reduction of *L. monocytogenes* compared to treatments of salad dressing or microwave heating alone.

8.4. CONCLUSIONS

Results indicated that a 30 s microwave oven heating treatment of frankfurters followed by immersion in salad dressings may potentially contribute to control of *L. monocytogenes* on RTE frankfurters in the home environment. Salad dressings tested in this study reduced

pathogen counts by 1.0 to 3.0 log CFU/cm^2 (with prior microwave oven heating for 30 s) depending on the contamination level. The reduction effects of salad dressings decreased in the order of sunflower or extra virgin olive oil plus vinegar $\geq$ sunflower or extra virgin olive oil plus lemon juice > Caesar $\geq$ Thousand island $\geq$ Ranch $\geq$ Vinaigrette. Further studies need to be conducted on more RTE meat products such as diced ham or turkey breast, which are more commonly used in salads.

Table 8.1. Mean pH values of frankfurters inoculated with *Listeria monocytogenes* and stored aerobically at 7°C for 14 days. Samples were untreated (CON) or immersed for 5 or 20 min in distilled water (DW), sunflower oil + lemon juice (SL) or vinegar (SV), extra virgin olive oil + lemon juice (EL) or vinegar (EV), Vinaigrette (V), Ranch (R), Thousand Island (T) or Caesar (C), without or with prior microwave heating for 30 s, on days 0, 7 and 14 of storage

Storage	Dipping Treatment	No microwave		With microwave 30 s	
		5 min	20 min	5 min	20 min
Day 0	CON	6.06 ± 0.12a		----------	
	MW	---------		6.00 ± 0.06a	
	DW	6.03 ± 0.12aX	6.06 ± 0.13aX	5.99 ± 0.07aX	5.98 ± 0.09aX
	SL	5.88 ± 0.20abX	5.89 ± 0.14abX	5.84 ± 0.08abX	5.79 ± 0.08abX
	SV	5.79 ± 0.19bcX	5.77 ± 0.10bcXY	5.77± 0.10bcXY	5.67 ± 0.15bcY
	EL	5.89 ± 0.16abX	5.84 ± 0.03abX	5.83 ± 0.04abX	5.80 ± 0.08abX
	EV	5.81 ± 0.14bcX	5.71 ± 0.20bcX	5.73 ± 0.05bcX	5.71 ± 0.10bcX
	V	5.63 ± 0.10cX	5.46 ± 0.04cY	5.52 ± 0.14cXY	5.47 ± 0.11cY
	R	5.70 ± 0.18cX	5.74 ± 0.10bcX	5.71 ± 0.07bcX	5.63 ± 0.16bcX
	T	5.71 ± 0.05cX	5.67 ± 0.08bcX	5.55 ± 0.08cY	5.57 ± 0.18bcY
	C	5.77 ± 0.08cX	5.69 ± 0.10bcX	5.66 ± 0.12bcX	5.69 ± 0.12bcX
Day 7	CON	5.99 ± 0.07a		----------	
	MW	---------		6.00 ± 0.08a	
	DW	6.01 ± 0.11aX	5.99 ± 0.12aX	6.04 ± 0.09aX	6.07 ± 0.10aX
	SL	5.91 ± 0.19abX	5.91 ± 0.14abX	5.92 ± 0.08abX	5.83 ± 0.13abX
	SV	5.80 ± 0.10bX	5.73 ± 0.11bX	5.84 ± 0.13bX	5.66 ± 0.08bY
	EL	5.88 ± 0.16abX	5.86 ± 0.16abX	5.87 ± 0.09abX	5.89 ± 0.11abX
	EV	5.84 ± 0.09abX	5.68 ± 0.09abY	5.83 ± 0.10bX	5.77 ± 0.08bX
	V	5.66 ± 0.13cX	5.49 ± 0.10cY	5.58 ± 0.13cXY	5.48 ± 0.07cY
	R	5.86 ± 0.07abX	5.77 ± 0.07bY	5.80 ± 0.08bXY	5.78 ± 0.07bY
	T	5.77 ± 0.09bcX	5.74 ± 0.08 bX	5.67± 0.08bcXY	5.63 ± 0.12bY
	C	5.79 ± 0.07bcX	5.75 ± 0.09bX	5.73 ± 0.14bcX	5.74 ± 0.06bX
Day 14	CON	6.01 ± 0.06a		---------	
	MW	----------		6.02 ± 0.07a	
	DW	6.00 ± 0.05aX	6.00 ± 0.08aX	5.99 ± 0.09aX	6.03 ± 0.08aX
	SL	5.93 ± 0.08abX	5.88 ± 0.05abX	5.86 ± 0.10abX	5.85± 0.07abX
	SV	5.75 ± 0.10bX	5.68 ± 0.09bX	5.74 ± 0.09bX	5.66 ± 0.04bX
	EL	5.94 ± 0.05abX	5.88 ± 0.04abX	5.90± 0.13abX	5.84± 0.08abX
	EV	5.79 ± 0.05bX	5.72 ± 0.08bXY	5.77 ± 0.04bX	5.69 ± 0.05bY
	V	5.58 ± 0.05cX	5.51 ± 0.07cX	5.66 ± 0.09bX	5.60 ± 0.10bX
	R	5.74 ± 0.04bX	5.73 ± 0.04bX	5.71 ± 0.19bX	5.72 ± 0.05bX
	T	5.68 ± 0.09bcX	5.61 ± 0.03bcX	5.67 ± 0.11bX	5.60 ± 0.11bX
	C	5.70 ± 0.06bcXY	5.70 ±0.05bcXY	5.78 ± 0.08bX	5.68 ± 0.09bY

a-c: Means within a column that have a common letter, within a storage day, are not different ($P \geq 0.05$).
X-Y: Means within a row that have a common letter, within a storage day, are not different ($P \geq 0.05$).

Table 8.2. Mean (± SD) counts (log CFU/cm^2, n=6) of total microbial flora on frankfurters inoculated and stored aerobically at 7°C for 14 days. Samples were untreated (CON) or immersed for 5 or 20 min in distilled water (DW), sunflower oil + lemon juice (SL) or vinegar (SV), extra virgin olive oil + lemon juice (EL) or vinegar (EV), Vinaigrette (V), Ranch (R), Thousand Island (T) or Caesar (C), without or with prior microwave heating for 30 s, on days 0, 7 and 14 of storage

Storage	Dipping Treatment	No microwave		With microwave 30 s	
		5 min	5 min	5 min	20 min
Day 0	CON	2.5 ± 0.1a		----------	
	MW	---------		1.7 ± 0.1b	
	DW	1.9 ± 0.1bX	1.6 ± 0.1bY	1.6 ± 0.1bY	1.3 ± 0.2bZ
	SL	1.8 ± 0.1bcX	1.0 ± 0.1cdY	1.0 ± 0.1cdY	0.9 ± 0.2deY
	SV	1.7 ± 0.1cX	0.9 ± 0.2cY	0.9 ± 0.2cY	0.8 ± 0.3dY
	EL	1.7 ± 0.2cX	0.9 ± 0.2cY	0.9 ± 0.2cY	0.9 ± 0.2deY
	EV	1.7 ± 0.1cX	1.0 ± 0.1cdZ	1.0 ± 0.1cdZ	0.8 ± 0.3dz
	V	1.8 ± 0.1bcX	1.1 ± 0.1deY	1.1 ± 0.1deY	1.0 ± 0.1efY
	R	1.9 ± 0.2bX	1.2 ± 0.1eZ	1.2 ± 0.1eZ	1.2 ± 0.2cfY
	T	1.6 ± 0.2cX	1.2 ± 0.1eY	1.2 ± 0.1eY	1.1 ± 0.1fY
	C	1.8 ± 0.1bcX	1.3 ± 0.2eY	1.3 ± 0.2eY	1.1 ± 0.2fZ
Day 7	CON	4.1 ± 0.6a		----------	
	MW	---------		2.9 ± 0.5b	
	DW	3.1 ± 0.8bX	2.4 ± 0.6bY	2.4 ± 0.6bY	2.4 ± 0.6bY
	SL	2.3 ± 0.4cX	1.6 ± 0.3cY	1.6 ± 0.3cY	1.4 ± 0.4cY
	SV	2.6 ± 0.4bcX	1.7 ± 0.3cY	1.7 ± 0.3cY	1.4 ± 0.4cY
	EL	2.8 ± 0.7bcX	1.6 ± 0.2cY	1.6 ± 0.2cY	1.6 ± 0.6cY
	EV	2.6 ± 0.7bcX	1.4 ± 0.2cY	1.4 ± 0.2cY	1.5 ± 0.5cY
	V	2.8 ± 0.9bcX	1.5 ± 0.2cY	1.5 ± 0.2cY	1.5 ± 0.2cY
	R	2.9 ± 0.4bX	1.8 ± 0.3cY	1.8 ± 0.3cY	1.6 ± 0.4cY
	T	2.7 ± 0.5bcX	1.8 ± 0.4bcY	1.8 ± 0.4bcY	1.6 ± 0.2cY
	C	2.5 ± 0.5bcX	1.8 ± 0.7bcY	1.8 ± 0.7bcY	1.7 ± 0.7cY
Day 14	CON	6.1 ± 0.5a		---------	
	MW	----------		4.9 ± 0.6b	
	DW	5.3 ± 0.5bX	4.4 ± 0.7bdY	4.4 ± 0.7bdY	4.4 ± 0.9bY
	SL	4.5 ± 0.5bcX	3.3 ± 0.7ceY	3.3 ± 0.7ceY	3.3 ± 0.5cdY
	SV	4.6 ± 0.4bcX	3.2 ± 1.1cY	3.2 ± 1.1cY	2.9 ± 0.6dY
	EL	4.5 ± 0.5bcX	3.6 ± 0.6cdY	3.6 ± 0.6cdY	3.3 ± 1.0cdY
	EV	4.2 ± 0.8cX	3.6 ± 0.5cdX	3.6 ± 0.5cdX	3.2 ± 0.9cdXY
	V	4.7 ± 0.5bcX	4.0 ± 0.6deX	4.0 ± 0.6deX	3.6 ± 0.3cdXY
	R	4.5 ± 0.7bcX	3.6 ± 0.6cY	3.6 ± 0.6cY	3.3 ± 1.0cdY
	T	4.7 ± 0.4bcX	3.4 ± 0.7cY	3.4 ± 0.7cY	3.7 ± 0.3bcY
	C	4.4 ± 0.8cX	3.5 ± 1.0cY	3.6 ± 1.0cY	3.6 ± 0.6bcdY

a-c: Means within a column that have a common letter, within a day of storage, are not different ($P \geq 0.05$).
X-Z: Means within a row that have a common letter, within a day of storage, are not different ($P \geq 0.05$).

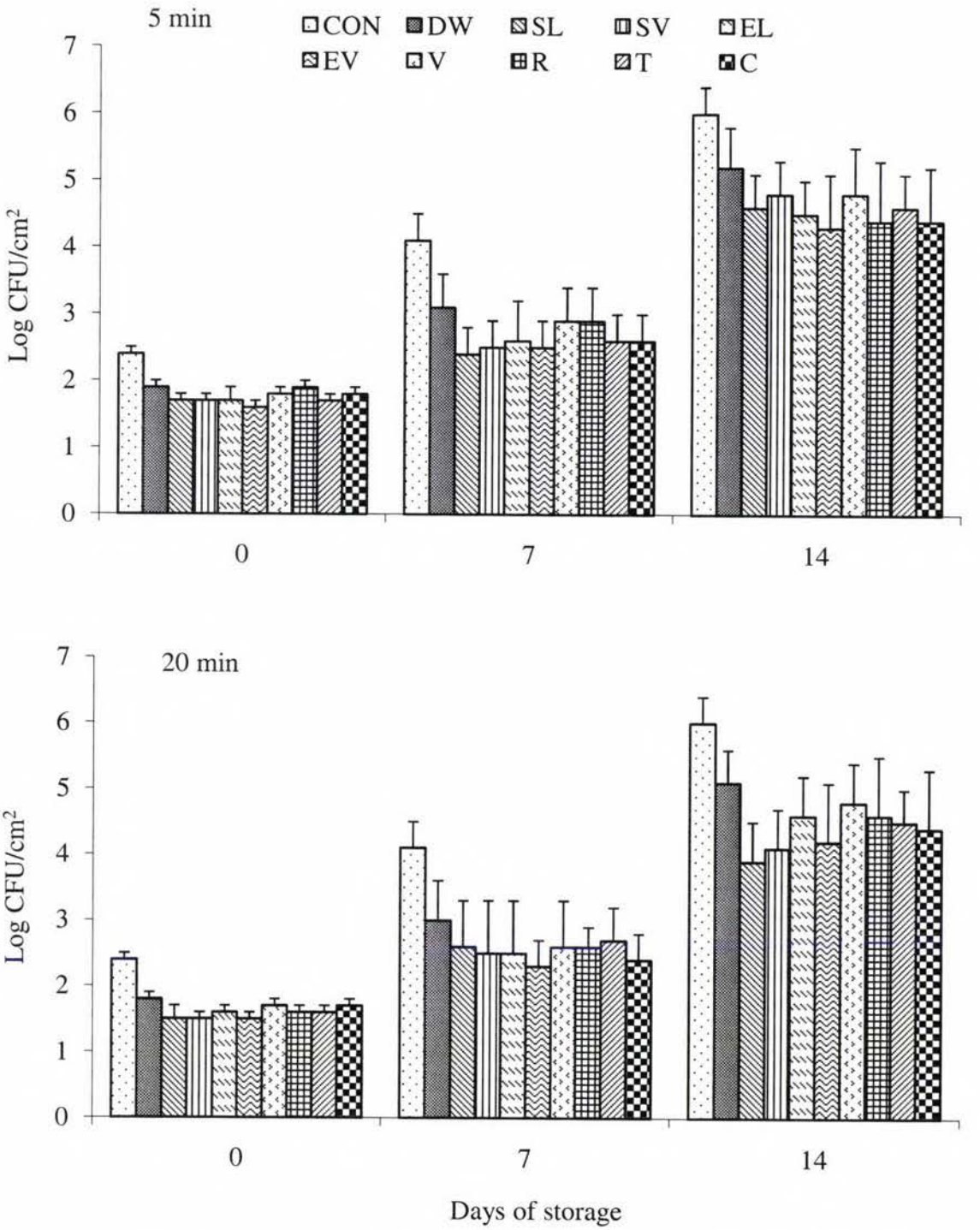


Figure 8.1(Appendix Table 17). Mean populations (log CFU/cm^2) of *Listeria monocytogenes* on frankfurters inoculated and stored aerobically at 7°C for 14 days. Samples were left untreated (CON) or immersed for 5 or 20 min in distilled water (DW), sunflower oil + lemon juice (SL) or vinegar (SV), extra virgin olive oil + lemon juice (EL) or vinegar (EV), Vinaigrette (V), Ranch (R), Thousand Island (T) or Caesar (C) on days 0, 7 and 14 of storage.

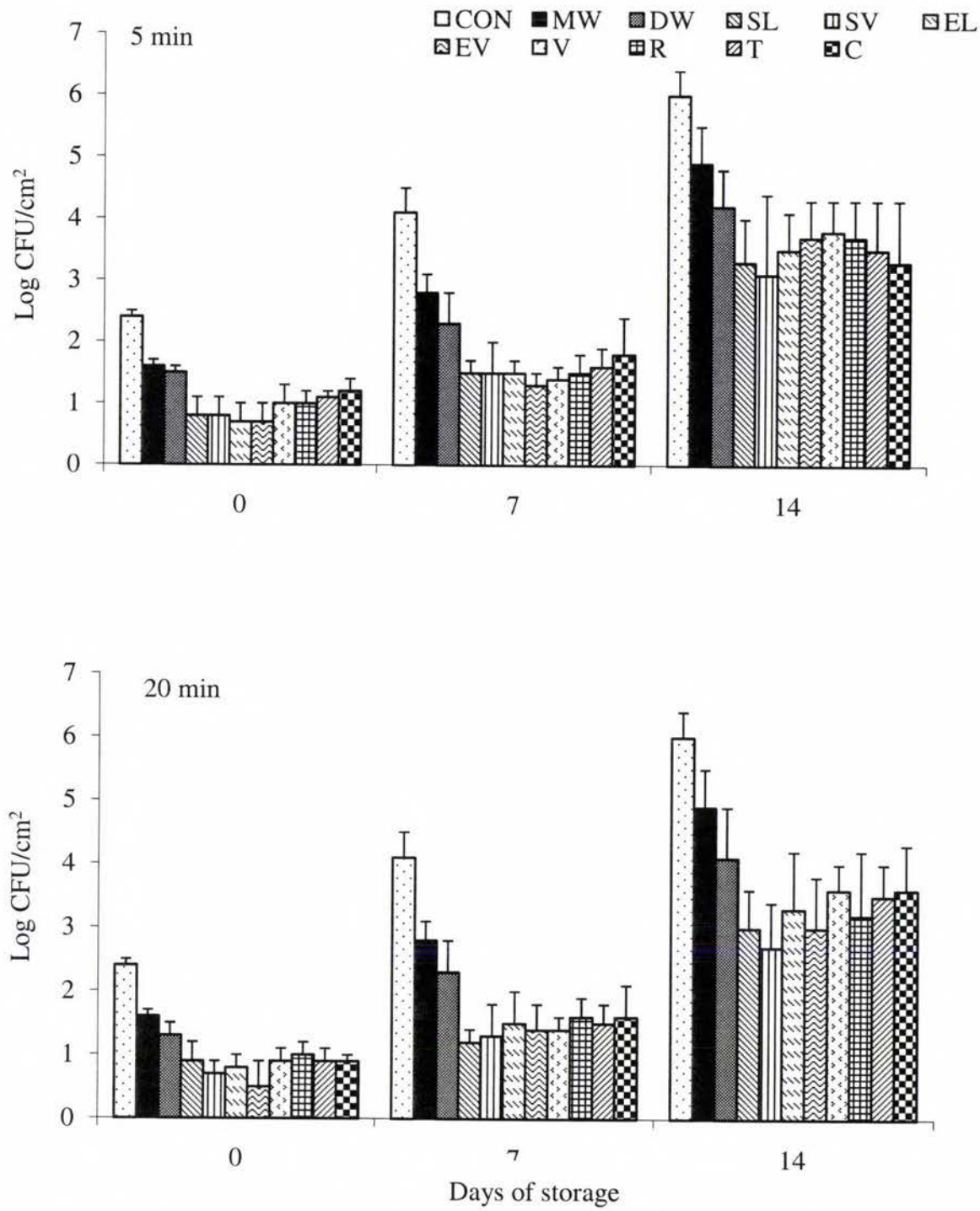


Figure 8.2 (Appendix Table 17). Mean populations (log CFU/cm^2) of *Listeria monocytogenes* on frankfurters inoculated and stored aerobically at 7°C for 14 days. Samples were left untreated (CON) or immersed for 5 or 20 min in distilled water (DW), sunflower oil + lemon juice (SL) or vinegar (SV), extra virgin olive oil + lemon juice (EL) or vinegar (EV), Vinaigrette (V), Ranch (R), Thousand Island (T) or Caesar (C), with prior microwave (MW) heating for 30 s, on days 0, 7 and 14 of storage.

CHAPTER 9

EVALUATION OF SALAD DRESSINGS FOR CONTROL OF *LISTERIA MONOCYTOGENES* ON DICED HAM AND TURKEY BREAST WITHOUT OR WITH PRIOR MICROWAVE OVEN HEATING

ABSTRACT

This study evaluated the antilisterial effects of salad dressings on diced ham and turkey breast during simulated home storage, without or with prior microwave oven heating of the delicatessen meats. Cured ham and uncured turkey breast were sliced and diced (1.5×1.5×1.5 cm), inoculated (2.1±0.1 log CFU/cm^2) with *Listeria monocytogenes* (10-strain mixture), and stored (7°C) aerobically in bags to simulate home storage. During storage, ham (days-0, 7, 14) and turkey breast (days-0, 5, 9) samples were left untreated or immersed (5 or 10 min, 25±2°C) in sunflower oil+vinegar, extra virgin olive oil+lemon juice, commercially-available

salad dressings (i.e., Vinaigrette, and Thousand Island) or distilled water (DW), each without or with prior microwave oven heating (MW, 1100 Watts, 2450 MHz, high power, 30 or 45 s). Similar ($P \geq 0.05$) *L. monocytogenes* reductions were obtained for ham and turkey breast samples when immersed in each of the salad dressing treatments without prior MW treatment, but the reductions obtained were higher than those of DW-treated samples (by 0.3 to 0.8 and 0.2 to 0.6 log CFU/cm^2 for ham and turkey breast, respectively). When the salad dressing treatments were applied following exposure of the samples to MW, the reductions increased from 0.5 to 1.2 or 0.2 to 0.8 (without MW) to 3.4 to 5.5 or 2.9 to 5.7 (with MW, 45 s) log CFU/cm^2 for ham and turkey breast, respectively. Reductions were not ($P \geq 0.05$) different between 5 and 10 min of immersion in most salad dressing treatments. Use of certain salad dressings and microwaving may contribute to the reduction of *L. monocytogenes*, if present, on diced delicatessen meats consumed with salads, especially when consumed by at-risk populations.

9.1. INTRODUCTION

Delicatessen meats have been involved in a number of listeriosis outbreaks in the United States (CDC, 1998; 1999; 2000; 2002; Lianou and Sofos, 2007), and have been identified as the highest predicted relative risk for listeriosis among 23 categories of ready-to-eat foods based on a risk assessment conducted by the U.S. Food and Drug

Administration and the U.S. Department of Agriculture Food Safety and Inspection Service in 2003 (USDHHS-FDA-CFSAN and USDA-FSIS, 2003). Although many foodborne illness outbreaks have been associated with eating outside the home, it has been reported that foodborne illness is three times more likely to originate in consumer homes than in commercial cafeterias (Borneff et al., 1988) due to poor food hygiene practices, improper temperature control and inappropriate refrigerator management (Kennedy et al., 2005; Yang et al., 2006; Kilonzo-Nthenge et al., 2008). *Listeria monocytogenes* is difficult to control in consumer homes because it may be present in a wide variety of foods including vegetables, seafood, dairy products, and meat and poultry products, and because it grows at refrigerated temperatures (Ryser and Marth, 2007). Thus, it is important to develop appropriate risk management strategies for consumers to control *L. monocytogenes* in their homes.

Chemical antimicrobials, such as benzoate, lactate, diacetate, sorbate, acetic acid, and lactic acid, are widely used in food processing (Sofos et al., 1998). In recent years, however, there has been an increasing demand by consumers for the use of natural antimicrobial systems to maintain food safety. Commercial salad dressings, such as vinegar, lemon juice, plant oil, Thousand Island, and Vinaigrette, are widely used in the United States for preparation of vegetable salads (Sengun and Karapinar, 2005), egg mayonnaise

(Smittle, 2000) and cooked fish (Tassou et al., 1995), and have a good safety record, because they contain acetic or citric acids as ingredients (Beuchat et al., 2006). Antimicrobial effects of salad dressings have been documented in vegetables (Sengun and Karapinar, 2004; 2005), egg or milk mayonnaise (Lock and Board, 1995; Medina et al., 2007), and stuffed mussels (Kisla, 2007). This indicates that exposing foods to salad dressings prior to consumption could potentially control microbial contamination, especially with foodborne pathogens.

Microwave ovens are remarkably popular for food preparation in the home (Fung and Cunningham, 1980; Giese, 1992) because of their speed of heating and convenience (Heddleson and Doores, 1994). According to the U.S. Department of Agriculture Food Safety and Inspection Service, more than 90% of homes in America have at least one microwave oven (USDA-FSIS, 2008d). Control of *L. monocytogenes* using microwave heating has been reported in lamb and quail meat (Dasdag et al., 1995) and beef frankfurters (Huang and Sites, 2007).

Diced ham and turkey breast are available for consumers to purchase in the local market and they may be consumed with salads. Since deli meats do require reheating before consumption, products potentially contaminated with *L. monocytogenes* represent a risk for consumers, especially to those individuals with a suppressed immune system. Thus,

the objective of this study was to evaluate inactivation of *L. monocytogenes* by salad dressings when applied to inoculated diced ham or turkey breast, without or with prior microwave oven heating, during simulated home storage of the products at 7°C for 14 (ham) or 9 (turkey breast) days.

9.2. MATERIALS AND METHODS

9.2.1. Product inoculation

Commercially manufactured cured ham and uncured turkey breast were purchased from a local supermarket. The ingredients of the cured ham included water, salt, sugar, sodium phosphate, sodium erythorbate, and sodium nitrite, while the formulation of the uncured turkey breast consisted of turkey breast meat, turkey broth, modified food starch, salt, sugar, sodium phosphate, and flavoring. A 10-strain mixture of *L. monocytogenes* was used for product inoculation, and was prepared according to the procedures described by Lianou et al. (2007a; 2007b). The strains included NA-1 (serotype 3b, pork sausage isolate); N-7150 (serotype 3a, meat isolate); 558 (serotype 1/2, pork meat isolate); N1-227, R2-500 and R2-764 (serotype 4b; food isolates), N1-225, R2-501 and R2-763 (serotype 4b; clinical isolates); and R2-765 (serotype 4b, environmental isolate). Strains N1-225, N1-227, R2-500, R2-501, R2-763, R2-764 and R2-765 were kindly provided by Dr. Martin Wiedmann (Cornell University, Ithaca, NY; Fugett et al., 2006). The mixture of *L.*

monocytogenes strains was serially diluted in freshly prepared ham (Lianou et al., 2007a) or turkey breast (Lianou et al., 2007b) homogenate to a concentration of 4-5 log CFU/ml.

Before inoculation, ham and turkey breast products were sliced (Hobart 2712 12" Semi Automatic Slicer, Hobart Mfg. Co., Troy, OH) and manually diced into pieces of 1.5×1.5×1.5 cm. Batches of 18 pieces of each product were immersed in 200 ml of the diluted inoculum for 5 min, with intermittent shaking. The pieces were then transferred to a tray covered with sterile aluminum foil and placed in a biosafety cabinet for 15 min to allow for cell attachment and drying of the excess liquid inoculum. The inoculated samples were placed into zip-top type vacuum bags (18 pieces per bag, Zip Vak 15.2 × 20.3 cm, nylon/EVA copolymer, Winpak Winnipeg, MB, Canada), vacuum-packaged (Hollymatic Corp., Countryside, IL), and then opened to remove six pieces for day-0 testing. The bags with the remaining 12 pieces were reclosed (zip-top) and stored at 7°C for up to 14 (ham) or 9 days (turkey breast). This procedure simulated opening of a vacuum-packaged product by a consumer, removing some of the pieces for consumption, reclosing the bag and storing the rest in a home refrigerator for consumption on another day.

9.2.2. Product treatments

On days-0, 7 and 14 (ham) or days-0, 5 and 9 (turkey breast) of storage, six pieces of diced ham or turkey breast from each bag were transferred to Whirl-Pak® bags (Nasco,

Modesto, CA) containing one of the following liquid treatments: (i) no treatment (control); (ii) sterile distilled water (DW, 20 ml); (iii) sunflower oil (18 ml, Kroger®, Kroger Co., Cincinnati, OH) mixed with vinegar (2 ml, Private Selection®, Inter-American Products, Inc. Cincinnati, OH) (pH 2.50); (iv) extra virgin olive oil (18 ml, Star®, Star Fine Foods, Fresno, CA) mixed with lemon juice (2 ml, Kroger®) (pH 3.50); and each of two commercial salad dressings: (v) Vinaigrette (20 ml, Kraft®, Kraft Foods Global Inc., Northfield, IL) (pH 3.30), and, (vi) Thousand Island (20 ml, Kraft®) (pH 3.44). Each of the DW or salad dressing treatments were applied for 5 or 10 min (25 ± 2°C), and to product previously exposed or not exposed to 30 or 45 s of prior microwave oven (Amana, model Radarange AMC5143AAW, Newton, IA) heating (1100 Watts, 2450 MHz, high power) treatment. The 45 s microwave heating treatment was not applied to day-0 samples as their contamination levels were low. Microwave oven exposure was applied to batches of six pieces of diced ham or turkey breast immersed in 150 ml of DW in sterile bowls (346 cm^3). All salad dressing immersion treatments were applied to product cubes within 5 to 10 s following the 30 or 45 s microwave treatment.

9.2.3. Microbiological, chemical and physical analyses

After treatment with salad dressings, without or with prior microwave heating, the six product pieces were transferred into a filter bag (24 oz, WhirlPak®) containing 20 ml of

maximum recovery diluent (MRD; 0.85% NaCl and 0.1% peptone). The samples were shaken for 30 s, serially diluted in 0.1% buffered peptone water (Difco, Becton Dickinson, Sparks, MD), and plated on tryptic soy agar (Difco, Becton Dickinson) supplemented with 0.6% yeast extract (Acumedia, Lansing, MI; TSAYE) and on PALCAM agar (Difco, Becton Dickinson) for enumeration of total microbial populations and *L. monocytogenes*, respectively (Byelashov et al., 2008). Colonies were counted manually after incubation of plates at 25°C for 72 h (TSAYE) and 30°C for 48 h (PALCAM agar). The pH and water activity (day-0) of each sample was measured using a digital pH meter with a glass electrode (Denver Instruments, Arvada, CO) and an AquaLab (model series 3, Decagon Devices Inc., Pullman, WA) water activity meter according to the procedures described by Lianou et al. (2007a; 2007b).

9.2.4. Statistical analysis

The experimental unit in this study was comprised of six pieces of diced ham or turkey breast. The study was repeated twice, and for each time, three samples (each consisting of six pieces) for each treatment were analyzed at each sampling time for each product. The pH and microbiological data (log CFU/cm^2) were analyzed using the Mixed Model Procedure of SAS® version 9.1 (SAS Institute, Cary, NC) to analyze the survivors and reductions of each treatment. For each product and each analysis day, independent

variables included immersion treatment, immersion time, and microwave treatment (without, or with 30 or 45 s microwave heating) and interactions of immersion treatment × immersion time, immersion treatment × microwave treatment, and immersion treatment × immersion time × microwave treatment. The interaction of immersion treatments × immersion time and immersion treatments × immersion time × microwave treatment were not significant ($P \geq 0.05$), while the interaction of immersion treatments × microwave treatment were significant ($P < 0.05$), which indicated that the antilisterial effects on diced ham or turkey breast depended on different salad dressing treatments and without or with prior microwave heating (30 s or 45 s). Means and standard deviations for microbiological data were calculated, and the mean differences were separated with the least significant difference procedure at the significance level of α=0.05.

9.3. RESULTS AND DISCUSSION

9.3.1. Physical and chemical properties of ham and turkey breast

Initial (day-0) water activity and pH of the inoculated diced ham samples were 0.975 ± 0.004 and 6.42 ± 0.04, respectively (Table 9.1). The pH values of the ham samples decreased ($P < 0.05$) following immersion in the salad dressings. On day-0, without prior microwave heating, immersion of samples in each of the four salad dressings for 5 or 10 min reduced ($P < 0.05$) the pH value by 0.37 to 1.13 (Table 9.1) compared to the controls

(pH 6.42). Ham samples stored at 7°C for 14 days had a lower ($P < 0.05$) pH than day-0 samples (Tables 9.2 and 9.3), and corresponded with an increase in microbial populations to more than 7 log CFU/cm^2 on these samples. The initial (day-0) water activity and pH of the inoculated diced turkey breast samples were 0.979 ± 0.002 and 6.36 ± 0.02, respectively (Table 9.4). On day-0, without prior microwave heating, dipping diced turkey breast samples in each of the four salad dressings for 5 or 10 min reduced ($P < 0.05$) the pH value by 0.43 to 1.00 (Table 9.4), compared to the controls (pH 6.36). During storage, turkey breast samples did not show ($P \geq 0.05$) large variation in pH values for most treatments as storage times progressed (Tables 9.5 and 9.6). In diced ham or turkey breast samples, the pH of the extra virgin olive oil plus lemon juice treated sample was higher ($P < 0.05$) than the other three salad dressings. These results were similar to those of our previous study (Chapter 8) which showed that immersion of frankfurters in various salad dressings reduced the pH of the product by 0.17 to 0.43 units. Salad dressings are known to act as acidifiers when applied to vegetables or egg mayonnaise (Smittle, 2000; Beuchat et al., 2006). Overall, microwave heating did not have large ($P \geq 0.05$) effects on the pH values of diced ham or turkey breast samples.

9.3.2. Microbial growth on diced ham and turkey breast during aerobic storage

Initial *L. monocytogenes* and total microbial counts on inoculated diced ham and turkey

breast samples were 2.1 ± 0.1 and 2.2 ± 0.1 log CFU/cm^2, respectively. During aerobic storage at 7°C, *L. monocytogenes* grew to 7.7 and 8.0 log CFU/cm^2 on ham and turkey breast control samples, respectively (Figures 9.1-9.3; Appendix Tables 19-23). On day-0 and day-7 (ham) or day-5 (turkey breast), total microbial counts on TSAYE (Tables 9.7 to 9.12) were similar ($P \geq 0.05$) to those observed on PALCAM agar, indicating that the majority of colonies found on TSAYE were *L. monocytogenes*. Total microbial populations reached 8.1 and 8.2 log CFU/cm^2 on ham (Table 9.9) and turkey breast (Table 9.12) control samples by the end of storage at 7°C, respectively. On day-14 (ham, Table 9.9) or day-9 (turkey breast, Table 9.12), total microbial counts on TSAYE were approximately 0.2 to 1.0 log CFU/cm^2 higher than the counts on PALCAM agar plates for most treatments of the two products, indicating the potential presence of spoilage microflora, such as lactic acid bacteria and yeasts (Lianou et al., 2007a; 2007b).

9.3.3. Antilisterial effects of salad dressings without prior microwave heating

On day-0 of storage, immersion of diced ham or turkey breast samples in each of the four salad dressings for 5 or 10 min reduced pathogen numbers by 0.7 to 1.0 and 0.5 to 0.6 log CFU/cm^2, respectively. These reductions were higher, but not statistically different ($P \geq 0.05$) to the reductions (0.5 to 0.6 and 0.3 log CFU/cm^2 for ham and turkey breast, respectively) caused by immersion in DW (Figure 9.1; Appendix Table 18). In a previous

study (Chapter 8), immersion of inoculated frankfurters in sunflower or extra virgin olive oil with lemon juice or vinegar, or four commercial salad dressings, reduced initial *L. monocytogenes* populations by 0.5 to 0.9 log CFU/cm^2, which is in agreement with the findings of the present study. Pathogen reductions were not different ($P \geq 0.05$) between 5 or 10 min of immersion of samples in the salad dressings (Figure 9.1; Appendix Tables 18 and 19).

As previously indicated, as storage time progressed from day-0 to day-14 (ham) or day-9 (turkey breast), *L. monocytogenes* contamination levels increased from 2.1 to 7.7 or 8.0 log CFU/cm^2, respectively. Reductions in pathogen counts following exposure of stored samples to the salad dressings were similar to those obtained for day-0 samples. Specifically, reductions of 0.5 to 1.0 and 0.8 to 1.2 log CFU/cm^2 were obtained for ham samples stored for 7 and 14 days, respectively. For turkey breast samples, reductions of 0.3 to 0.8 and 0.5 to 0.7 log CFU/cm^2 were obtained for product stored for 5 and 9 days, respectively. Among the four salad dressing treatments, sunflower oil plus vinegar generally caused slightly higher reductions on the two products at all storage times, and pathogen numbers were reduced by 1.0 to 1.2 and 0.6 to 0.8 log CFU/cm^2 on ham and turkey breast, respectively (Figure 9.1; Appendix Tables 18-20). As for day-0 samples, reductions in pathogen levels were similar ($P \geq 0.05$) for 5 or 10 min exposure to the salad

dressings.

9.3.4. Antilisterial effects of salad dressings with prior microwave heating

As expected, microwave heating of the deli meats resulted in reductions of *L. monocytogenes* populations (Figures 9.2 and 9.3; Appendix Tables 18-23). Compared to the untreated controls, microwave treatment for 30 s reduced pathogen counts by approximately 0.8 to 0.9 or 0.8 log CFU/cm^2 on ham and turkey breast samples, respectively (Figure 9.2; Appendix Tables 18 to 23), whereas treatment for 45 s increased the reductions to 1.8 to 2.3 and 2.0 to 2.7 log CFU/cm^2, respectively (Figure 9.3; Appendix Tables 19, 20, 22, and 23). The average surface temperature of samples of ham or turkey breast immediately after microwaving for 30 or 45 s was 52 and 62.5°C, respectively, as measured with a TempTestr® IR laser thermometer (Oaklon, Gainesville, FL). As previously reported (Fung and Cunningham, 1980; Giese, 1992; Heddleson and Doores, 1994), reductions of microbial counts by microwaving are due to heat generation.

Immersion (5 or 10 min) of ham or turkey breast samples in DW after microwave heating caused additional pathogen reductions of only 0.1 to 0.3 (30 s) and 0.1 to 0.8 (45 s) log CFU/cm^2 at all storage times. In comparison, samples immersed in salad dressings after 30 or 45 s of microwave heating resulted in lower ($P < 0.05$) numbers of pathogen survivors compared to those of corresponding DW-treated samples. Specifically, for stored

samples of ham and turkey breast, microwave heating for 30 s followed by treatment with salad dressings reduced *L. monocytogenes* counts by a total of 1.4 to 2.3 and 1.3 to 2.7 log CFU/cm^2, respectively (Figure 9.2; Appendix Tables 18 to 23). When the salad dressing treatments were preceded by a 45 s microwave treatment, total reductions of 3.4 to 5.5 and 2.9 to 5.7 log CFU/cm^2 were obtained for ham and turkey breast samples, respectively (Figure 9.3; Appendix Tables 19, 20, 22, and 23).

On day-0, microwave heating of ham and turkey breast samples for 30 s followed by treatment with sunflower oil plus vinegar for 10 min resulted in the lowest pathogen numbers (-0.1 and 0.2 log CFU/cm^2, respectively) compared to samples treated with the other three salad dressings (Figure 9.2; Appendix Tables 18 and 21). Pathogen survivors on samples treated with the other three salad dressings were 0.4 to 0.7 (ham) and 0.7 to 0.8 (turkey breast) log CFU/cm^2. On day-7 and day-14, microwave heating of ham samples for 30 or 45 s followed by immersion in the salad dressings resulted in total reductions of 1.5 to 2.3 (Figure 9.2; Appendix Tables 19 and 20) or 3.4 to 5.5 log CFU/cm^2 (Figure 9.3; Appendix Tables 19 and 20), respectively. Similarly, on day-5 and day-9 of storage, total reductions of 1.3-2.4 (Figure 9.2: Appendix Table 22) or 2.9 to 5.7 log CFU/cm^2 (Figure 9.3; Appendix Table 23) were obtained on turkey breast samples that were exposed to the salad dressing treatments after microwave heating for 30 or 45 s, respectively. Maximum

pathogen reductions were observed on day-7 (ham) and day-5 (turkey breast) on samples treated with sunflower oil plus vinegar (5 or 10 min) with prior microwave heating for 45 s, which reduced counts by a total of 5.5 and 5.7 log CFU/cm^2, respectively. Reductions were not different ($P \geq 0.05$) between 5 and 10 min of immersion for most of the salad dressing treatments applied to stored product samples; only 9-day old turkey breast samples treated with Thousand Island dressing had higher ($P<0.05$) total reductions at 10 min (4.2 log CFU/cm^2) as compared to those obtained after 5 min (2.9 log CFU/cm^2).

9.4. CONCLUSIONS

Results suggested that microwaving and salad dressing treatments in sequence resulted in higher reductions of *L. monocytogenes* as compared to individual treatments. Salad dressings, and especially sunflower oil plus vinegar, with prior microwave heating for 45 s may contribute to reduction of *L. monocytogenes* counts on deli meat products, such as ham or turkey breast, in the home environment. Salad dressings tested in this study reduced pathogen numbers by approximately 1.0 to 5.0 log CFU/cm^2, depending on product storage time, when preceded by a 45 s microwave oven treatment. It is recommended that consumers consider using microwave oven heating and salad dressings to prepare ham or turkey breast dices for use in salads, especially if they are at risk for listeriosis.

Table 9.1. Mean (± SD) pH values of diced ham samples inoculated with *Listeria monocytogenes* and stored (day-0) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s.

Dipping Treatment	No microwave		With microwave 30s	
	5 min	10 min	5 min	10 min
Control	6.42 ± 0.04a		----------	
Microwave 30 s	---------		6.38 ± 0.03a	
Distilled Water	6.40 ± 0.04aX	6.38 ± 0.04aX	6.40 ± 0.04aX	6.41 ± 0.04aX
Sunflower oil + vinegar	5.41 ± 0.10bX	5.29 ± 0.08bY	5.50 ± 0.03cX	5.33 ± 0.09cY
Extra virgin olive oil + lemon juice	6.05 ± 0.07cX	5.85 ± 0.03cY	5.83 ± 0.07bY	5.73 ± 0.09bZ
Vinaigrette	5.39 ± 0.16bX	5.35 ± 0.05bX	5.42 ± 0.05cX	5.26 ± 0.05cY
Thousand island	5.68 ± 0.15bX	5.57 ± 0.05dX	5.58 ± 0.09cX	5.59 ± 0.05cX

a-d: Means within a column that have a common letter are not different ($P \geq 0.05$).

X-Z: Means within a row that have a common letter are not different ($P \geq 0.05$).

Table 9.2. Mean (± SD) pH values of diced ham samples inoculated with *Listeria monocytogenes* and stored (day-7) aerobically at 7°C and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s or 45 s

Dipping Treatment	No microwave		With microwave 30 s		With microwave 45 s	
	5 min	10 min	5 min	10 min	5 min	10 min
Control	6.43 ± 0.06a		----------		----------	
Microwave 30s	----------		6.40 ± 0.07a		----------	
Microwave 45s	----------		----------		6.41 ± 0.09a	
Distilled Water	6.45 ± 0.05aX	6.43 ± 0.05aX	6.42 ± 0.07aX	6.41 ± 0.13aX	6.37 ± 0.07aX	6.38 ± 0.04aX
Sunflower oil + vinegar	5.43 ± 0.12bX	5.36± 0.09bXY	5.45 ± 0.04cX	5.31 ± 0.11cY	5.30 ± 0.05bY	5.28 ± 0.06bY
Extra virgin olive oil + lemon juice	6.04 ± 0.07cX	5.92 ± 0.08cX	5.84 ± 0.12bX	5.83 ± 0.15bX	5.88 ± 0.09cX	5.86 ± 0.08cX
Vinaigrette	5.35 ± 0.15bX	5.42 ± 0.07bX	5.34± 0.13cXY	5.26 ± 0.12cY	5.34±0.07bXY	5.21 ± 0.13bY
Thousand Island	5.53 ± 0.15bX	5.54 ± 0.09bX	5.50 ± 0.14cX	5.53± 0.14bcX	5.50± 0.12bcX	5.54 ± 0.04cX

a-c: Means within a column that have a common letter are not different ($P \geq 0.05$).

X-Y: Means within a row that have a common letter are not different ($P \geq 0.05$).

Table 9.3. Mean (± SD) pH values of diced ham samples inoculated with *Listeria monocytogenes* and stored (day-14) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s or 45 s

Dipping Treatment	No microwave		With microwave 30s		With microwave 45s	
	5 min	10 min	5 min	10 min	5 min	10 min
Control	5.79 ± 0.26a		----------		----------	
Microwave 30s	----------		5.86 ± 0.23a		----------	
Microwave 45s	----------		----------		5.88 ± 0.34a	
Distilled Water	5.88 ± 0.24aX	5.85 ± 0.35aX	5.93 ± 0.24aX	5.86 ± 0.18aX	5.80 ± 0.28aX	5.83 ± 0.24aX
Sunflower oil + vinegar	5.24 ± 0.36bX	5.06 ± 0.06bX	4.86 ± 0.17bY	4.98 ± 0.10bXY	5.10 ± 0.12cdX	4.93 ± 0.14bY
Extra virgin olive oil + lemon juice	5.50 ± 0.24cX	5.53 ± 0.12cX	5.39 ± 0.13cXZ	5.08 ± 0.22cY	5.29 ± 0.20cZ	5.27 ± 0.11cZ
Vinaigrette	5.01 ± 0.20dX	4.91 ± 0.14dX	4.96 ± 0.16bX	4.90 ± 0.16cX	4.96 ± 0.25dX	4.96 ± 0.13bX
Thousand Island	5.09 ± 0.17bdX	5.11 ± 0.10bdX	5.04 ± 0.18bX	5.02 ± 0.17cX	5.11 ± 0.20cX	5.24 ± 0.10cY

a-d: Means within a column that have a common letter are not different ($P \geq 0.05$).

X-Y: Means within a row that have a common letter are not different ($P \geq 0.05$).

Table 9.4 Mean (± SD) pH values of diced turkey breast samples inoculated with *Listeria monocytogenes* and stored (day-0) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s

Dipping Treatment	No microwave		With microwave 30 s	
	5 min	10 min	5 min	10 min
Control	6.36 ± 0.02a		----------	
Microwave 30 s	---------		6.41 ± 0.05a	
Distilled Water	6.39 ± 0.04aX	6.38 ± 0.02aX	6.40 ± 0.04aX	6.42 ± 0.07aX
Sunflower oil + vinegar	5.42 ± 0.10bX	5.36 ± 0.04bX	5.44 ± 0.16bX	5.33 ± 0.13bX
Extra virgin olive oil + lemon juice	5.93 ± 0.17cX	5.87 ± 0.08cX	5.79 ± 0.05cY	5.76 ± 0.09cY
Vinaigrette	5.47 ± 0.07bX	5.39 ± 0.08bX	5.42 ± 0.12bX	5.29 ± 0.06bY
Thousand island	5.54 ± 0.08bX	5.45 ± 0.05bY	5.45 ± 0.16bY	5.30 ± 0.11bZ

a-c: Means within a column that have a common letter are not different ($P \geq 0.05$).

X-Z: Means within a row that have a common letter are not different ($P \geq 0.05$).

Table 9.5. Mean (± SD) pH values of diced turkey breast samples inoculated with *Listeria monocytogenes* and stored (day-5) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s or 45 s

Dipping Treatment	No microwave		With microwave 30 s		With microwave 45 s	
	5 min	10 min	5 min	10 min	5 min	10 min
Control	6.18 ± 0.17a		----------		----------	
Microwave 30 s	----------		6.27 ± 0.11a		----------	
Microwave 45 s	----------		----------		6.33 ± 0.09a	
Distilled Water	6.14 ± 0.25aX	6.16 ± 0.24aX	6.26 ± 0.12aX	6.31 ± 0.13aX	6.36 ± 0.08aY	6.30 ± 0.05aX
Sunflower oil + vinegar	5.37 ± 0.10bX	5.41 ± 0.07bX	5.33 ± 0.04cX	5.32± 0.11bcX	5.35 ±0.09bX	5.21± 0.10bcY
Extra virgin olive oil + lemon juice	5.95 ± 0.17aX	5.80 ± 0.20aX	5.72 ± 0.12dXY	5.64 ± 0.15cY	5.69 ± 0.07cY	5.52 ± 0.15bZ
Vinaigrette	5.38 ± 0.23bX	5.46 ± 0.23bX	5.32 ± 0.13bX	5.18 ± 0.12bY	5.35 ± 0.08bX	5.10 ± 0.15cY
Thousand Island	5.41 ± 0.10bX	5.36 ± 0.06bX	5.42 ± 0.14bX	5.31± 0.14bcY	5.31 ± 0.08bY	5.28± 0.10bcY

a-c: Means within a column that have a common letter are not different ($P \geq 0.05$).

X-Z: Means within a row that have a common letter are not different ($P \geq 0.05$).

Table 9.6. Mean (± SD) pH values of diced ham samples inoculated with *Listeria monocytogenes* and stored (day-9) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s or 45 s.

Dipping Treatment	No microwave		With microwave 30 s		With microwave 45 s	
	5 min	10 min	5 min	10 min	5 min	10 min
Control	5.94 ± 0.14b		----------		----------	
Microwave 30 s	----------		6.40 ± 0.29a		----------	
Microwave 45 s	----------		----------		6.32 ± 0.12a	
Distilled Water	6.33 ± 0.28aX	6.32 ± 0.40aX	5.94 ± 0.40bY	6.43 ± 0.26aX	6.22 ± 0.09aZ	6.38 ± 0.21aX
Sunflower oil + vinegar	5.69 ± 0.17bcX	5.72 ± 0.17bX	5.57 ± 0.22cY	5.51 ± 0.24cY	5.67± 0.19cdX	5.39 ± 0.24cZ
Extra virgin olive oil + lemon juice	5.91 ± 0.09bX	5.98 ± 0.06bX	5.80 ± 0.16bY	5.76 ± 0.14bY	5.84 ± 0.25bY	5.83 ± 0.22bY
Vinaigrette	5.60 ± 0.15cX	5.46 ± 0.13cY	5.45 ± 0.13cY	5.50 ± 0.17cXY	5.65 ± 0.32cdX	5.33 ± 0.22cZ
Thousand Island	5.47 ± 0.17cXY	5.40 ± 0.17cX	5.56 ± 0.14cY	5.47 ± 0.12cXY	5.48 ± 0.17dXY	5.47 ± 0.23cXY

a-d: Means within a column that have a common letter are not different ($P \geq 0.05$).

X-Z: Means within a row that have a common letter are not different ($P \geq 0.05$).

Table 9.7. Mean (± SD) counts (log CFU/cm^2, n=6) of total microorganisms on diced ham that were inoculated with *Listeria monocytogenes* and stored (day-0) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s

Dipping Treatment	No microwave		With microwave 30 s	
	5 min	10 min	5 min	10 min
Control	2.2 ± 0.1a		----------	
Microwave 30 s	---------		1.2 ± 0.1b	
Distilled Water	1.6 ± 0.1bX	1.6 ± 0.2bX	1.2 ± 0.2bY	1.1 ± 0.2bY
Sunflower oil + vinegar	1.2 ± 0.1cX	1.3 ± 0.2bcX	0.2 ± 0.4cY	0.0 ± 0.3cY
Extra virgin olive oil + lemon juice	1.4 ± 0.1bcX	1.3 ± 0.2bcX	0.5 ± 0.1dY	0.4 ± 0.4dY
Vinaigrette	1.3 ± 0.2bcX	1.2 ± 0.2cX	0.8 ± 0.2dY	0.8 ± 0.3dY
Thousand island	1.2 ± 0.2cX	1.3 ± 0.2bcX	0.8 ± 0.2dY	0.6 ± 0.2dY

a-d: Means within a column that have a common letter are not different ($P \geq 0.05$).

X-Y: Means within a row that have a common letter are not different ($P \geq 0.05$).

Table 9.8. Mean (± SD) counts (log CFU/cm^2, n=6) of total microorganisms on diced ham that were inoculated with *Listeria monocytogenes* and stored (day-7) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s or 45 s.

Dipping Treatment	No microwave		With microwave 30s		With microwave 45s	
	5 min	10 min	5 min	10 min	5 min	10 min
Control	5.5 ± 0.4a		----------		----------	
Microwave 30 s	----------		4.8 ± 0.8b		----------	
Microwave 45 s	----------		----------		3.1 ± 0.7c	
Distilled Water	5.3 ± 0.4aX	5.4 ± 0.5aX	4.6 ± 0.6bY	4.3 ± 0.5bY	2.2 ± 0.7dZ	2.2 ± 0.6dZ
Sunflower oil + vinegar	4.6 ± 0.2bX	4.9 ± 0.5bX	3.9 ± 0.7cY	3.4 ± 0.3dY	0.1 ±1.0eZ	0.3 ± 0.8eZ
Extra virgin olive oil + lemon juice	5.0 ± 0.2abX	5.2 ± 0.7abX	3.8 ± 0.4cY	4.0 ± 0.5cY	1.0 ± 0.8fZ	1.0 ± 1.0fZ
Vinaigrette	5.2 ± 0.4abX	5.2 ± 0.9abX	3.9 ± 0.3cY	3.3 ± 0.5dY	1.2 ± 0.4fZ	1.2 ± 0.5fZ
Thousand Island	5.1 ± 0.3abX	5.0 ± 0.4abX	3.6 ± 0.3cY	3.4 ± 0.4dY	1.4 ± 0.6fZ	1.3 ± 0.5fZ

a-f: Means within a column that have a common letter are not different ($P \geq 0.05$).

X-Z: Means within a row that have a common letter are not different ($P \geq 0.05$).

Table 9.9. Mean (± SD) counts (log CFU/cm^2, n=6) of total microorganisms on diced ham that were inoculated with *Listeria monocytogenes* and stored (day-14) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s or 45 s.

Dipping Treatment	No microwave		With microwave 30 s		With microwave 45 s	
	5 min	10 min	5 min	10 min	5 min	10 min
Control	8.1 ± 0.1a		----------		----------	
Microwave 30 s	----------		7.1 ± 0.1b		----------	
Microwave 45 s	----------		----------		5.9 ± 0.6c	
Distilled Water	7.7 ± 0.2aX	7.7 ± 0.1bX	7.0 ±0.2bY	7.0 ± 0.2bY	6.1 ± 0.3cZ	5.8 ± 0.6cZ
Sunflower oil + vinegar	7.3 ± 0.1bX	7.3 ± 0.2cX	6.0 ±0.7cY	5.8 ± 0.8cY	3.1 ± 0.5dZ	3.7 ± 0.7dZ
Extra virgin olive oil + lemon juice	7.6 ± 0.1abX	7.3 ± 0.1cX	6.4 ±0.2cdY	6.5 ± 0.1dY	4.2 ± 1.4efZ	4.5 ± 1.0eZ
Vinaigrette	7.4 ± 0.2bX	7.4 ± 0.1cX	6.4 ±0.3cdY	6.5 ± 0.2dY	4.7 ± 1.1eZ	4.8 ± 0.7eZ
Thousand Island	7.6 ± 0.1abX	7.5 ± 0.1bcX	6.6 ±0.2dY	6.6 ± 0.2dY	3.8 ± 0.8fZ	4.3 ± 1.4edZ

a-f: Means within a column that have a common letter are not different ($P \geq 0.05$).

X-Z: Means within a row that have a common letter are not different ($P \geq 0.05$).

Table 9.10. Mean (± SD) counts (log CFU/cm^2, n=6) of total microorganisms on diced turkey breast that were inoculated with *Listeria monocytogenes* and stored (day-0) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s.

Dipping Treatment	No microwave		With microwave 30 s	
	5 min	10 min	5 min	10 min
Control	2.1 ± 0.1a		----------	
Microwave 30 s	---------		1.4 ± 0.2b	
Distilled Water	1.8 ± 0.2bX	1.7 ± 0.1bX	1.3 ± 0.2bY	1.4 ± 0.2bY
Sunflower oil + vinegar	1.6 ± 0.2bcX	1.6 ± 0.1bcX	0.7 ± 0.2cY	0.4 ± 0.4cY
Extra virgin olive oil + lemon juice	1.6 ± 0.1bcX	1.5 ± 0.1cX	1.0 ± 0.1bY	0.9 ± 0.2dY
Vinaigrette	1.6 ± 0.1bcX	1.7 ± 0.1bX	1.1 ± 0.2bY	0.8 ± 0.2dY
Thousand island	1.5 ± 0.1cX	1.5 ± 0.1cX	0.8 ± 0.2cY	0.9 ± 0.2dY

a-d: Means within a column that have a common letter are not different ($P \geq 0.05$).

X-Y: Means within a row that have a common letter are not different ($P \geq 0.05$).

Table 9.11 Mean (± SD) counts (log CFU/cm^2, n=6) of total microorganisms on diced turkey breast that were inoculated with *Listeria monocytogenes* and stored (day-5) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s or 45 s.

Dipping Treatment	No microwave		With microwave 30 s		With microwave 45 s	
	5 min	10 min	5 min	10 min	5 min	10 min
Control	5.9 ± 0.2a		----------		----------	
Microwave 30 s	----------		5.2 ± 0.7b		----------	
Microwave 45 s	----------		----------		3.3 ± 0.7c	
Distilled Water	6.0 ± 0.1aX	5.9 ± 0.3aX	5.0 ± 0.1bY	5.0 ± 0.2bY	3.2 ± 0.7cZ	3.1 ± 1.1cZ
Sunflower oil + vinegar	5.6 ± 0.3bX	5.6 ± 0.5aX	4.0 ± 0.6cY	4.3 ± 0.4cdY	1.4 ± 1.0dZ	1.4 ± 1.0dZ
Extra virgin olive oil + lemon juice	5.6 ± 0.2bX	5.8 ± 0.3aX	4.5 ± 0.4dY	4.4 ± 0.5cdY	1.5 ± 1.1dZ	1.5 ± 1.1dZ
Vinaigrette	5.7 ± 0.2abX	5.8 ± 0.2aX	4.3 ± 0.4cdY	4.3 ± 0.8cdY	1.9 ± 1.3dZ	1.9 ± 1.3dZ
Thousand Island	5.7 ± 0.2abX	5.9 ± 0.2aX	4.6 ± 0.7cdY	3.8 ± 0.5dY	2.0 ± 1.1dZ	2.0 ± 1.1dZ

a-d: Means within a column that have a common letter are not different ($P \geq 0.05$).

X-Z: Means within a row that have a common letter are not different ($P \geq 0.05$).

Table 9.12. Mean (± SD) counts (log CFU/cm^2, n=6) of total microorganisms on diced turkey breast that were inoculated with *Listeria monocytogenes* and stored (day-9) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s or 45 s.

Dipping Treatment	No microwave		With microwave 30 s		With microwave 45 s	
	5 min	10 min	5 min	10 min	5 min	10 min
Control	8.2 ± 0.3a		----------		----------	
Microwave 30 s	----------		7.7 ± 0.4b		----------	
Microwave 45 s	----------		----------		6.2 ± 0.6c	
Distilled Water	8.1 ± 0.1aX	8.1 ± 0.2aX	7.1 ± 0.5cY	7.0 ± 0.3cY	5.8 ± 0.4cZ	5.7 ± 0.4cZ
Sunflower oil + vinegar	7.7 ± 0.2bX	7.8 ± 0.1bX	6.7 ± 0.4cY	6.5 ± 0.6cY	4.6 ± 1.2dZ	4.8 ± 1.2dZ
Extra virgin olive oil + lemon juice	7.7 ± 0.2bX	7.8 ± 0.2abX	6.8 ± 0.3cY	6.7 ± 0.1cY	4.9 ± 0.6deZ	5.6 ± 0.3cZ
Vinaigrette	7.9 ± 0.1abX	7.9 ± 0.1abX	6.9 ± 0.2cY	6.8 ± 0.1cY	4.0 ± 1.2fZ	4.3 ± 0.6dZ
Thousand Island	7.9 ± 0.4abX	7.9 ± 0.1abX	7.0 ± 0.3cY	6.7 ± 0.1cY	5.0 ± 1.0eZ	4.1 ± 0.8dZ

a-f: Means within a column that have a common letter are not different ($P \geq 0.05$).

X-Z: Means within a row that have a common letter are not different ($P \geq 0.05$).

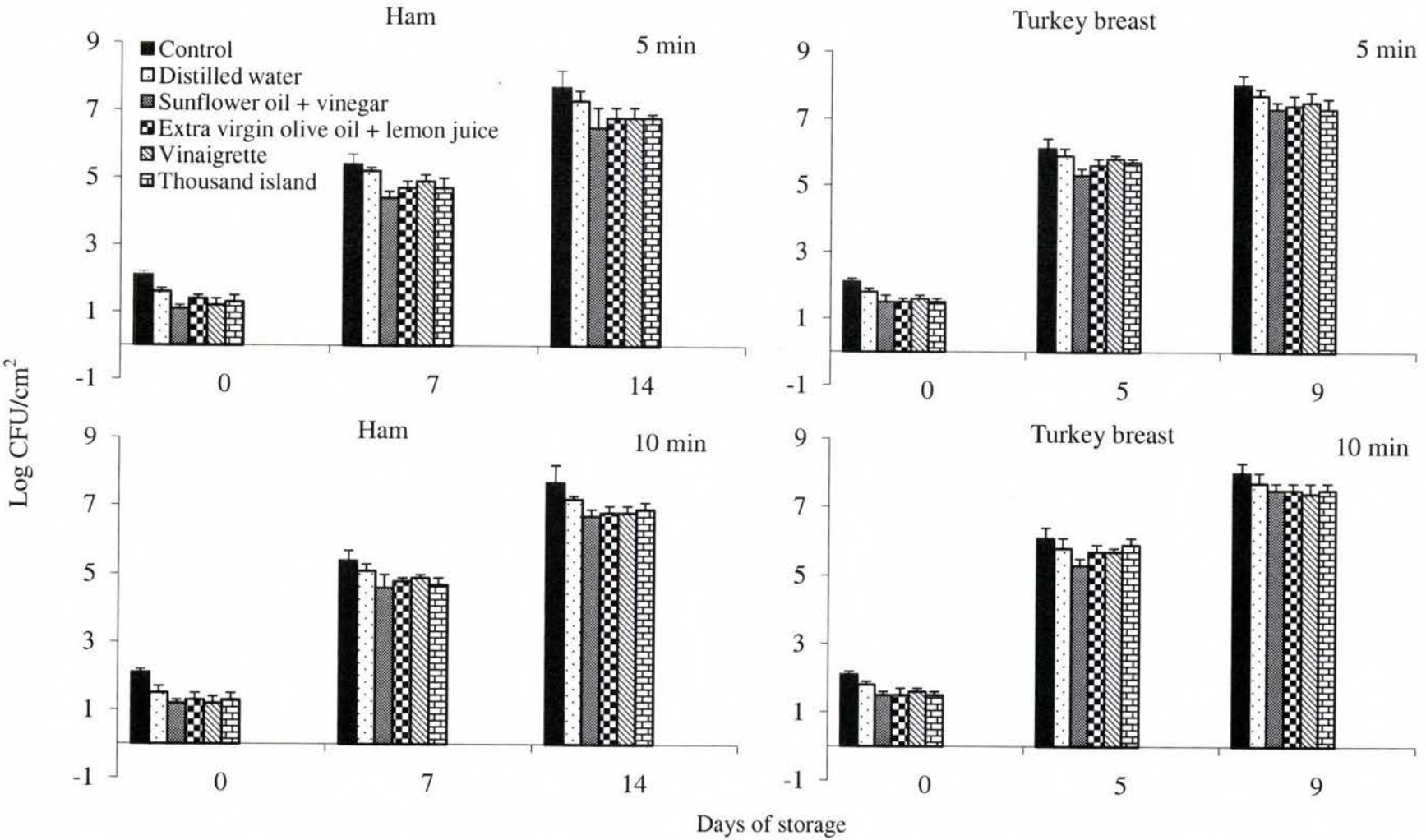


Figure 9.1(Appendix Tables 18-23). Mean populations (log CFU/cm^2) of *Listeria monocytogenes* inoculated on diced ham or turkey breast, stored aerobically at 7°C for 14 or 9 days, respectively, and left untreated or immersed for 5 or 10 min in distilled water, sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette or Thousand Island.

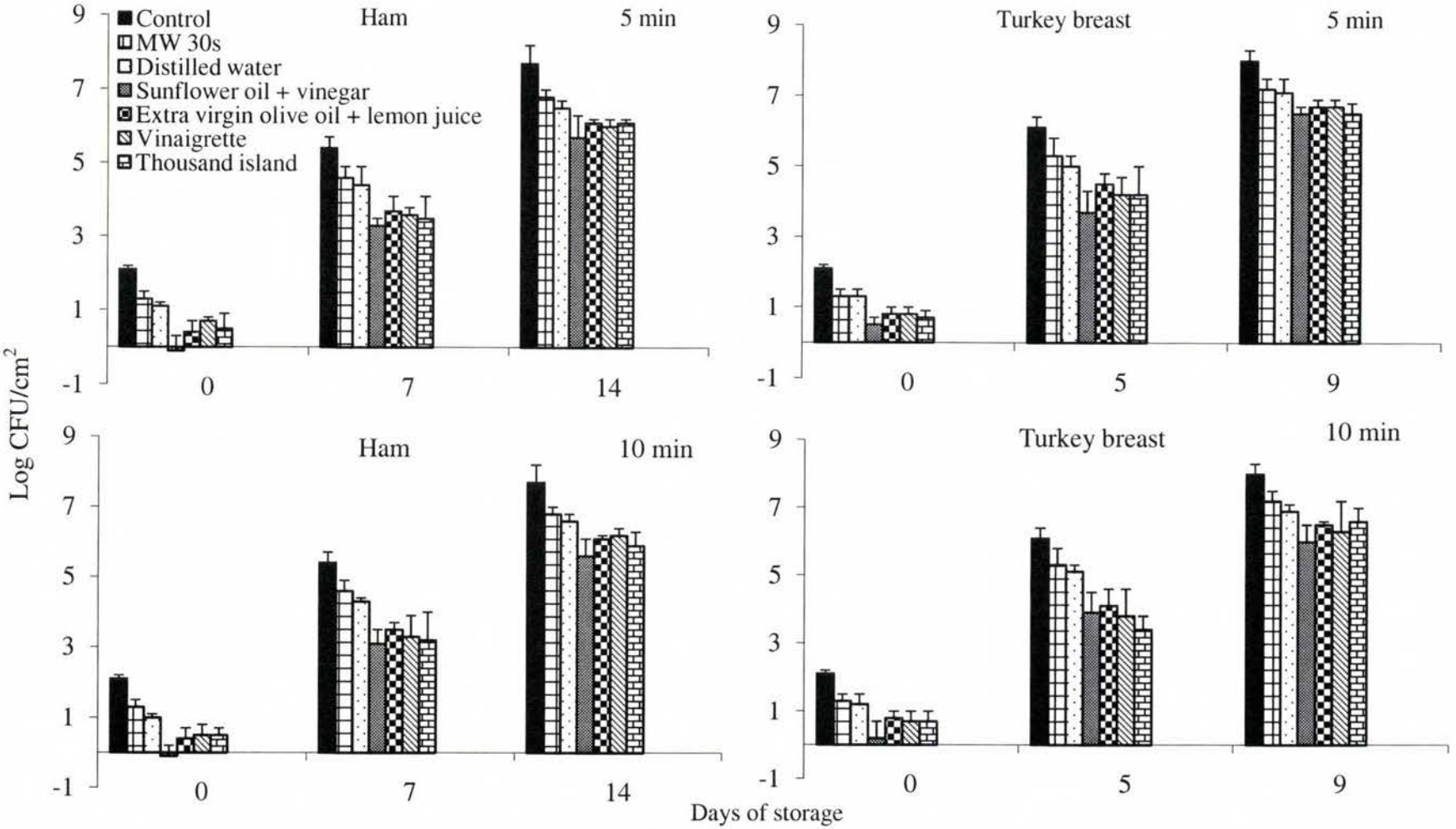


Figure 9.2 (Appendix Tables 18-23). Mean populations (log CFU/cm^2) of *Listeria monocytogenes* inoculated on diced ham or turkey breast, stored aerobically at 7°C for 14 or 9 days, respectively, and left untreated or immersed for 5 or 10 min in distilled water, sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette or Thousand Island, with prior microwave heating (MW) for 30 s.

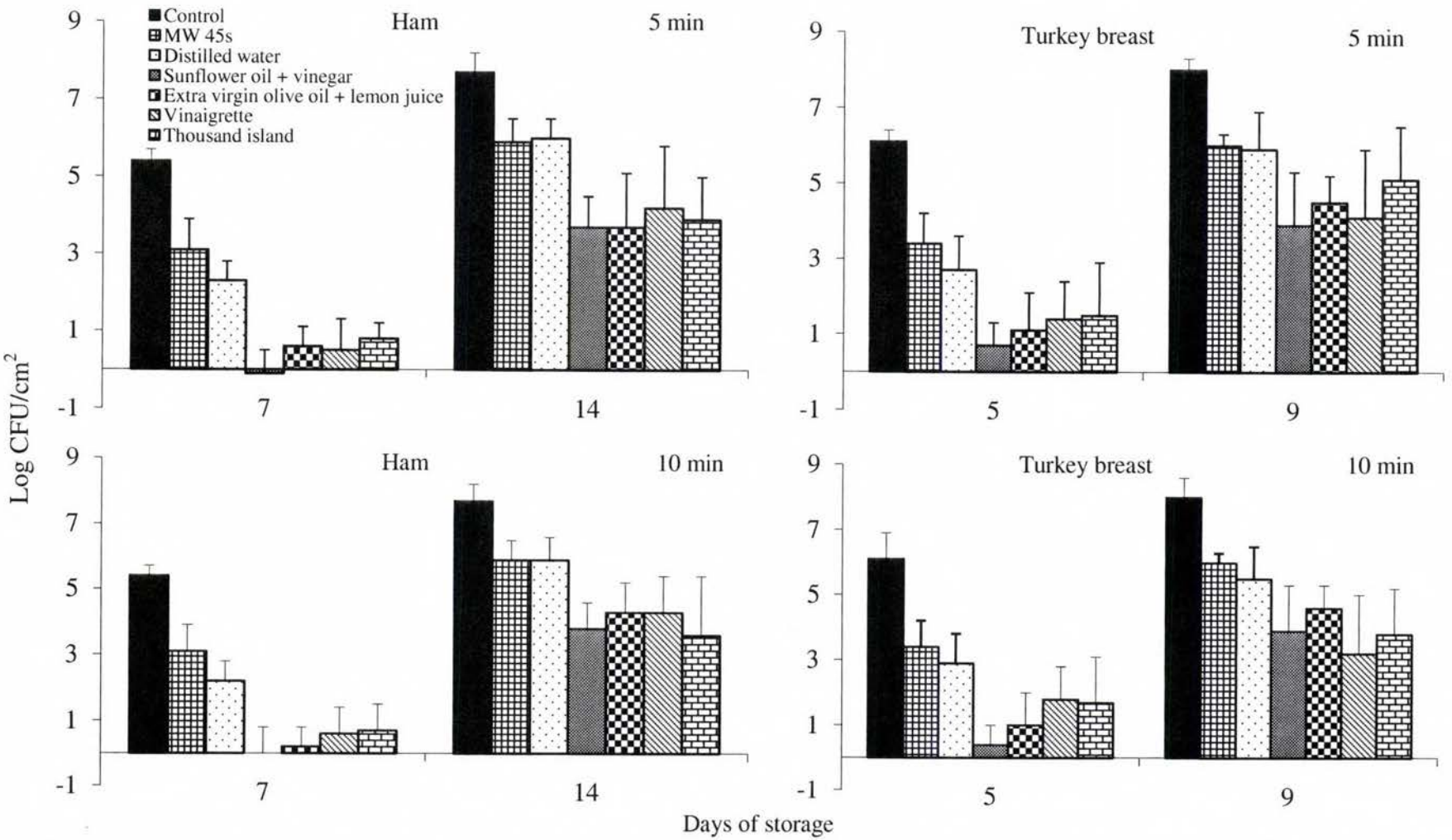


Figure 9.3 (Appendix Tables 19, 20, 22, and 23). Mean populations (log CFU/cm^2) of *Listeria monocytogenes* inoculated on diced ham or turkey breast, stored aerobically at 7°C for 14 or 9 days, respectively, and left untreated or immersed for 5 or 10 min in distilled water, sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette and Thousand Island, with prior microwave heating (MW) for 45 s.

CHAPTER 10

SUMMARY OF DISSERTATION

Escherichia coli O157:H7 is a highly virulent foodborne pathogen that causes approximately 62,000 cases of symptomatic infections, resulting in about 1,800 hospitalizations and 52 deaths annually in the U.S. (Mead et al., 1999). Nonintact beef products, including mechanically tenderized or reconstructed products, have been implicated as the source for outbreaks of *E. coli* O157:H7 due to translocation of pathogen cells from the beef muscle surface to the interior part by mechanical tenderization or restructuring processes (Hajmeer et al., 2000; Ortega-Valenzuela et al., 2001). According to the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS), nonintact beef products have been involved in three relatively recent (2000, 2003, and 2004) *E. coli* O157:H7 outbreaks in the U.S. (USDA-FSIS, 2005a; 2005b), and these outbreaks have been attributed to inadequate cooking (USDA-FSIS, 2005a; 2005b; 2007a) or cooking products directly from the frozen state (Laine et al., 2005). Because proper cooking renders non-intact products microbial safe, it is important to develop effective cooking protocols for non-intact beef products. In addition,

the fresh beef processing environment (Samelis et al., 2002b; Samelis and Sofos, 2003) of non-intact beef products may create potential stress-adapted *E. coli* O157:H7, thus, the responses of different stress-adapted pathogen cells in nonintact beef products of brine formulations with various antimicrobials needs to be evaluated during brining and cooking.

Listeria monocytogenes, the causative agent of listeriosis, is a postprocessing contaminant of ready-to-eat (RTE) meat products and has been associated with multistate outbreaks of listeriosis in the United States through consumption of frankfurters and poultry deli meats (CDC 1998; 1999; 2000; 2002). Although listeriosis outbreaks are not common (3.3 cases per 1,000,000 individuals per year), its fatality rate is high (20 to 40%) for high risk groups such as neonates, and immunocompromised adults (ILSI Research Foundation/Risk Science Institute, 2005). According to the USDA-FSIS, there were 147,208, 21,215, 88,484 and 157,347 kg of ready-to-eat meat products recalled for possible *L. monocytogenes* contamination in 2005, 2006, 2007, and 2008, respectively (USDA-FSIS, 2005b; 2006; 2007e; 2008e). Therefore, *L. monocytogenes* continues to be a major concern to the food industry and public health. Recently, interest has grown in the possible use of natural antimicrobials to control foodborne pathogens in foods at the processing and consumer environment. Thus, it is important to evaluate new natural antilisterial agents to be potentially used by the food industry and to develop appropriate risk management strategies to control *L. monocytogenes*

in the consumers' home.

The overall goal of this dissertation was to evaluate the effects of chemical antimicrobial agents and heat treatments to control *E. coli* O157:H7 in moisture enhanced nonintact beef products and *L. monocytogenes* on RTE meat and poultry products. The first three studies evaluated the thermal inactivation of unstressed or stress-adapted *E. coli* O157:H7 inocula in nonintact beef steaks by different methods and types of cooking appliances. The last four studies were focused on control of *L. monocytogenes* on RTE meat and poultry products such as frankfurters, diced ham and turkey breast, which are considered as high risk for transmission of the pathogen.

The first study evaluated thermal inactivation effects of *E. coli* O157:H7 in noninact beef steaks of different thickness as that were cooked by pan-broiling, double pan-broiling or roasting using different types of cooking appliances. The fresh beef was first coarse ground, inoculated with an eight-strain mixture of rifampin-resistant *E. coli* O157:H7 (6 to 7 log CFU/g) and then moisture-enhanced with sodium chloride (0.5%) plus sodium tripolyphosphate (0.23%). The meat was extruded into 10-cm diameter bags, semi-frozen (-20°C, 6 h), and cut into 1.5, 2.5 and 4.0 cm thick steaks. Samples were individually vacuum packaged, frozen (-20°C, 42 h), and tempered (4°C, 2.5 h) before cooking. The partially thawed steaks were cooked by pan-broiling in Presto® electric skillet and Sanyo® grill,

double pan-broiling in George Foreman® grill, and roasting in Oster® toaster oven and Magic chef® kitchen oven to the internal target temperature of 65°C. The temperatures of appliances and steaks were monitored with thermocouples. Results confirmed that the thicker the steaks, the higher the extent of reduction of *E. coli* O157:H7 cells. It also showed that roasting in a standard kitchen oven was most effective for pathogen inactivation.

The second study evaluated thermal inactivation of *E. coli* O157:H7 in nonintact beef steaks by pan-broiling and roasting in appliances set at different temperatures. The preparion of strains and steaks, monitoring temperatures of appliances and samples in this study was same as in the first study, and the only difference was that the beef was cut into 2.54 cm thick before cooking. The cooking appliances were preheated and set and maintained at different temperature settings, including 149 or 204°C for the electric skillet, 149 or 218°C for the grill, 149 or 232°C for the toaster oven, and 149, 204, or 260°C for the kitchen oven. Higher temperatures (204 to 260°C) resulted greater pathogen reductions than those of lower one (149°C). Results of the first two studies should be useful to the food service industry for establishing effective cooking protocols for noninact beef, also should be useful in risk assessments of nonintact as well as intact beef products.

The third study evaluated thermal inactivation of stress-adapted *E. coli* O157:H7 in noninact beef steaks by pan-broiling on an electric skillet. The materials and methods used in

this study were based on the first study. Differences included inoculation in moisture enhanced beef steaks with acid, cold, heat, starvation and desiccation stress-adapted or unstressed *E. coli* O157:H7, and moisture enhancing with different bringing solutions including distilled water, sodium chloride (NaCl, 0.5%) plus sodium tripolyphosphate (STP, 0.25%), NaCl(0.5%)+STP(0.25%) combined with cetylpyridinium chloride (0.2%), NaCl (0.5%)+STP(0.25%) combined with lactic acid (0.3%), and NaCl (0.5%)+STP(0.25%) combined with AvGard®XP (0.2%, active ingredient: sodium metasilicate). Results indicated that acid stress-adapted cells were more resistant to thermal inactivation, while cold or desiccation stress-adapted cells were more sensitive to thermal inactivation. Heat or starvation stressed cells did not show differences in sensitivity to heat treatment compared to the unstressed cells. It was also observed that the lowest surviving pathogen counts were in the cetylpyridinium chloride or lactic acid treated samples. This study clearly verified the difference in behavior of different stress-adapted cells during cooking, and that cetylpyridinium chloride or lactic acid could be used as antimicrobials in the brining solutions when preparing nonintact beef products.

The antilisterial activity of hops beta acids (HBA) in culture broth medium and on frankfurters was evaluated in the fourth and fifth study. In the fourth study, a 10-strain mixture of *L. monocytogenes* (2-3 log CFU/ml) was inoculated into tryptic soy broth

supplemented with 0.6% yeast extract (TSBYE) without or with 0.5 to 5.0 μg/ml of HBA, 1.0% of potassium lactate, 0.25% of sodium diacetate, or 0.1% of acetic acid alone or in combination with HBA (0.5 to 3.0 μg/ml). The survival/growth of the pathogen during storage at 4, 10 and 25°C for 35, 20, and 2 days, respectively, was periodically monitored. Antilisterial activity was observed by HBA solutions in the range of 1.0 to 5.0 μg/ml, which was increased with increasing HBA concentrations at the lower temperature (4°C), and when HBA were combined with other antimicrobials. In the fifth study, frankfurters artificially inoculated with *L. monocytogenes* (10-strain mixture, 1.9 log CFU/cm^2) were dipped into 0.03, 0.06, and 0.10% solution of HBA or distilled water, and then vacuum packaged and stored at 4°C or 10°C for up to 90 and 48 days, respectively. Results showed that pathogen growth was completely suppressed for up to 30 to 50 (4°C) or 20 to 28 days (10°C), and their antilisterial effects increased with increasing concentrations ranging from 0.03 to 0.10%. Based on these two studies, it is possible that HBA could be used to improve the safety of ready-to-eat meat products as a post-processing dipping treatment, based on economic feasibility, and provided that future studies find no adverse effects on sensory qualities.

In the sixth study, frankfurters were surface inoculated with 2.4 log CFU/cm^2 of *L. monocytogenes* (10-strain mixture) and stored aerobically at 7°C for 14 days. At 0, 7 and 14 days, frankfurters were immersed for 5 or 20 min in sunflower oil plus lemon juice or vinegar,

extra virgin olive oil plus lemon juice or vinegar, or commercial salad dressings including Vinaigrette, Ranch, Thousand island, and Cesar, or distilled water, without or with prior microwave heating (high power) for 30 s. Results showed that pathogen cells reducing effects of salad dressings decreased in the order of sunflower or extra virgin olive oil plus vinegar ≥ sunflower or extra virgin olive oil plus lemon juice > Caesar ≥ Thousand island ≥ Ranch ≥ Vinaigrette. Results also indicated that the antilisterial activities of salad dressings increased after microwave heating of inoculated samples.

The last study examined the antilisterial activities of salad dressings, chosen based on the results of the third study, on diced ham (days-0, 7, 14) and turkey breast (days-0, 5, 9) during simulated home storage, without or with prior microwave heating for 30 or 45 s of high power. Reductions of pathogen counts by different salad dressing treatments were also observed in these products, especially with sunflower oil plus vinegar, and they increased, to as high as 5.5 to 5.7 log CFU/cm^2 with prior microwave heating of inoculated samples for 45 s. According to the results of the last two studies, it is reasonable to conclude that use of certain salad dressings and microwaving may contribute to control of *L. monocytogenes* on ready-to-eat meat products or diced deli delicatessen meats, especially when consumed in salads by at-risk populations at their home.

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APPENDIX

Appendix Table 1 (Figure 3.1). Cooking time (time) and temperature (°C) for nonintact beef steaks of different thickness cooked by pan-broiling using the Presto® electric skillet.

Place	Thickness (cm)	Cooking time (minutes)													
		0	5	10	15	16.3	20	25	27.2	30	35	40	45	50	55
Lower surface	1.5	-0.7	70.7	57.1	48.6	49.5									
	2.5	-0.9	73.7	93.4	90.1	103.8	107.5	64.6	54.3						
	4.0	-0.7	45.1	66.9	76.5	78.2	81.2	82.3	83.4	84.7	86.9	88.5	96.8	69.5	65.0
Upper surafce	1.5	-3.0	28.3	75.5	75	80.1									
	2.5	-2.0	0.4	5.3	14.6	23.6	70.9	94.5	95.2						
	4.0	-2.0	3.5	20.4	28.6	30.2	33.1	35.4	36.6	38.6	44.8	49.3	79.6	92.2	91.9
Edge	1.5	-0.5	43.5	61.3	72.9	74.0									
	2.5	-1.4	46.5	63.9	70.4	70.6	80	73.9	72.6						
	4.0	-1.0	15.1	42.0	56.5	59.9	65.9	69.4	71.2	72.0	75.3	76.6	74.3	71.1	65.0
Center	1.5	-1.6	38.8	54.7	63.8	65.0									
	2.5	-1.7	-0.7	12.1	31.1	33.9	43.9	53.3	65.0						
	4.0	-2.0	-2.1	-2.2	-1.0	-0.3	3.9	8.9	17.2	27.0	34.8	42.2	52.7	55.5	65.0

Appendix Table 2 (Figure 3.2). Cooking time (min) and temperature (°C) for nonintact beef steaks of different thickness cooked by pan-broiling using the Sanyo® grill.

Place	Thickness (cm)	Cooking time (minutes)													
		0	5	10	15	20	25	30	35	40	45	50	55	60	65
Lower surface	1.5	-2.7	52.5	63.0	54.6										
	2.5	-2.1	60.5	68.4	71.0	80.7	86.7	62.9							
	4.0	-1.3	67.6	77.5	88.7	93.1	96.1	101.9	105.6	107	107.9	101.8	88.5	67.2	55.5
Upper surafce	1.5	-1.7	4.2	25.9	88.2										
	2.5	-2.5	-2.8	2.8	10.0	21.8	45.1	90.4							
	4.0	-2.5	-2.3	-2.2	-1.6	-1.4	-1.4	-1.0	-0.2	4.5	25.8	37.8	62.8	86.7	93.4
Edge	1.5	0.4	69.7	72.0	76.5										
	2.5	-0.2	32.4	54.4	58.4	57.7	65.3	66.1							
	4.0	-1.2	8.7	25.4	34.6	43.2	46.9	50.7	55.4	57.9	57.8	59.6	57.8	53.2	51.8
Center	1.5	-2.1	15.9	36.2	65.0										
	2.5	-1.6	-1.6	8.6	20.6	31.3	45.8	65.0							
	4.0	-2.0	-2.4	-2.3	-1.8	2.2	7.5	13.9	20.4	26.4	29.4	37.9	44.2	44.5	65.0

Appendix Table 3 (Figure 3.3). Cooking time (min) and temperature (°C) for nonintact beef steaks of different thickness cooked by double pan-broiling using the Geogre Forman® grill.

Place	Thickness (cm)	Cooking time (minutes)							
		0	2	4	5	7	10	15	20
Surface	1.5	-1.7	84.6	91.4					
	2.5	-1.8	55.1	82.1	85.9	91.8	99.7		
	4.0	-2.1	45.7	75.2	83.9	90.1	96.7	103.6	117.7
Edge	1.5	-1.6	53.4	90.0					
	2.5	-1.7	23.8	64.8	73.3	83.1	89.1		
	4.0	-1.8	23.9	52.8	61.0	68.7	73.8	77.6	81.8
Center	1.5	-2.0	15.9	65.0					
	2.5	-2.2	-2.3	4.9	11.2	35.9	65.0		
	4.0	-2.3	-2.6	-2.1	-1.6	0.7	12.0	38.0	65.0

Appendix Table 4 (Figure 3.4). Cooking time (min) and temperature (°C) for nonintact beef steaks of different thickness cooked by roasting using the Oyster® toaster oven.

Place	Thickness (cm)	Cooking time (minutes)										
		0	5	10	11.3	15	20	25	30	35	40	45
Surface	1.5	-1.1	48.6	67.6	77.8							
	2.5	-1.7	20.6	33.6	36.3	44.1	52.5	74.9	84.4			
	4.0	-2.2	23.2	40.5	45.2	51.3	60.2	68.4	76.5	82.8	88.5	93.2
Edge	1.5	-1.4	47.8	66.1	70.2							
	2.5	0.4	10.8	29.6	33.1	43.1	69.1	72.5	81.2			
	4.0	-1.6	4.0	20.1	24.7	34.9	44.4	52.1	55.5	63.3	71.2	84.9
Center	1.5	-1.6	21.6	57.6	65.0							
	2.5	-2.1	-1.9	1.5	3.5	13.2	32.0	51.3	65.0			
	4.0	-1.5	-1.9	-1.9	-1.9	-1.9	-1.1	8.0	25.2	41.5	55.7	65.0

Appendix Table 5 (Figure 3.5). Cooking time (min) and temperature (°C) for nonintact beef steaks of different thickness cooked by roasting using the Magic Chef ®kitchen oven.

Place	Thickness (cm)	Cooking time (minutes)													
		0	5	10	15	20	25	30	35	40	45	50	55	60	65
Surface	1.5	1.25	32.3	49.1	75.8	82.2									
	2.5	-2.1	32.6	42.2	58.9	71.1	78.4	88.2	90.3						
	4.0	-2.2	45.2	53.1	66.9	74.1	78.7	86.5	86.8	91.0	92.6	92.8	93.8	93.8	92.6
Edge	1.5	-2.4	28.3	50.2	67.5	76.0									
	2.5	-1.9	21.3	40.2	55.7	65.1	72.9	78.1	80.3						
	4.0	-2.0	6.0	18.9	31.8	41.7	50.2	56.1	60.5	65.9	69.0	72.3	74.5	75.8	83.9
Center	1.5	-2.4	8.9	54.7	28.7	49.9	65.0								
	2.5	-2.5	-2.5	-0.0	10.1	27.9	42.8	52.6	65.0						
	4.0	-2.5	-2.9	-2.9	-2.8	-2.0	2.6	14.1	29.3	39.1	46.4	53.0	58.4	62.9	65.0

Appendix Table 6 (Figure 4.1). Cooking time (min) and temperature (°C) of pan-broiling and roasting appliances set and maintained at different temperatures setting during cooking of nonintact beef steaks.

Appliances	Set temperature (°C)	Cooking time (minutes)												
		0	5	10	15	20	25	30	35	40	45	50	55	60
Presto® electric	149	168.5	150.0	156.5	161.5	151.0	165.0	160.5	188.5	187.0	182.0	170.0	167.0	161.0
skillet	204	216.0	232.0	241.0	202.0	215.0	213.0	218.0	199.5	208.0	221.0	223.0	217.5	224.5
Sanyo® grill	149	192.0	165.0	179.0	154.5	171.6	158.7	145.8	155.0	148.8	182.0	147.0	185.0	167.0
	218	242.5	241.1	250.8	242.3	243.5	232.5	235.5	241.0	235.5	242.0	233.5	245.5	213.6
Oster® toaster	149	136.0	147.0	138.5	135.0	145.0	132.5	142.0	141.5	147.5	135.5	133.0	137.0	138.5
oven	232	228.0	238.4	239.5	203.6	215.4	218.9	234.2	235.5	238.0	242.8	221.5	225.0	235.5
Magic Chef®	149	144.5	142.0	144.5	136.0	149.5	131.5	142.0	141.5	137.5	150.5	155.0	137.0	142.0
Kitchen oven	204	163.0	173.6	175.5	178.0	177.5	173.3	173.5	173.3	189.5	181.2	182.5	179.5	184.5
	260	206.0	200.5	203.5	205.5	207.3	207.0	196.5	198.8	207.5	208.9	209.8	210.5	213.5

Appendix Table 7 (Figure 4.2). Cooking time (min) and temperature (°C) of nonintact beef steaks cooked by pan-broiling using the Presto® electric skillet setting at 149 or 204°C.

Place	Set temperature (°C)	Cooking time (minutes)							
		0	5	10	15	20	25	30	35
Upper surface	149	-2.5	-3.0	-1.7	2.6	8.4	13.3	51.4	78.6
	204	-1.9	-1.7	-1.0	1.0	15.9	21.2	70.4	91.3
Lower surface	149	-1.8	55.2	70.1	80.5	85.5	91.0	69.2	63.7
	204	3.6	86.3	94.1	97.7	101.1	103.8	72.6	67.0
Edge	149	-1.6	22.2	44.5	58.0	61.2	65.8	63.8	61.7
	204	-1.4	42.9	56.5	70.9	79.7	75.2	72.1	66.5
Center	149	-3.2	-2.5	7.8	15.9	23.9	35.6	49.3	65.0
	204	-2.5	-2.5	3.1	11.1	22.1	36.1	49.6	65.0

Appendix Table 8 (Figure 4.3). Cooking time (min) and temperature(°C) of nonintact beef steaks cooked by pan-broiling using the Sanyo® grill setting at 149 or 218°C.

Place	Set temperature (°C)	Cooking time (minutes)								
		0	5	10	15	20	25	32	40	45
Upper surface	149	-2.2	-2.1	-1.3	-0.9	1.3	5.7	25.4	77.7	94.0
	218	-1.4	-1.4	1.7	16.6	30.4	61.7	84.7		
Lower surface	149	0.3	66.4	77.9	80.9	84.8	87.4	103.4	82.6	61.5
	218	0.0	73.6	80.1	105.8	90.1	82.1	58.1		
Edge	149	-1.5	-0.2	33.8	53.4	57.3	58.6	61.8	64.4	57.0
	218	-1.6	29.9	55.4	64.4	65.7	62.0	64.0		
Center	149	-2.2	-2.2	0.9	6.5	13.2	21.5	30.3	49.3	65.0
	218	-2.3	-1.3	5.3	5.3	18.0	42.3	65.0		

Appendix Table 9 (Figure 4.4). Cooking time (min) and temperature (°C) of nonintact beef steaks cooked by roasting using the Oyster® toaster oven setting at 149 or 232°C.

Place	Set temperature (°C)	Cooking time (minutes)										
		0	5	10	15	20	25	27	35	40	45	46
Surface	149	-2.0	3.9	27.2	35.6	42.0	48.6	57.2	69.3	76.9	80.9	81.3
	232	-1.3	37.2	50.8	59.3	77.9	87.5	89.7				
Edge	149	-2.0	15.5	23.0	34.8	43.7	52.8	60.8	66.2	69.0	72.5	73.3
	232	-0.9	23.3	42.5	61.7	73.4	81.9	78.1				
Center	149	-2.3	-2.4	-2.1	-1.0	2.6	17.3	32.9	44.3	54.9	62.5	65.0
	232	-1.9	-2.2	-0.7	9.7	41.0	60.0	65.0				

Appendix Table 10 (Figure 4.5). Cooking time (min) and temperature (°C) of nonintact beef steaks cooked by pan-broiling using the Magic Chef® kitchen oven setting at 149, 204, or 260°C.

Place	Set temperature (°C)	Cooking time (minutes)												
		0	5	10	15	20	25	30	33	40	45	50	55	60
Surface	149	-2.4	7.6	8.8	20.4	28.6	35.9	43.9	52.8	56.6	63.7	65.6	70.7	73.7
	204	-3.0	0.6	5.7	27.7	43.1	48.3	62.1	75.0					
	260	-1.3	3.9	9.9	30.8	52.8	62.8	85.5						
Edge	149	-1.8	1.1	16.6	29.6	38.5	45.7	51.9	56.6	61.3	64.1	66.7	68.4	70.0
	204	-2.5	5.9	26.8	42.8	56.6	68.6	75.1	79.1					
	260	-1.8	26.3	49.7	65.1	76.5	82.4	85.5						
Center	149	-1.3	-0.9	-0.4	1.3	5.3	16.3	28.4	37.9	46.2	52.4	57.7	61.6	65.0
	204	-1.8	-1.0	0.7	6.7	25.9	44.3	56.7	65.0					
	260	-1.0	-0.5	1.7	14.2	28.7	46.5	65.0						

Appendix Table 11 (Figures 4.6; 4.7). Mean (± SD, log CFU/g, n=9) of *Escherichia coli* O157:H7 and total microbial counts recovered with tryptic soy agar plus pyruvate (0.1%, TSAP) plus rifampicin (100μg/ml) and TSAP from uncooked and cooked nonintact beef steaks to internal temperature of 65°C with a Presto® Electric skillet, a Sanyo® grill, an Oyster® toaster oven, and a Magic Chef® kitchen oven set at different temperatures

Cooking method	Appliance	Set cooking temperature (°C)	TSAP+Rif	TSAP
Uncooked		----	6.4 ± 0.1a	6.5 ± 0.2a
Pan broiling	Presto® Electric skillet	149	4.9 ± 0.4b	4.9 ± 0.5b
		204	3.1 ± 1.0d	3.4 ± 1.0c
	Sanyo® grill	149	4.7 ± 0.2bc	4.7 ± 0.2b
		218	3.1 ± 0.9d	3.1 ± 0.8c
Roasting	Oyster® toaster oven	149	4.5 ± 0.2bc	4.5 ± 0.4b
		232	2.4 ± 1.2e	3.3 ± 1.4c
	Magic Chef® Kitchen oven	149	4.0 ± 0.6c	4.6 ± 0.4b
		204	2.7 ± 1.2ed	2.9 ± 1.4c
		260	0.9 ± 0.7f	1.0 ± 0.7d

a-f: Means with a common letter within a column are not different ($P \geq 0.05$).

Appendix Table 12 (Figure 5.1). Temperature changes of the surface of Presto® electric skillet and lower, and upper surface, edge and geometric center of nonintact beef steaks during cooking to the internal geometric center temperature of 65°C.

Place	Cooking time (minutes)						
	0	5	10	15	20	25	30
Electric skillet	176.0	187.5	170.5	165.0	177.0	180.0	183.0
Upper surface	-2.4	-1.9	0.7	9.0	35.5	63.0	91.0
Lower surface	5.9	66.1	78.2	82.2	76.9	70.3	56.6
Edge	-0.5	31.9	58.6	70.2	68.5	66.4	57.1
Center	-1.8	1.5	12.4	24.2	38.9	50.6	65.0

Appendix Table 13 (Figure 5.2). Mean (± SD, log CFU/g, n=6) unstressed, acid, cold, heat, starvation or desiccation stress adapted *Escherichia coli* O157:H7 recovered with tryptic soy agar plus pyruvate (0.1%, TSAP) plus rifampicin (100μg/ml) from uncooked and cooked nonintact beef steaks with brines containing different antimicrobials and cooked to internal temperature of 65°C

Type of Stress	Treatment	After frozen 4.5 h	Before cooking	After cooking
Unstressed	Distilled water	5.9 ± 0.1aX	5.7 ± 0.1aX	3.4 ± 0.1adgY
	NaCl+STP	5.8 ± 0.1aX	5.6 ± 0.1aX	3.5 ± 0.5adgY
	NaCl+STP+CPC	5.4 ± 0.1bX	4.9 ± 0.3bY	2.2 ± 0.2bfZ
	NaCl+STP+LA	5.5 ± 0.1bX	5.4 ± 0.1acX	2.8 ± 0.7bgY
	NaCl+STP+AvGard®XP	5.5 ± 0.1bX	5.3 ± 0.1cX	3.2 ± 0.4agY
Acid	Distilled water	5.9 ± 0.1aX	5.8 ± 0.1aX	4.2 ± 0.1cdY
	NaCl+STP	5.8 ± 0.1aX	5.7 ± 0.1aX	4.4 ± 0.4cY
	NaCl+STP+CPC	5.5 ± 0.1bX	5.0 ± 0.3bY	3.6 ± 0.6adX
	NaCl+STP+LA	5.6 ± 0.1bX	5.4 ± 0.1cX	3.5 ± 0.6adY
	NaCl+STP+AvGard®XP	5.5 ± 0.1bX	5.4 ± 0.1cX	3.9 ± 0.3dY
Cold	Distilled water	5.5 ± 0.1aX	5.3 ± 0.1aX	2.2 ± 0.2bfY
	NaCl+STP	5.5 ± 0.2aX	5.3 ± 0.1aX	2.3 ± 0.5bfY
	NaCl+STP+CPC	4.5 ± 0.4bX	4.2 ± 0.2bX	0.8 ± 0.4eY
	NaCl+STP+LA	5.2 ± 0.1aX	5.3 ± 0.1aX	0.8 ± 0.4eY
	NaCl+STP+AvGard®XP	5.3 ± 0.1aX	5.1 ± 0.1aX	2.1 ± 0.6bfY
Heat	Distilled water	5.8 ± 0.1aX	5.7 ± 0.1aX	3.3 ± 0.6adY
	NaCl+STP	5.7 ± 0.1aX	5.8 ± 0.1aX	4.1 ± 0.2cdY
	NaCl+STP+CPC	5.1 ± 0.4bX	4.8 ± 0.1bX	1.8 ± 0.8fhY
	NaCl+STP+LA	5.4 ± 0.2bX	5.4 ± 0.1cX	2.9 ± 0.7gY
	NaCl+STP+AvGard®XP	5.3 ± 0.3bX	5.4 ± 0.1cX	3.8 ± 0.3dY
Starvation	Distilled water	5.5 ± 0.1aX	5.5 ± 0.3aX	3.4 ± 0.8adgY
	NaCl+STP	5.5 ± 0.1aX	5.3 ± 0.1acX	3.0 ± 0.4gY
	NaCl+STP+CPC	5.1 ± 0.1bX	4.5 ± 0.1bY	1.9 ± 0.6fhZ
	NaCl+STP+LA	5.3 ± 0.1aX	5.1 ± 0.1cX	2.0 ± 0.5fhY
	NaCl+STP+AvGard®XP	5.3 ± 0.1aX	5.1 ± 0.1cX	2.9 ± 1.0gY
Desiccation	Distilled water	5.5 ± 0.1aX	5.4 ± 0.1aX	2.5 ± 0.5bgY
	NaCl+STP	5.5 ± 0.1aX	5.7 ± 0.2bX	2.5 ± 0.3bgY
	NaCl+STP+CPC	4.9 ± 0.3bX	4.7 ± 0.3cX	0.8 ± 0.6eY
	NaCl+STP+LA	5.3 ± 0.2aX	5.1 ± 0.2aX	1.5 ± 0.7hY
	NaCl+STP+AvGard®XP	5.2 ± 0.3aX	5.1 ± 0.1aX	2.5 ± 0.5bgY

a-f: Means with a common letter within a column are not different ($P \geq 0.05$).

X-Y: Means with a common letter within a column are not different ($P \geq 0.05$).

NaCl: sodium chloride (0.5%); STP: sodium tripolyphosphate (0.25%); CPC: cetylpyridinium chloride (0.5%); LA: lactic acid (0.2%).

Appendix Table 14 (Figure 7.1). Mean pH values of untreated (control) frankfurters, and frankfurters dipped (2 min) in distilled water (DW) or hops beta acids (HBA) and stored for 90 or 48 days under vacuum at 4 (A) or 10°C (B), respectively.

A)

	Day of storage							
Treatment	0	5	10	20	30	50	70	90
Control	6.04 ± 0.01a	5.93 ± 0.06a	6.06 ± 0.09a	6.02 ± 0.06a	6.07 ± 0.05a	6.07 ± 0.05a	5.87 ± 0.08b	5.68 ± 0.03b
DW	6.04 ± 0.01a	5.95 ± 0.08a	6.10 ± 0.07a	6.02 ± 0.04a	6.07 ± 0.09a	6.07 ± 0.09a	5.87 ± 0.04b	5.58 ± 0.03b
HBA 0.03%	6.01 ± 0.07a	5.95 ± 0.07a	6.11 ± 0.08a	6.03 ± 0.03a	6.13 ± 0.02a	6.13 ± 0.02a	6.03 ± 0.05a	5.82 ± 0.09b
HBA 0.06%	6.08 ± 0.02a	5.93 ± 0.07a	6.12 ± 0.10a	6.10 ± 0.09a	6.03 ± 0.11a	6.03 ± 0.11a	6.07 ± 0.02a	5.95 ± 0.05a
HBA 0.1%	6.02 ± 0.06a	5.92 ± 0.09a	6.10 ± 0.11a	6.03 ± 0.06a	6.05 ± 0.06a	6.05 ± 0.06a	6.03 ± 0.07a	6.00 ± 0.04a

B)

	Day of storage							
Treatment	0	4	8	12	20	28	36	48
Control	6.04 ± 0.01a	5.94 ± 0.08a	6.05 ± 0.06a	6.09 ± 0.03a	6.04 ± 0.05a	5.88 ± 0.07b	5.46 ± 0.17c	5.29 ± 0.13c
DW	6.04 ± 0.01a	5.92 ± 0.03a	6.03 ± 0.06a	6.07 ± 0.06a	6.01 ± 0.06a	5.66 ± 0.10b	5.49 ± 0.05b	4.98 ± 0.14c
HBA 0.03%	6.01 ± 0.07a	6.00 ± 0.08a	6.02 ± 0.08a	6.04 ± 0.02a	5.98 ± 0.13a	5.99 ± 0.01a	5.91 ± 0.11a	5.55 ± 0.13b
HBA 0.06%	6.08 ± 0.02a	6.02 ± 0.04a	6.10 ± 0.08a	6.08 ± 0.03a	6.05 ± 0.04a	6.04 ± 0.02a	6.05 ± 0.05a	6.02 ± 0.05a
HBA 0.1%	6.02 ± 0.06a	5.97 ± 0.02a	6.10 ± 0.08a	6.07 ± 0.06a	6.03 ± 0.05a	5.99 ± 0.05a	5.98 ± 0.09a	6.01 ± 0.05a

a-c: Means with a common letter within a row are not different ($P \geq 0.05$)

Appendix Table 15 (Figure 7.2). Mean (log CFU/cm^2) *L. monocytogenes* (PALCAM agar; A) and total microbial (TSAYE; B) populations on the surface of untreated (control) frankfurters, and frankfurters dipped (2 min) in distilled water (DW) or hops beta acids (HBA) during storage in vacuum packages at 4°C for 90 days.

A)

	Day of storage							
Treatment	0	5	10	20	30	50	70	90
Control	1.9 ± 0.1a	1.8 ± 0.1a	1.8 ± 0.2a	2.4 ± 0.3a	3.9 ± 0.2a	6.2 ± 0.7a	7.1 ± 0.1a	7.4 ± 0.1a
DW	0.9 ± 0.3b	0.9 ± 0.3b	1.2 ± 0.5b	1.7 ± 0.4b	2.7 ± 1.0b	6.1 ± 0.4a	7.0 ± 0.1a	7.4 ± 0.2a
HBA 0.03%	0.6 ± 0.1c	0.1 ± 0.2c	0.2 ± 0.2c	0.4 ± 0.2c	0.8 ± 0.4c	2.5 ± 1.4b	5.9 ± 0.2b	6.4 ± 0.5b
HBA 0.06%	0.3 ± 0.1d	0.2 ± 0.2c	0.0 ± 0.4c	0.2 ± 0.3cd	0.3 ± 0.3d	0.1 ± 0.4c	4.2 ± 0.4c	6.2 ± 0.4b
HBA 0.1%	0.3 ± 0.3cd	-0.1 ± 0.3c	0.1 ± 0.3c	0.1 ± 0.2d	0.2 ± 0.4d	0.3 ± 0.4c	4.2 ± 0.3c	5.6 ± 0.2c

B)

	Day of storage							
Treatment	0	5	10	20	30	50	70	90
Control	2.0 ± 0.1a	1.8 ± 0.2a	1.8 ± 0.1a	2.5 ± 0.3a	3.9 ± 0.1a	6.2 ± 0.7a	7.1 ± 0.1a	7.4 ± 0.1a
DW	1.0 ± 0.3b	1.0 ± 0.3b	1.2 ± 0.5b	1.8 ± 0.4b	2.7 ± 1.0b	6.0 ± 0.4a	7.2 ± 0.1a	7.4 ± 0.1a
HBA 0.03%	0.6 ± 0.1c	0.4 ± 0.2c	0.6 ± 0.4c	0.5 ± 0.4c	0.6 ± 0.6c	2.4 ± 1.4b	6.0 ± 0.2b	6.5 ± 0.4b
HBA 0.06%	0.5 ± 0.2c	0.4 ± 0.2c	0.2 ± 0.3c	0.3 ± 0.3c	0.3 ± 0.5d	0.2 ± 0.4c	4.3 ± 0.3c	6.3 ± 0.4b
HBA 0.1%	0.5 ± 0.1c	0.0 ± 0.2d	0.4 ± 0.2c	0.3 ± 0.4c	0.3 ± 0.4d	0.3 ± 0.4c	4.2 ± 0.3c	5.9 ± 0.1c

a-d: Means with a common letter within a column are not different ($P \geq 0.05$)

Appendix Table 16 (Figure 7.2). Mean (log CFU/cm^2) *L. monocytogenes* (PALCAM agar; A) and total microbial (TSAYE; B) populations on the surface of untreated (control) frankfurters, and frankfurters dipped (2 min) in distilled water (DW) or hops beta acids (HBA) during storage in vacuum packages at 10°C for 48 days.

A)

	Day of storage							
Treatment	0	4	8	12	20	28	36	48
Control	1.9 ± 0.1a	1.8 ± 0.3a	2.3 ± 0.5a	3.1 ± 0.8a	5.6 ± 1.3a	6.7 ± 0.7a	7.6 ± 0.2a	7.6 ± 0.3a
DW	0.9 ± 0.3b	1.0 ± 0.6b	1.9 ± 0.9a	2.7 ± 1.2a	5.2 ± 1.7a	6.9 ± 0.8a	7.7 ± 0.1a	7.6 ± 0.1a
HBA 0.03%	0.6 ± 0.1bd	0.2 ± 0.2c	0.3 ± 0.5b	0.8 ± 0.4b	0.8 ± 0.4b	4.4 ± 1.6b	6.8 ± 0.1b	7.5 ± 0.1a
HBA 0.06%	0.3 ± 0.1c	0.0 ± 0.3c	0.2 ± 0.2b	0.4 ± 0.2bc	0.6 ± 0.5b	4.0 ± 1.3b	5.8 ± 0.6c	6.2 ± 1.2b
HBA 0.1%	0.3 ± 0.3cd	-0.1± 0.3d	-0.1 ± 0.4b	0.2 ± 0.6c	0.3 ± 0.3b	0.8 ± 0.7c	4.0 ± 0.6d	5.7 ± 0.2b

B)

	Day of storage							
Treatment	0	4	8	12	20	28	36	48
Control	2.0 ± 0.1a	2.0 ± 0.3a	2.3 ± 0.6a	3.1 ± 0.8a	5.5 ± 1.4a	6.6 ± 0.7a	7.6 ± 0.2a	7.7 ± 0.3ab
DW	1.0 ± 0.3a	1.2 ± 0.5b	1.8 ± 0.8a	2.9 ± 1.2a	5.2 ± 1.7a	6.9 ± 0.9b	7.8 ± 0.2a	7.8 ± 0.1a
HBA 0.03%	0.6 ± 0.1b	0.3 ± 0.2c	0.6 ± 0.5b	0.8 ± 0.2b	1.0 ± 0.2b	4.4 ± 1.4c	6.7 ± 0.1b	7.5 ± 0.1b
HBA 0.06%	0.5 ± 0.2b	0.2 ± 0.3cd	0.2 ± 0.2b	0.5 ± 0.2b	0.6 ± 0.5bc	4.0 ± 1.2c	5.8 ± 0.6c	6.0 ± 1.2c
HBA 0.1%	0.5 ± 0.1b	0.1± 0.3d	0.1 ± 0.3b	0.3 ± 0.4b	0.4 ± 0.3c	1.1 ± 0.5d	4.8 ± 0.1d	5.6 ± 0.2c

a-d: Means with a common letter within a column are not different ($P \geq 0.05$)

Appendix Table 17 (Figures 8.1; 8.2). Mean (± SD) populations (log CFU/cm^2, n=6) of *Listeria monocytogenes* on frankfurters inoculated and stored aerobically at 7°C for 14 days. Samples were left untreated (CON) or immersed for 5 or 20 min in distilled water (DW), sunflower oil + lemon juice (SL) or vinegar (SV), extra virgin olive oil + lemon juice (EL) or vinegar (EV), Vinaigrette (V), Ranch (R), Thousand Island (T) or Caesar (C), without or with prior microwave heating for 30 s, on days 0, 7 and 14 of storage

Storage	Dipping Treatment	No microwave		With microwave 30 s	
		5 min	5 min	5 min	20 min
Day 0	CON	2.4 ± 0.1a		----------	
	MW	---------		1.6 ± 0.1b	
	DW	1.9 ± 0.1bx	1.8 ± 0.1bx	1.5 ± 0.1by	1.3 ± 0.2cz
	SL	1.7 ± 0.1cx	1.5 ± 0.2cx	0.8 ± 0.3cdy	0.9 ± 0.3dy
	SV	1.7 ± 0.1cx	1.5 ± 0.1cx	0.8 ± 0.3cdy	0.7 ± 0.2ey
	EL	1.7 ± 0.2cx	1.6 ± 0.1bcx	0.7 ± 0.3dy	0.8 ± 0.2dey
	EV	1.6 ± 0.1cx	1.5 ± 0.1cx	0.7 ± 0.3dy	0.5 ± 0.4fy
	V	1.8 ± 0.1bcx	1.7 ± 0.1bcx	1.0 ± 0.3cey	0.9 ± 0.2dy
	R	1.9 ± 0.1bx	1.6 ± 0.1bcy	1.0 ± 0.2cez	1.0 ± 0.2dz
	T	1.7 ± 0.1cx	1.6 ± 0.1bcx	1.1 ± 0.1efy	0.9 ± 0.2dz
	C	1.8 ± 0.1bcx	1.7 ± 0.1bcx	1.2 ± 0.2fy	0.9 ± 0.1dz
Day 7	CON	4.1 ± 0.4a		----------	
	MW	---------		2.8 ± 0.3b	
	DW	3.1 ± 0.5bx	3.0 ± 0.6bx	2.3 ± 0.5by	2.3 ± 0.5by
	SL	2.4 ± 0.4cx	2.6 ± 0.7bcx	1.5 ± 0.2cy	1.2 ± 0.2cy
	SV	2.5 ± 0.4cx	2.5 ± 0.8cx	1.5 ± 0.5cy	1.3 ± 0.5cy
	EL	2.6 ± 0.6bcx	2.5 ± 0.8cx	1.5 ± 0.2cy	1.5 ± 0.5cy
	EV	2.5 ± 0.4cx	2.3 ± 0.4cx	1.3 ± 0.2cy	1.4 ± 0.4cy
	V	2.9 ± 0.5bcx	2.6 ± 0.7bcx	1.4 ± 0.2cy	1.4 ± 0.2cy
	R	2.9 ± 0.5bcx	2.6 ± 0.3bcx	1.5 ± 0.3cy	1.6 ± 0.3cy
	T	2.6 ± 0.4bcx	2.7 ± 0.5bcx	1.6 ± 0.3cy	1.5 ± 0.3cy
	C	2.6 ± 0.4bcx	2.4 ± 0.4cx	1.8 ± 0.6bcy	1.6 ± 0.5cy
Day 14	CON	6.0 ± 0.4a		---------	
	MW	----------		4.9 ± 0.6b	
	DW	5.2 ± 0.6abx	5.1 ± 0.5bx	4.2 ± 0.6bdy	4.1 ± 0.8cy
	SL	4.6 ± 0.5bcx	3.9 ± 0.6cxy	3.3 ± 0.7cyz	3.0 ± 0.6dez
	SV	4.8 ± 0.5bcx	4.1 ± 0.6cx	3.1 ± 1.3cy	2.7 ± 0.7ey
	EL	4.5 ± 0.5bcx	4.6 ± 0.6bcx	3.5 ± 0.6cdy	3.3 ± 0.9cdey
	EV	4.3 ± 0.8cx	4.2 ± 0.9cx	3.7 ± 0.6cdxy	3.0 ± 0.8dey
	V	4.8 ± 0.7bcx	4.8 ± 0.6bcx	3.8 ± 0.5cdy	3.6 ± 0.4cdy
	R	4.4 ± 0.9cxy	4.6 ± 0.9bcx	3.7 ± 0.6cdy	3.2 ± 1.0dey
	T	4.6 ± 0.5bcx	4.5 ± 0.5bcx	3.5 ± 0.8cdy	3.5 ± 0.5cdy
	C	4.4 ± 0.8cx	4.4 ± 0.9bcx	3.3 ± 1.0cy	3.6 ± 0.7cdyx

a-c: Means within a column that have a common letter, within a day of storage, are not different ($P \geq 0.05$).
x-z: Means within a row that have a common letter, within a day of storage, are not different ($P \geq 0.05$).

Appendix Table 18 (Figures 9.1; 9.2) Mean (± SD) populations (log CFU/cm^2, n=6) of *Listeria monocytogenes* on diced ham that were inoculated and stored (day-0) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s.

Dipping Treatment	No microwave		With microwave 30 s	
	5 min	10 min	5 min	10 min
Control	2.1 ± 0.1a		----------	
Microwave 30 s	---------		1.3 ± 0.2b	
Distilled Water	1.6 ± 0.1bX	1.5 ± 0.2bX	1.1 ± 0.1bY	1.0 ± 0.1bY
Sunflower oil + vinegar	1.1 ± 0.1cX	1.2 ± 0.1cX	-0.1 ± 0.4cY	-0.1 ± 0.3cY
Extra virgin olive oil + lemon juice	1.4 ± 0.1bcX	1.3 ± 0.2bcX	0.4 ± 0.3dY	0.4 ± 0.3dY
Vinaigrette	1.2 ± 0.2bcX	1.2 ± 0.2bcX	0.7 ± 0.1dY	0.5 ± 0.3dY
Thousand island	1.3 ± 0.2bcX	1.3 ± 0.2bcX	0.5 ± 0.4dY	0.5 ± 0.2dY

a-d: Means within a column that have a common letter are not different ($P \geq 0.05$).

X-Y: Means within a row that have a common letter are not different ($P \geq 0.05$).

Appendix Table 19 (Figures 9.1; 9.2; 9.3). Mean (± SD) populations (log CFU/cm^2, n=6) of *Listeria monocytogenes* on diced ham that were inoculated and stored (day-7) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s or 45 s

Dipping Treatment	No microwave		With microwave 30s		With microwave 45s	
	5 min	10 min	5 min	10 min	5 min	10 min
Control	5.4 ± 0.3a		----------		----------	
Microwave 30 s	----------		4.6 ± 0.3b		----------	
Microwave 45 s	----------		----------		3.1 ± 0.8c	
Distilled Water	5.2 ± 0.1bX	5.1 ± 0.2bX	4.3 ± 0.1bY	4.3 ± 0.1bY	2.3 ± 0.5dZ	2.2 ± 0.6dZ
Sunflower oil + vinegar	4.4 ± 0.2cX	4.6 ± 0.4cX	3.1 ± 0.4cY	3.1 ± 0.4cY	0.1 ±0.6eZ	0.0 ± 0.8eZ
Extra virgin olive oil + lemon juice	4.7 ± 0.2cX	4.8 ± 0.1cX	3.5 ± 0.2cY	3.5 ± 0.2cY	0.6 ± 0.5eZ	0.2 ± 0.6fZ
Vinaigrette	4.9 ± 0.2bcX	4.9 ± 0.1bcX	3.3 ± 0.6cY	3.3 ± 0.6cY	0.5 ± 0.8eZ	0.6 ± 0.8fZ
Thousand Island	4.7 ± 0.3cX	4.7 ± 0.2cX	3.2 ± 0.8cY	3.2 ± 0.8cY	0.8 ± 0.4eZ	0.7 ± 0.8fZ

a-f: Means within a column that have a common letter are not different ($P \geq 0.05$).

X-Z: Means within a row that have a common letter are not different ($P \geq 0.05$).

Appendix Table 20 (Figures 9.1; 9.2; 9.3).Mean (± SD) populations (log CFU/cm^2, n=6) of *Listeria monocytogenes* on diced ham that were inoculated and stored (day-14) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s or 45 s

Dipping Treatment	No microwave		With microwave 30s		With microwave 45s	
	5 min	10 min	5 min	10 min	5 min	10 min
Control	7.7 ± 0.5a		----------		----------	
Microwave 30 s	----------		6.8 ± 0.2b		----------	
Microwave 45 s	----------		----------		5.9 ± 0.6c	
Distilled Water	7.3 ± 0.3aX	7.2 ± 0.1bX	6.5 ±0.2bY	6.6 ± 0.2bY	6.0 ± 0.5cZ	5.9 ± 0.7cZ
Sunflower oil + vinegar	6.5 ± 0.6bX	6.7 ± 0.2cX	5.7 ±0.6cY	5.6 ± 0.5cY	3.7 ± 0.8dZ	3.8 ± 0.8dZ
Extra virgin olive oil + lemon juice	6.8 ± 0.3bX	6.8 ± 0.2cX	6.1 ±0.1dY	6.1± 0.1dcY	3.7 ± 1.4dZ	4.3 ± 0.9dZ
Vinaigrette	6.8 ± 0.3bX	6.8 ± 0.2cX	6.0 ±0.2dcY	6.2 ± 0.2dcY	4.2 ± 1.6dZ	4.3 ± 1.1dZ
Thousand Island	6.8 ± 0.1bX	6.9 ± 0.2bcX	6.1 ±0.1dY	5.9 ± 0.4dcY	3.9 ± 1.1dZ	3.6 ± 1.8dZ

a-f: Means within a column that have a common letter are not different ($P \geq 0.05$).

X-Z: Means within a row that have a common letter are not different ($P \geq 0.05$).

Appendix Table 21 (Figures 9.1; 9.2). Mean (± SD) populations (log CFU/cm^2, n=6) of *Listeria monocytogenes* on diced turkey breast that were inoculated and stored (day-0) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s.

Dipping Treatment	No microwave		With microwave 30 s	
	5 min	10 min	5 min	10 min
Control	2.1 ± 0.1a		----------	
Microwave 30 s	---------		1.3 ± 0.2b	
Distilled Water	1.8 ± 0.1bX	1.8 ± 0.1bX	1.3 ± 0.2bY	1.2 ± 0.3bY
Sunflower oil + vinegar	1.5 ± 0.2cX	1.5 ± 0.1cX	0.5 ± 0.2cY	0.2 ± 0.5cY
Extra virgin olive oil + lemon juice	1.5 ± 0.1cX	1.5 ± 0.2cX	0.8 ± 0.2cY	0.8 ± 0.2dY
Vinaigrette	1.6 ± 0.1bcX	1.6 ± 0.1bcX	0.8 ± 0.2cY	0.7 ± 0.3dY
Thousand island	1.5 ± 0.1cX	1.5 ± 0.1cX	0.7 ± 0.2cY	0.7 ± 0.3dY

a-d: Means within a column that have a common letter are not different ($P \geq 0.05$).

X-Z: Means within a row that have a common letter are not different ($P \geq 0.05$).

Appendix Table 22 (Figures 9.1; 9.2; 9.3). Mean (± SD) populations (log CFU/cm^2, n=6) of *Listeria monocytogenes* on diced turkey breast that were inoculated and stored (day-5) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s or 45 s

Dipping Treatment	No microwave		With microwave 30s		With microwave 45s	
	5 min	10 min	5 min	10 min	5 min	10 min
Control	6.1 ± 0.3a		----------		----------	
Microwave 30 s	----------		5.3 ± 0.5b		----------	
Microwave 45 s	----------		----------		3.4 ± 0.8c	
Distilled Water	5.9 ± 0.2aX	5.8 ± 0.3aX	5.0 ± 0.3bY	5.1 ± 0.2bY	2.7 ± 0.8dZ	2.9 ± 0.9dZ
Sunflower oil + vinegar	5.3 ± 0.2bX	5.3 ± 0.2bX	3.7 ± 0.6cY	3.9 ± 0.6cdY	0.7 ± 0.9eZ	0.4 ± 0.6eZ
Extra virgin olive oil + lemon juice	5.6 ± 0.2abX	5.7 ± 0.2abX	4.5 ± 0.3dY	4.1 ± 0.5cY	1.1 ± 0.6efZ	1.0 ± 1.0efZ
Vinaigrette	5.8 ± 0.1abX	5.7 ± 0.1abX	4.2 ± 0.5cdY	3.8 ± 0.8cdY	1.4 ± 1.0efZ	1.8 ± 1.0fZ
Thousand Island	5.7 ± 0.1abW	5.9 ± 0.2acW	4.2 ± 0.8cdX	3.4 ± 0.4dY	1.5 ± 0.9fZ	1.7 ± 1.4fZ

a-f: Means within a column that have a common letter are not different ($P \geq 0.05$).

W-Z: Means within a row that have a common letter are not different ($P \geq 0.05$).

Appendix Table 23 (Figures 9.1; 9.2; 9.3). Mean (± SD) populations (log CFU/cm^2, n=6) of *Listeria monocytogenes* on diced turkey breast that were inoculated and stored (day-9) aerobically at 7°C, and then left undipped or dipped for 5 or 10 min in distilled water (DW), sunflower oil + vinegar, extra virgin olive oil + lemon juice, Vinaigrette, Thousand Island, without or with prior microwave heating 30 s or 45 s

Dipping Treatment	No microwave		With microwave 30s		With microwave 45s	
	5 min	10 min	5 min	10 min	5 min	10 min
Control	8.0 ± 0.3a		----------		----------	
Microwave 30 s	----------		7.2 ± 0.3b		----------	
Microwave 45 s	----------		----------		6.0 ± 0.3c	
Distilled Water	7.7 ± 0.2aX	7.7 ± 0.3aX	7.1 ± 0.4bY	6.9 ± 0.2bY	5.9 ± 0.2cZ	5.5 ± 1.0cZ
Sunflower oil + vinegar	7.3 ± 0.2bX	7.5 ± 0.2abX	6.5 ± 0.2cY	6.0 ± 0.5cY	3.9 ± 1.1dZ	3.9 ± 1.4dZ
Extra virgin olive oil + lemon juice	7.4 ± 0.3abX	7.5 ± 0.2abX	6.7 ± 0.2cY	6.5 ± 0.1bcY	4.5 ± 0.9deZ	4.6 ± 0.7dZ
Vinaigrette	7.5 ± 0.3abW	7.4 ± 0.3abW	6.7 ± 0.2cX	6.3 ± 0.9cX	4.1 ± 0.7dY	3.2 ± 1.8dZ
Thousand Island	7.3 ± 0.3bW	7.5 ± 0.2abW	6.5 ± 0.3cX	6.6 ± 0.4bcX	5.1 ± 0.3eY	3.8 ± 1.4dZ

a-f: Means within a column that have a common letter are not different ($P \geq 0.05$).

W-Z: Means within a row that have a common letter are not different ($P \geq 0.05$).