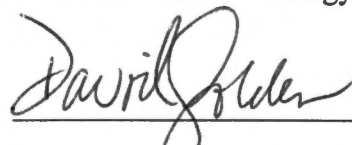


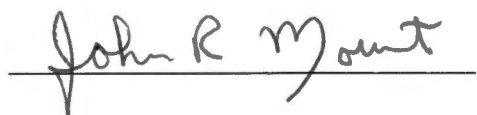
To the Graduate Council:

I am submitting herewith a thesis written by Jeffrey J. Semanchek entitled "Influence of Package Atmosphere on Growth and Survival of Uninjured and Sublethally Heat-injured *Escherichia coli* O157:H7." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.



David A. Golden, Major Professor

We have read this thesis
and recommend its acceptance:



Accepted for the Council:



Associate Vice Chancellor and
Dean of the Graduate School

**INFLUENCE OF PACKAGE ATMOSPHERE ON
GROWTH AND SURVIVAL OF UNINJURED AND SUBLETHALLY
HEAT-INJURED *ESCHERICHIA COLI* O157:H7**

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee

Jeffrey J. Semanchek
May 1997

AD-VET-MED.

Thesis

97

.S49

ACKNOWLEDGMENTS

There are many people to whom I would like to express my gratitude for making this thesis and my graduate study possible. I am thankful for my junior committee members, Dr. F.A. Draughon and Dr. J.R. Mount for their suggestions and editorial comments regarding my research and thesis. I wish to thank my major professor, mentor, and friend, Dr. David A. Golden, for his immeasurable knowledge and guidance throughout my graduate endeavors. My success at The University of Tennessee and undoubtedly my success in the future, is due largely to Dr. Golden's contribution to my knowledge inside and outside of the classroom.

I would also like to thank several friends within the department that contributed to my work, and made my time at UT a memorable experience, Tomeka Fisher, Rebecca Ohmes, Lilliam Arroyo-Gallyoun, Paul Angelino, Derike Smiley, and Rob Williams.

I want to thank my brothers John and Gary for their friendship and support during my venture at UT. Most of all I wish to thank my father and mother for their love, encouragement, and confidence.

ABSTRACT

The research portion of this thesis focuses on two main aspects of *Escherichia coli* O157:H7. The first investigation was an examination concerning the influence of growth temperature on heat-, lactic acid-, and freeze- induced inactivation and injury of *E. coli* O157:H7. Three strains of *E. coli* O157:H7 isolated from ground beef, apple cider, and salami were grown at optimum (37°C) and suboptimum (10°C) growth temperatures for 16 hr and 7 days, respectively. Strains were inoculated into 0.1% peptone water (PW) and heated at 52, 54, and 56°C or stored at -20°C for heat or freeze-induced inactivation and injury, respectively. Lactic acid induced inactivation and injury was performed by inoculating and holding test strains. Growth of strains at 10°C as compared with 37°C had a significant impact on reducing ($P<0.01$) D-values obtained for heating, acid exposure, and freezing, with the exception of the cider strain stored in lactic acid. When strains were cultivated at 10 and 37°C and heated at 54 and 56°C, the salami strain possessed the highest ($P<0.01$) D-values. When grown at 10°C, the beef strain had the lowest ($P<0.01$) D-values after heating at 52, 54, and 56°C. The salami strain grown at 10°C had the highest ($P<0.01$) D-values in all concentrations of lactic acid. After growth at 37°C, the salami strain had the highest ($P<0.01$) D-values after storage in 0.1 and 0.25% lactic acid, while D-values for salami and beef strains did not differ ($P>0.05$) when stored in 0.5% lactic acid. The cider strain grown at 37°C had lower ($P<0.01$) D-values than the beef strain after storage in 0.25 and 0.5% lactic acid. Substantial portions of strain populations were sublethally injured after heat and lactic acid treatments, as

evidenced by the inability of injured organisms to form colonies on tryptone soya agar containing 2% NaCl. Strains cultured at 10°C were more susceptible to sublethal injury, as compared with strains cultured at 37°C. Storage of test strains at -20°C for 7 months resulted in a 4 to 6 log CFU/ml reduction in viable population, but induced only minimal sublethal injury. After 5 months at -20°C, strains cultured at 10°C were more sensitive to freeze-inactivation than strains cultured at 37°C. When grown at 10 and 37°C and stored at -20 for 7 months, the cider strain possessed higher ($P<0.01$) D-values than beef and salami strains. Results of this investigation indicate that the environment from which *E. coli* O157:H7 is isolated, as well as growth temperature, can influence resistance of the organism to heat-, lactic acid-, and freeze-stress.

The second investigation was an examination of the influence of package atmosphere on growth and survival of *E. coli* O157:H7 stored at 37, 10, and 4°C. The salami strain used in the previous study was used for investigation due to its resistance to heat- and lactic acid-induced inactivation and injury. The strain was heated for 10 min at 56°C, as described in the previous study, to achieve approximately 90% injury within the test population. Populations of uninjured and heat-injured *E. coli* O157:H7 were inoculated onto brain heart infusion agar containing 0.3% beef extract, packaged in barrier bags in air, 100% CO₂, 100% N₂, 20% CO₂/80% N₂, and vacuum and stored at 37, 10, and 4°C. Package atmosphere and inoculum status (i.e., uninjured or heat-injured) influenced ($P<0.01$) growth and survival of *E. coli* O157:H7 stored at all test temperatures. Growth of heat-injured *E. coli* O157:H7 was slower ($P<0.01$) than

grew during 10 days of storage at 10°C, while heat-injured populations declined during 20 days of storage at 10°C. Uninjured *E. coli* O157:H7 stored at 10°C reached stationary phase growth within approximately 10 days in all packaging atmospheres except CO₂. Populations of uninjured and heat-injured *E. coli* O157:H7 declined throughout storage for 20 days at 4°C. Survival of uninjured populations stored at 4°C, as well as heat-injured populations stored at 4 and 10°C, was enhanced in CO₂ atmosphere. Survival of heat-injured *E. coli* O157:H7 at 4 and 10°C was not different ($P>0.05$). Results of this investigation indicate that uninjured and heat-injured *E. coli* O157:H7 are able to survive at low temperatures in the modified atmospheres used in this study. Therefore, packaging treatments commonly applied to fresh beef may be ineffective at inhibiting growth and survival of this pathogen.

TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION	1
Rationale	2
Objectives of thesis	3
References	5
II. LITERATURE REVIEW	7
General characteristics	8
Distinguishing characteristics	9
Heat resistance	10
Resistance to preservatives	11
Acid and pH tolerance	11
<i>Escherichia coli</i> O157:H7 human illness	13
<i>Escherichia coli</i> O157:H7 outbreaks	15
Presence in food and animals	17
Control of <i>Escherichia coli</i> O157:H7 in food	19
Refrigeration	19
Freezing	20
Organic acid rinses	20
Heat	21
Modified atmosphere packaging	23
Bacterial injury	25
Recovery of injured bacteria	27
Factors influencing injury repair	29
References	31

III.	INACTIVATION AND INJURY OF <i>ESCHERICHIA COLI</i> O157:H7 CULTURED AT 10 AND 37°C, AS INDUCED BY HEATING, LACTIC ACID AND FREEZING.	42
	Abstract	42
	Introduction	43
	Materials and methods	46
	Results	49
	Discussion	62
	References	67
IV.	INFLUENCE OF PACKAGE ATMOSPHERE ON GROWTH AND SURVIVAL OF UNINJURED AND SUBLETHALLY HEAT-INJURED <i>ESCHERICHIA COLI</i> O157:H7	71
	Abstract	71
	Introduction	72
	Materials and methods	74
	Results	78
	Discussion	86
	References	92
V.	SUMMARY AND CONCLUSIONS	96
	VITA	98

LIST OF TABLES

TABLE		PAGE
LITERATURE REVIEW		
1.	Summary of <i>E. coli</i> O157:H7 outbreaks and implicated foods.	16
INACTIVATION AND INJURY OF <i>ESCHERICHIA COLI</i> O157:H7 CULTURED AT 10 AND 37°C, AS INDUCED BY HEATING, LACTIC ACID AND FREEZING		
1.	D-values for <i>E. coli</i> O157:H7 strains grown at 10 and 37°C and subjected to heat, lactic acid, and freezing.	54
2.	Maximum injury in <i>E. coli</i> O157:H7 strains grown at 10 and 37°C and subjected to heat, lactic acid, and freezing.	56
INFLUENCE OF PACKAGE ATMOSPHERE ON GROWTH AND SURVIVAL OF UNINJURED AND SUBLETHALLY HEAT-INJURED <i>ESCHERICHIA COLI</i> O157:H7		
1.	pH of packaged BEM samples stored at 37, 10, and 4°C.	79
2.	Survival of sublethally heat-injured <i>E. coli</i> O157:H7 stored at 10 and 4°C in different gaseous atmospheres.	85

LIST OF FIGURES

FIGURE		PAGE
INACTIVATION AND INJURY OF <i>ESCHERICHIA COLI</i> O157:H7 CULTURED AT 10 AND 37°C, AS INDUCED BY HEATING, LACTIC ACID AND FREEZING		
1.	Inactivation and injury of three strains of <i>E. coli</i> O157:H7 heated at 52°C in 0.1% peptone water.	51
2.	Inactivation and injury of three strains of <i>E. coli</i> O157:H7 heated at 54°C in 0.1% peptone water.	52
3.	Inactivation and injury of three strains of <i>E. coli</i> O157:H7 heated at 56°C in 0.1% peptone water.	53
4.	Inactivation and injury of three strains of <i>E. coli</i> O157:H7 in 0.1% peptone water containing 0.1% of an 85% lactic acid solution.	57
5.	Inactivation and injury of three strains of <i>E. coli</i> O157:H7 in 0.1% peptone water containing 0.25% of an 85% lactic acid solution.	58
6.	Inactivation and injury of three strains of <i>E. coli</i> O157:H7 in 0.1% peptone water containing 0.5% of an 85% lactic acid solution.	59
7.	Inactivation and injury of three strains of <i>E. coli</i> O157:H7 stored at -20°C in 0.1% peptone water.	61
INFLUENCE OF PACKAGE ATMOSPHERE ON GROWTH AND SURVIVAL OF UNINJURED AND SUBLETHALLY HEAT-INJURED <i>ESCHERICHIA COLI</i> O157:H7		
1.	Growth of uninjured <i>E. coli</i> O157:H7 at 37°C on beef extract medium packaged in air, 100% CO ₂ , 100% N ₂ , 20% CO ₂ /80% N ₂ , and vacuum	81

2.	Growth of sublethally heat-injured <i>E. coli</i> O157:H7 at 37°C on beef extract medium packaged in air, 100% CO ₂ , 100% N ₂ , 20% CO ₂ /80% N ₂ , and vacuum.	82
3.	Growth of uninjured <i>E. coli</i> O157:H7 at 10°C on beef extract medium packaged in air, 100% CO ₂ , 100% N ₂ , 20% CO ₂ /80% N ₂ , and vacuum.	84
4.	Survival of uninjured <i>E. coli</i> O157:H7 at 4°C on beef extract medium packaged in air, 100% CO ₂ , 100% N ₂ , and 20% CO ₂ /80% N ₂ , and vacuum.	87

CHAPTER I

INTRODUCTION

In January of 1993, an unusually high number of patients with symptoms consistent with *Escherichia coli* O157:H7 infection became apparent to physicians at the Children's Hospital and Medical Center in Seattle, Washington (Tarr, 1994). Over 600 patients with hemorrhagic colitis and/or hemolytic uremic syndrome were diagnosed. Seventy five percent of the infections were linked to consumption of ground beef; 3 died, and approximately 40% of the infected individuals acquired hemolytic uremic syndrome (Tarr, 1994). Over half of these patients required kidney dialysis or hemofiltration for acute kidney failure or electrolyte balance, and most required transfusions to overcome the hemolytic anemia and thrombocytopenia (Tarr, 1994).

Strains of *E. coli* O157:H7 can cause severe human illnesses such as hemorrhagic colitis and hemolytic uremic syndrome, although less severe symptoms may include nausea, vomiting, watery diarrhea, abdominal cramps, fever, and malaise (Molenda, 1994). Unlike most foodborne bacterial pathogens, *E. coli* O157:H7 can induce illness in humans when as few as 10 organisms are ingested. In the United States, over 250 deaths and 20,000 infections each year are attributed to *E. coli* O157:H7 (Boyce et al., 1995a). However, only 59% of 230 randomly selected North American clinical laboratories that perform stool cultures routinely screen for *E. coli* O157:H7 in cases of bloody or non-bloody diarrhea (Boyce et al., 1995b).

Domestic cattle have been suggested to be important reservoirs of *E. coli* O157:H7, and in 1994, the United States Department of Agriculture (USDA) declared that raw ground beef harboring *E. coli* O157:H7 would be considered "adulterated" and prohibited from sale. The USDA employs several methods to reduce contamination of *E. coli* O157:H7 and other bacteria present on beef. These include organic acid rinses, hot water rinses, and steam pasteurization, but the effectiveness of such measures is questionable, as evidenced by recent *E. coli* O157:H7 outbreaks linked to beef. Foods such as apple cider (Besser et al., 1993), roast beef (Rodrigue et al., 1995), salami (Alexander et al., 1995), yogurt (Morgan et al., 1993), and turkey roll (Salmon et al., 1989) have also been implicated vehicles of *E. coli* O157:H7 infection.

RATIONALE

Although minimal processes applied to foods can substantially reduce bacterial populations present in food, bacteria are often rendered injured by these processes (Busta, 1994). Injury within bacterial populations can be beneficial in extending shelf-life, due to extending the lag phase of populations present in the food. However, problems can arise because these injured bacteria often exhibit increased sensitivity to compounds commonly used in selective enumeration media (Busta, 1994). As a result, typical enumeration protocols can give misleading results regarding the population of viable but injured bacteria present, and if storage conditions are suitable for repair, bacteria can grow to substantial populations rendering the food potentially dangerous for

consumption.

Modified atmosphere packaging (MAP) is an effective method of extending shelf-life of foods. Approximately 80% of fresh beef in the United States is vacuum packaged before leaving the packaging facility, primarily due to its effectiveness at increasing shelf-life (Jay, 1996). However, the vulnerability of MAP foods from a safety standpoint is that with many modified atmospheres containing moderate to high levels of carbon dioxide, the aerobic spoilage organisms which usually warn consumers with signs of spoilage (i.e., off-odors and flavors) are inhibited, while growth of pathogenic bacteria may be unaffected or even stimulated (Farber, 1991). Modified atmospheres commonly used for fresh vegetables have no effect on the growth and survival of *E. coli* O157:H7 (Hao and Brackett, 1993), while anaerobic conditions increase tolerance of *E. coli* O157:H7 to heat stress (Gadzella and Ingram, 1994; Murano and Pierson, 1992). Golden et al. (1989) reported that survival of uninjured and heat-injured *Aeromonas hydrophila* at 4°C is enhanced by atmospheres of 100%N₂.

OBJECTIVES

The objectives of this thesis are:

- (1) To determine the influence of growth temperature on inactivation and development of injury in *E. coli* O157:H7, as induced by heating, lactic acid, and freezing.

- (2) To assess the general heat-, lactic acid-, and freeze-resistance of *E. coli* O157:H7.
- (3) To evaluate the variability exhibited among *E. coli* O157:H7 strains isolated from different food environments to the stresses noted above.
- (4) To determine the influence of modified atmosphere on survival and growth of uninjured and sublethally heat-injured *E. coli* O157:H7 stored at 37, 10, and 4°C.

REFERENCES

- Alexander, E.R., J. Boase, M. Davis, L. Kirchner, C. Osaki, T. Tanino, M. Samadpour, P. Tarr, M. Goldoft, S. Lankford, J. Kobyashi, P. Stehr-Green, P. Bradley, B. Hinton, P. Tghe, B. Pearson, G.R. Flores, R. Bryant, S.B. Werner, and D.J. Vugia. 1995. *Escherichia coli* O157:H7 outbreak linked to commercially distributed dry-cured salami- Washington and California. MMWR. 44(9):157-159.
- Besser, R.E., S.M. Lett, J.T. Beber, M.P. Doyle, T.J. Barret, J.G. Wells, and P.M. Griffin. 1993. An outbreak of diarrhea and hemorrhagic uremic syndrome from *Escherichia coli* O157:H7 in fresh pressed apple cider. JAMA. 269:2217-2220.
- Busta, F.F. 1994. Importance of injured food-borne microorganisms in minimal processing. In Minimal processing of foods and process optimization. Edited by R.P. Singh and F.A.R. Oliveira. CRC Press, Boca Raton. pp. 227-231.
- Boyce, T.G., D.L. Swerdlow, and P.M. Griffin. 1995a. *Escherichia coli* O157:H7 and the hemolytic-uremic-syndrome. New Eng. J. Med. 333:364-368.
- Boyce, T.A., A.G. Pemberton, J.G. Wells, and P.M. Griffin. 1995b. Screening for *Escherichia coli* O157:H7-a nationwide survey of clinical laboratories. 1995. J. Clin. Microbiol. 33:3275-3277.
- Gadzella, T.A. and S.C. Ingram. 1994. Heat shock, anaerobic jar incubation and fluid thioglycollate medium have contrasting effects on D-values of *Escherichia coli*. J. Food Prot. 57:671-673.
- Golden, D.A., M.J. Eyles, and L.R. Beuchat. 1989. Influence of modified-atmosphere storage on the growth of uninjured and heat injured *Aeromonas hydrophila*. Appl. Environ. Microbiol. 55:3012-3015.
- Farber, J.M. 1991. Microbiological aspects of modified-atmosphere packaging technology - a review. J. Food Prot. 54:58-70.
- Hao, Y.Y., and R.E. Brackett. 1993. Growth of *Escherichia coli* O157:H7 in modified atmosphere. J. Food Prot. 56:330-332.
- Jay, J.M. 1996. Modern food microbiology. 5th ed. Chapman and Hall, New York, NY.
- Molenda, J.R. 1994. *Escherichia coli* (including O157:H7): an environmental health perspective. Dairy Food Environ. Sanit. 14:742-747.

- Morgan, D., C.P. Newman, D.N. Hutchinson, A.M. Walker, B. Rowe, and F. Majid. 1993. Verotoxin producing *Escherichia coli* O157 infections associated with consumption of yogurt. *Epidemiol. Infect.* 111:181-187.
- Murano, E.A. and M.D. Pierson. 1993. Effect of heat shock and incubation atmosphere on injury and recovery of *Escherichia coli* O157:H7. *J. Food Prot.* 56:568-572.
- Rodrigue, D.C., E.E. Mast, K.D. Greene, J.P. Davis, M.A. Hutchinson, J.G. Wells, T.J. Barret, P.M. Griffin. 1995. A University outbreak of *Escherichia coli* O157:H7 infections associated with roast beef and an unusually benign clinical course. *J. Infect. Dis.* 172:1122-1125.
- Salmon, R.L., I.D. Farrell, J.G.P. Hutchinson, D.J. Coleman R.J. Gross, N.K. Fry, B. Rowe, and S.R. Palmer. 1989. A christening party outbreak of hemorrhagic colitis and haemolytic uraemic syndrome associated with *Escherichia coli* O157:H7. *Epidem. Inf.* 103:249-254.
- Tarr, P.I. 1994. *Escherichia coli* O157:H7: overview of clinical and epidemiological issues. *J. Food Prot.* 57:632-636.

CHAPTER II

LITERATURE REVIEW

In 1885, Theodore Escherich discovered and described the properties of *Bacterium coli commune*, which later was renamed *Escherichia coli*, from infant feces, (Sussman, 1985). *E. coli* was first regarded as non-pathogenic, but Escherich later demonstrated that it was present in the bladder of young girls suffering from urinary tract infections (Sussman, 1985). In the late 1950's, interest in *E. coli* resurged due to its role in enteric and septicemic diseases in animals (Loir, 1994a). Serotyping emerged as an important tool in describing *E. coli*, and was instrumental in the differentiation and specification of different groups of *E. coli* based on proteins present in the outer membrane, flagella, and capsule of the bacterium.

Two outbreaks of bloody diarrhea in Oregon and Michigan (Riley et al., 1983; Wells et al., 1983) led to the recognition of *Escherichia coli* O157:H7 as a foodborne pathogen in 1982. Although it had not been identified, *E. coli* O157:H7 is thought to have been responsible in a 1980 outbreak involving apple juice (Steele et al., 1982), when several individuals experienced the same characteristic symptoms as those infected with *E. coli* O157:H7 in 1982. O'Brien et al. (1993) reported that five strains isolated from the 1993 *E. coli* O157:H7 outbreak in the Pacific Northwestern United States were indistinguishable from each other and were consistent with the *E. coli* O157:H7 strain EDL933, first isolated in 1982.

GENERAL CHARACTERISTICS

E. coli O157:H7 is a Gram-negative, rod shaped bacillus, and member of the Enterobacteriaceae family of bacteria. *E. coli* O157:H7 is a non-sporulating, facultative anaerobe approximately 1 x 2 µm in size (Neidhardt et al., 1990). It is non-fastidious in its nutritional requirements and is easily cultivated on standard laboratory culture media. All isolated strains of *E. coli* O157:H7 are motile, possessing six flagella (Molenda, 1994).

E. coli O157:H7 grows well at 30 to 42°C, with 37°C being the optimum growth temperature (Doyle and Schoeni, 1984). The minimum reported growth temperature for *E. coli* O157:H7 in laboratory culture medium is 8°C (Rajkowski and Marmer, 1995; Weeratna and Doyle, 1991). Doyle and Schoeni (1984) reported generation times for *E. coli* O157:H7 in culture medium ranging from 0.49 hr at 37°C to 0.64 hr at 42°C.

E. coli O157:H7 is a natural inhabitant of the intestinal tracts of warm blooded animals and is commonly used as an indicator of fecal contamination in water and foods, but unlike typical fecal coliforms, *E. coli* O157:H7 does not grow at 45°C (Doyle and Schoeni, 1984). Typical enumeration procedures for fecal coliforms in water and foods require incubation at 44.6°C in *E. coli* medium after screening for lactose fermentation and gas production in lauryl sulfate tryptose broth at 35°C (Hitchins et al., 1992). Growth and gas production within 48 hr in *E. coli* medium at 44.5°C is presumptive for the presence of fecal coliforms, but the minimum and maximum temperatures at which *E.*

coli O157:H7 exhibits growth and gas production after 48 hr in *E. coli* medium is 19.3 and 41°C (Raghubeer and Matches, 1990).

DISTINGUISHING CHARACTERISTICS

Several studies have illustrated differences between *E. coli* O157:H7 and non-pathogenic strains of *E. coli*. *E. coli* O157:H7 represents 7% of *Escherichia* spp. of human origin which lack the ability to rapidly ferment sorbitol (Padhye and Doyle, 1992). A commonly used differential/selective recovery medium for *E. coli* is sorbitol macConkey agar (March and Ratnam, 1986), in which *E. coli* O157:H7 is distinguished from other serotypes of *E. coli* by its inability to ferment sorbitol within 24 to 48 hr. Reportedly, *E. coli* O157:H7 will ferment sorbitol after 6 to 7 days at 37°C, compared to utilization within 24 hr for typical *E. coli*. However, some *E. coli* O157:H7 isolates can rapidly ferment sorbitol (Fratamico et al., 1993), indicating the ability of the organism to mutate under suitable conditions.

Another distinguishing characteristic between *E. coli* and *E. coli* O157:H7 is the presence of the enzyme β -D-glucuronidase (GUD), which serves as the basis for the rapid fluorogenic assay in which glucuronide is hydrolyzed forming a fluorogenic compound (Okrend et al., 1990). Unlike nonpathogenic *E. coli*, *E. coli* O157:H7 does not possess GUD. Okrend et al. (1990) reported that false positive identifications for *E. coli* O157:H7 were reduced by 36% when GUD was used. However, GUD positive *E. coli* O157:H7 strains have been identified in the United States, suggesting that phenotypic variants may

be a concern. In Germany, Gunzer et al. (1992) reported that 17 of 44 SLT-II producing strains isolated from patients with diarrhea rapidly fermented sorbitol and were GUD positive.

The presence of the O157:H7 antigen is exclusive from all other strains of *E. coli* and can be detected by several agglutination methods, as reviewed by Padhye and Doyle (1992). Identification should not be solely based on serology, as 17% of *Escherichia hermannii* strains will test positive for presence of the O157 antigen (Borczyk et al., 1987; Rice et al., 1992). Similarly, Notenboom et al. (1986) reported that *Brucellus abortus* and *E. coli* O157:H7 are antigenically related and may also result in O157 agglutination cross reaction.

HEAT RESISTANCE

E. coli O157:H7 possesses no unusual heat resistance, and is more sensitive to elevated temperatures than certain *Salmonella* spp. (Doyle and Schoeni, 1984). The organism is easily killed by pasteurization temperatures (Doyle and Schoeni, 1984). Heat resistance of the *E. coli* O157:H7 in meat has been of particular interest due to *E. coli* O157:H7 outbreaks involving improperly cooked ground beef. D-values of 4.5 min at 57.2°C and 0.02 min at 60°C have been reported for *E. coli* O157:H7 in ground beef (Doyle and Schoeni, 1984). Riordan et al. (1996) reported D-values for *E. coli* O157:H7 in pepperoni of 15.5 min at 55°C and 1.4 min at 60°C.

RESISTANCE TO PRESERVATIVES

The maximum concentration of NaCl in tryptone soya broth in which *E. coli* O157:H7 exhibits growth at 37°C is 6.5%, whereas inactivation occurs at 8.5% NaCl (Gibson and Roberts, 1986; Glass et al., 1992). Enteropathogenic *E. coli* can grow in broth medium (pH 5.6) containing up to 200 ppm nitrite and 4% NaCl (Gibson and Roberts, 1986). Glass et al. (1992) reported that populations of *E. coli* O157:H7 were reduced in fermented sausage (pH 4.8) containing 3.5% NaCl and 69 ppm sodium nitrite after storage for 2 months at 4°C. Throughout standard pepperoni (2.5% salt, 100 ppm sodium nitrite, pH 4) production, fermentation, and drying, only minimal reductions in *E. coli* O157:H7 were observed (Riordan et al., 1996). At 10°C, *E. coli* O157:H7 can grow in soft Hispanic-type cheese; however, addition of 0.3 % sodium benzoate or potassium sorbate to cheese made from milk acidified with propionic acid has a significant impact on delaying or preventing growth of the organism (Kasrazadeh and Genigeorgis, 1995).

ACID AND pH TOLERANCE

The "low acid canned food regulations" of the U.S. Food and Drug Administration state that foods possessing pH below 4.6 are safe from the growth and survival of pathogenic bacteria (Food and Drug Administration, 1993). Nevertheless, *E. coli* O157:H7 outbreaks linked to apple cider (Besser et al., 1993) and mayonnaise (Weagant et al., 1994) present substantial evidence for tolerance of *E. coli* O157:H7 to reduced pH environments. The addition of 40% mayonnaise to ground roasted beef held

at 4°C had no effect on the survival of *E. coli* O157:H7, and at 21 and 30°C, the organism grew in ground roasted beef containing 16 to 32% mayonnaise (Abdoul-Raouf et al., 1993b). Glass et al. (1992) reported that the minimum pH at which *E. coli* O157:H7 can grow at 37°C is between 4.0 and 4.5 or 4.6 when tryptone soya broth is adjusted with HCl or lactic acid, respectively.

E. coli O157:H7 can survive in culture medium at pH of 4.0 (Conner and Kotrola, 1995), although survival is greatly influenced by the type of organic acid and incubation temperature. Glass et al. (1992) reported that reducing pH with lactic acid as compared to HCl resulted in a more inhibitory response from *E. coli* O157:H7. Conner and Kotrola (1995) also observed that, at 4°C, organic acids enhanced the survival of *E. coli* O157:H7 when compared to non-acidulated controls. This "protective effect" was also noted in apple cider, in which populations of *E. coli* O157:H7 survived for 10 to 31 days or 2 to 3 days after storage at 8 or 25°C, respectively (Zhao et al., 1993).

The process of fermentation has long been used as an effective means of preserving foods via the rapid decrease in pH from acids produced during the fermentation process. Feresu and Nyati (1990) reported that pathogenic *E. coli* grew during the fermentation of pasteurized and unpasteurized milk, and decreased only minimally during storage of fermented milk held at 5 and 20°C. Glass et al. (1992) reported only slight decreases in *E. coli* O157:H7 during the fermentation, drying and storage of dry sausage at 4°C. However, Semanchek and Golden (1996) demonstrated

that alcoholic fermentation of apple cider results in destruction of *E. coli* O157:H7 within 3 days.

***ESCHERICHIA COLI* O157:H7 HUMAN ILLNESS**

E. coli infections can be divided into two broad categories: intestinal infections, also referred to as diarrheagenic, and extra-intestinal infections (Molenda, 1994).

Diarrheagenic *E. coli* can be further subdivided into five types, based on the type of infection that the strain produces in the host and the somatic and flagellar antigenic profile of the strain (Molenda, 1994). The five types of diarrheagenic *E. coli* are: enteropathogenic *E. coli*, enterotoxigenic *E. coli*, enteroinvasive *E. coli*, enteroadherent *E. coli*, and enterohemorrhagic *E. coli*.

Enterohemorrhagic *E. coli* (EHEC) have recently been responsible for several foodborne borne outbreaks in the United States, including the “Jack-in-the-Box” outbreak of *E. coli* O157:H7 in the western United States (Dorn, 1993). The most frequently implicated serotype is O157:H7, but others such as O26:H11 (Levine, 1987), O111:H8 (Molenda, 1994), O4:NM, O45:H2, O111:NM, and O145:NM (Tzipori et al., 1988) also have caused illness in humans.

Typically, EHEC infected individuals develop hemorrhagic colitis, which is characterized by severe abdominal cramps, vomiting, little or no fever, and watery stools which may progress to bloody diarrhea (Loir, 1994b). Less severe symptoms, such as nausea and malaise, have also been reported (Molenda, 1994). Advanced illnesses

associated with EHEC strains involve hemolytic uremic syndrome (HUS) which develops in 2 to 7% of individuals with EHEC intestinal infections (Molenda, 1994). HUS is characterized by renal failure, microangiopathic hemolytic anemia (i.e., anemia in which red blood cells are smaller than normal and have shortened survival) and severe thrombocytopenia purpura (i.e., reduction in number of platelets) (Molenda, 1994). Onset of HUS ensues symptoms of non-bloody diarrhea and severe abdominal cramps, and leads to bloody stools by day 2 or 3 that continue for 6 to 8 days (Griffin and Tauxe, 1991). HUS can also lead to central nervous system complications and death in 15% of infected individuals (Tarr, 1994). More frequently, individuals with HUS often require kidney dialysis or need blood transfusions (Molenda, 1994).

According to an epidemiological review by Griffin and Tauxe (1991), individuals high at risk for developing HUS include females, the young and elderly, the mentally retarded, persons with recent antimicrobial use, and individuals with bloody diarrhea or fever. In a nursing home related outbreak, the highest rate of diarrhea was present in the very old; diarrhea was also present in persons undergoing antimicrobial therapy, and those who had previous gastrectomy (Carter et al., 1987). Based on sporadic cases, the highest attack rates have involved children under five years of age, with the attack rate decreasing in individuals aged 45 to 64 years, then increasing in elderly persons (Griffin and Tauxe, 1991).

Treatment for *E. coli* O157:H7 infections rarely involves antibiotic therapy, because there is concern that toxin liberation from antibiotic-damaged cells will be more

available for systemic absorption (Tarr, 1993). Mild cases of *E. coli* infection commonly are treated by replacing body fluids and electrolytes. For individuals with HUS, transfusions and dialysis are the only therapies (Tarr, 1993).

***ESCHERICHIA COLI* O157:H7 OUTBREAKS**

The threat associated with *E. coli* O157:H7 lies not only in the pathogenicity of the organism, but in the great variability exhibited within strains, thus allowing the organism to persist in numerous foods. Table 1 contains a summary of *E. coli* O157:H7 outbreaks and implicated foods.

In addition to food, recreational lakes have been implicated as sources of *E. coli* O157:H7 transmission, stressing concern for proper monitoring of recreational lakes and other bodies of water contributing to the water supply. Twenty-one children in Oregon were infected with *E. coli* O157:H7 due to swimming in a fecally contaminated lakeside park (Keene et al., 1994). Another lake associated *E. coli* O157:H7 outbreak occurred in Illinois in 1995, when 12 individuals became symptomatic after swimming in a common recreational lake (Warner et al., 1996).

Person-to-person contact also has been responsible for *E. coli* O157:H7 outbreaks, as evidenced by several illnesses within nursing homes. In September 1984, 34 of 101 nursing home residents developed diarrheal illness due to *E. coli* O157:H7 infections; 14 were hospitalized and 4 died (Ryan et al., 1986).

Table 1. Summary of *E. coli* O157:H7 outbreaks and implicated foods.

Year	Vehicle	Cases	Location	Ref.
1980	Apple juice ^a	14	Canada	Steele et al., 1982
1982	Hamburger meat	>47	Oregon, Michigan	Riley et al., 1983 Wells et al., 1983
1985	Raw potatoes	24	United Kingdom	Jay, 1996
1986	Raw milk	46	Canada	Jay, 1996
1987	Turkey roll sandwiches	26	Birmingham	Salmon et al., 1989
1988	Roast beef and salad	61	Wisconsin	Rodrigue et al., 1995
1988	Precooked meat patties	32	Minnesota	Jay, 1996
1990	Well water	243	Missouri	Swerdlow et al., 1992
1990	Roast beef	>70	North Dakota	Jay, 1996
1991	Yogurt	9	England	Morgan et al., 1993
1991	Apple cider	4	Massachusetts	Besser et al., 1993
1992- 93	Hamburger meat	>600	Washington, Idaho, California, Nevada	Davis et al., 1993 Dorn, 1993 Tarr, 1993
1993	Home-cooked hamburgers	10	California	Jay, 1996
1994	Rare ground beef	18	Virginia	Frost et al., 1995
1994	Dry-cured salami	12	Washington, California	Alexander et al., 1995
1995	Hamburger meat	10	Georgia, Tennessee	Cannon et al., 1996

^aLinked to *E. coli* O157:H7 because symptoms were characteristic of later outbreaks.

One year later, *E. coli* O157:H7 was responsible for another nursing home outbreak, when 55 of 169 residents and 18 of 187 staff members in Ontario became ill; 17 patients died (Carter et al., 1987; Krishnan, 1987).

PRESENCE IN FOOD AND ANIMALS

Approximately 176 serogroups of *E. coli*, many of which are harmless natural inhabitants of the human intestinal tract, have been identified in the last 50 years. Many serogroups benefit humans by suppressing growth of harmful bacteria and synthesizing vitamins necessary for growth and well-being (Duncan and Hackney, 1994). Although most strains of *E. coli* typically found in humans are harmless, some types are pathogenic, such as *E. coli* O157:H7. Antigenic proteins present on the cell surface (O antigen) and flagellum (H antigen) are phenotypically distinguishing characteristics between *E. coli* and *E. coli* O157:H7. These antigenic determinants are responsible for inducing human immune responses when present in the human body.

Doyle and Schoeni (1987) investigated the prevalence of *E. coli* O157:H7 in meats and reported that 3.7% of 164 beef, 1.5% of 264 pork, 1.5% of 263 poultry, and 2.0% of 205 lamb samples tested positive for *E. coli* O157:H7. More recently, a survey of meat and seafood was conducted in Seattle following the 1993 *E. coli* O157:H7 outbreak. *E. coli* O157:H7 was present in 63% of 8 veal, 48% of 21 lamb, 23% of 60 beef, 18% of 51 pork, 12% of 33 chicken, 10% of 62 fish, 7% of 15 turkey, and 4.5% of 44 shellfish samples tested (Samadpour et al., 1994). In 1995, the Food Safety and Inspection Service

of the United States Department of Agriculture (USDA) conducted a nationwide survey of ground beef and reported that only 3 of 5291 samples (0.05%) tested positive for *E. coli* O157:H7 (Jay, 1996).

Studies in the United States reveal that the prevalence of *E. coli* O157:H7 in cattle ranges from <1 to 5% (Martin et al., 1986). Wells et al. (1991) isolated fecal specimens of *E. coli* O157:H7 in 5 of 210 calves, 12 of 394 heifers, and 1 of 662 cows, from a herd linked to two human cases of *E. coli* O157:H7 infection. In a similar investigation representing 14 states, young cattle again seemed to be more susceptible to *E. coli* O157:H7 infections, as 4.9 to 5.3% of weaned calves and 1.5 to 2.9% of preweaned calves shed *E. coli* O157:H7 in their feces; young cattle are probably more susceptible to *E. coli* O157:H7 colonization due to undeveloped intestinal flora (Zhao et al., 1995). It has been reported that in experimentally infected cattle, calves shed *E. coli* O157:H7 longer than cows, and that most cattle infected with *E. coli* O157:H7 will remain free of clinical signs of illness (Cray and Moon, 1995).

Cray and Moon (1995) reported that *E. coli* O157:H7 experimentally inoculated into cattle were restricted to the gastrointestinal tract. Rasmussen et al. (1993) investigated the effect of cattle rumen fermentation as a barrier to *E. coli* O157:H7 and found that the organism exhibited unrestricted growth in rumen fluid collected from fasted cattle, whereas inhibition of growth occurred in rumen fluid from well-fed cattle. Also, like the rumen, the colon and cecum house anaerobic fermentations that respond to

dietary manipulations, and if conditions turn favorable, fecal shedding can be more prevalent (Grau et al., 1969).

CONTROL OF *ESCHERICHIA COLI* O157:H7 IN FOOD

Because of outbreaks linked to animal products (e.g., beef and milk), domestic cattle have been suggested to be important reservoirs of *E. coli* O157:H7. In 1994, the USDA declared that raw ground beef harboring *E. coli* O157:H7 would be considered "adulterated" and prohibited from sale. This bold initiative marked the first time a raw food has been rendered hazardous by the USDA for containing pathogenic bacteria. Although retail beef free of pathogenic bacteria may not be a reasonable goal for the food industry, current methods of beef decontamination have proved to be ineffective, as evidenced by sporadic *E. coli* O157:H7 outbreaks involving hamburger meat.

Refrigeration. Refrigeration is an essential tool for extending shelf-life of semi-perishable and perishable foods. Refrigerated storage is based on the principle that certain activities of all organisms can be slowed at low temperatures and virtually inhibited at freezing temperatures (Jay, 1996). Also, enzymatic activities that may affect shelf-life are either inhibited or greatly reduced at refrigeration temperatures. Although refrigeration is often used to restrict bacterial growth, many pathogens such as *Listeria monocytogenes* and *Yersinia enterocolitica* grow at refrigerated temperatures (Jay, 1996).

Several studies have reported on the survival of *E. coli* O157:H7 during low temperature storage (Conner and Hall, 1994; Rajkowski and Marmer, 1995), with

refrigeration enhancing survival of *E. coli* O157:H7 in apple cider (Besser et al., 1993; Miller and Kaspar, 1994; Zhao et al., 1993). *E. coli* O157:H7 survived for up to 35 days in mayonnaise-based sauces held at 7°C, and up to 51 and 79 days in reduced-calorie and real mayonnaise, respectively, held at 5°C (Weagant et al., 1994)

Freezing. In a laboratory study with ground beef stored at -20°C, *E. coli* O157:H7 exhibited little reduction in population during nine months of storage (Padhye and Doyle, 1992). Smith (1995) reported that stationary phase *E. coli* were not injured by freezing, and concluded that injury due to freeze stresses may be a factor associated with the growth phase of the organism and nutrient-limiting media.

Hartsell (1951) reported that when frozen on peas and beef, several Gram-negative pathogens became sensitive to selective media and were more effectively recovered on media with increased nutrients, and therefore stresses due to freezing may alter nutritional requirements of Gram-negative pathogens. Hanna et al. (1977) reported a significant reduction of *Y. enterocolitica* in beef roasts stored at -18 to -20°C. When frozen in phosphate buffer at -18 and -75°C, *Y. enterocolitica* exhibited 7 and 42% reductions, respectively (Grecz and El-Zawahry, 1984). Although common foodborne pathogens may become injured during freezer storage, their survival can present a great threat when foods are improperly cooked after freezing or when thawing conditions permit injury repair and growth.

Organic acid rinses. In 1992, the USDA approved the application of organic acid rinses to beef carcasses, to reduce contamination by foodborne pathogens (Reagan, 1993).

Common organic acids that are applied to animal carcasses after slaughter include acetic, citric, lactic, and to a lesser extent formic and ascorbic acids (Jay, 1996). Beneficial aspects of applying organic acid rinses appear to result from the decrease in surface pH and possibly to some direct effect of the acid molecules (Jay, 1996). Undissociated acid molecules interfere with cellular metabolism or decrease biological activity as a result of pH changes of the cell environment (Doores, 1983).

Hamby et al. (1987) reported that beef sides intermittently sprayed with 1% lactic or acetic acid for up to 12 hr resulted in extensive reductions in aerobically growing organisms of selected cuts of beef after vacuum packaged storage at 2°C for 28 days. Brackett et al. (1994) found no significant reduction in *E. coli* O157:H7 when heated and non-heated treatments of 0.5%, 1.0%, and 1.5% concentrations of acetic, lactic, and citric acids were applied to fresh beef. However, upon treatment of beef with 1.5% lactic acid/1.5% gluconic acid and 1.5% lactic acid/3.0% gluconic acid, Zepeda et al. (1994) observed 2.0 and 2.5 log reductions, respectively, in viable *E. coli* O157:H7. When concentrations of 5% lactic, acetic, or citric acid were used, Cutter and Siragusa (1994) reported significant reductions in *E. coli* O157:H7, but total inactivation of the organism was not achieved. Anderson and Marshall (1989) and Greer and Dilts (1992) reported that increasing temperature and concentration of acetic acid sprays independently had significant effects on reducing populations of pathogens on beef.

Heat. The USDA recently approved a steam pasteurization process in order to reduce beef carcass contamination. When steam pasteurization was applied to beef sides,

significant reductions in overall bacteria resulted immediately after treatment and after 24 hr (Nutsch et al., 1996). Rinsing beef carcasses with hot water (92°C) is also an effective means of reducing contamination (Barkate et al. 1993). Anderson and Marshall (1989) reported that increasing organic acid concentrations applied to beef carcasses were only effective at lower temperatures, whereas hot water (70°C) alone reduced *E. coli* populations as much as any concentration of acetic acid tested.

Heat induced inactivation of *E. coli* can be described in terms of cell death with lysis and death in the absence of lysis. Cell death with lysis has been attributed to DNA damage (Woodcock and Grigg, 1972), whereas cell death without cell lysis is probably due to the presence of heat-sensitive wall structures in cells near division or to inhibiting enzymes responsible for peptidoglycan synthesis near the cell septum (Membrillo-Hernandez et al., 1995). Heat stress affects catalase enzyme systems, ribosomes, teichoic acids containing structures, and the outer membrane of *E. coli* (Ray, 1986). Bacterial catalase enzyme systems are important to aerobically living cells, functioning to degrade harmful peroxides and free oxygen radicals. When these enzyme systems are damaged, peroxides and oxygen radicals are free to react with cellular components often resulting in cell lysis and death. Martin et al. (1976) found that bacterial stress due to heat and NaCl resulted in decreased catalase activity in *Staphylococcus aureus*. The observation by Harries and Russell (1966) that heat stressed *E. coli* grew better when pour plating (reduced oxygen), as compared to surface plating (aerobic) on the same agar, may possibly be explained by the role catalase plays in degrading peroxides.

Modified atmosphere packaging. The use of modified atmosphere packaging (MAP) has become increasingly popular in North America as food manufacturers attempt to meet consumer demands for fresh refrigerated foods with extended shelf-life (Farber, 1991). MAP, although relatively new in North America, has been used overseas for fresh meats dating back to the 1930's (Farber, 1991; Jay, 1996). The popularity of MAP has evolved such that many fruits, vegetables, and fresh meats are currently packaged under modified atmospheres (Farber, 1991).

MAP characteristically involves the use of three gases: oxygen (O_2), carbon dioxide (CO_2), and nitrogen (N_2). O_2 plays a key role in oxygenating myoglobin, forming the deep red color associated with fresh meat (Farber, 1991; Jay, 1996). Oxygen can often stimulate growth of aerobic organisms and inhibit growth of strict anaerobes such as *Clostridium* spp. (Jay, 1996). The presence of O_2 in perishable, packaged foods supports growth of indigenous microflora which provides consumers with signs of spoilage, such as the appearance of off-odors or colors.

CO_2 functions in MAP by imparting a bacteriostatic effect on microorganisms (Farber, 1991). The overall effect of high concentrations of CO_2 is to shift the heterogeneous microflora consisting of Gram-negative bacteria to microflora consisting primarily of lactic acid bacteria (Jay, 1996). The inhibitory effect of CO_2 is not fully understood; however, CO_2 is believed to affect nutrient uptake, enzymatic activity, and cell membrane integrity, and causes adverse changes in the physio-chemical properties of proteins in the cell (Farber, 1991). The effects of CO_2 are temperature dependent, in that

inhibitory effects of CO₂ increase with increasing temperature, primarily due to the increased solubility of CO₂ at lower temperatures (Wolfe, 1980). Also, the pH of beef stored in high CO₂ environments is often lower than beef stored in air, due to carbonic acid formation (Jay, 1996). The presence of CO₂ in a package selects for growth of facultative anaerobes, such as *E. coli* O157:H7, and because visible signs of spoilage are not present, false conclusions can be made concerning the freshness and safety of the food (Hintlian and Hotchkiss, 1986).

N₂ is an inert, tasteless gas, functioning primarily as a filler (Farber, 1991; Jay, 1996) in MAP foods. It is believed to delay oxidative rancidity and growth of certain microorganisms by displacing O₂ present in the package (Farber, 1991). Although Blickstad and Molin (1983) reported that packaging pork loins and frankfurters in 100% N resulted in a longer shelf-life than packaging under vacuum, other investigators have reported just the opposite (Jay, 1996). Packaging salad vegetables inoculated with *E. coli* O157:H7 in 3% O₂ and 97% N₂ had no effect on the organism (Abdul-Raouf et al., 1993a).

Approximately 80% of fresh beef in the United States is vacuum packaged before leaving the processing facility, primarily due to its effectiveness in extending shelf-life (Jay, 1996). Vacuum packaging serves to extend food shelf-life by eliminating most of the O₂ in the package. Residual O₂ is consumed by aerobic microflora or respiratory activities of the food, resulting in decreased levels of O₂ and increased levels of CO₂ (Jay, 1996).

Several investigators have reported that MAP extends the shelf-life of fresh foods, but has little or no effect on pathogenic organisms such as *L. monocytogenes* (Berrang et al. 1989). Beuchat and Brackett (1990) reported that *L. monocytogenes* was capable of growth on lettuce subjected to packaging and distribution procedures commonly used in the food industry. Hao and Brackett (1993) observed minimal growth of *E. coli* O157:H7 during refrigerated storage, and found that gas compositions within the tolerable range for vegetables had no inhibitory effect on the organism. Storage of steak in 100% CO₂ resulted in significantly lower bacterial counts 16 to 27 days after slaughter, compared to steaks stored in 100% N₂, 100% O₂, or air (Huffman, 1974). Seideman et al. (1979) reported that packaging fresh red meats in 20%CO₂/ 80%N₂ is as effective as or superior to vacuum packaging.

BACTERIAL INJURY

Currently, the beef industry utilizes several minimal processing techniques to lengthen shelf-life of meats without sacrificing quality. These processes include steam sterilization, organic acid and hot water rinses, low temperature storage, and vacuum and MAP in films varying in gas permeability. These minimal processes are used in the food industry to inhibit degradative enzymes and inactivate or reduce the amount of spoilage or pathogenic organisms present in food. However, in the process, bacteria in or on the surface of the food may become metabolically or structurally injured (Busta, 1994). The importance of bacterial injury from a food safety standpoint involves sublethally injured

pathogenic organisms that with time can repair and grow, rendering the food potentially dangerous for human consumption.

Injured bacteria can be present in foods that have been exposed to heat, refrigeration, freezing, acid, low pH, drying, and sanitizers (Ray, 1986; 1989). Several foods possess characteristics such as the reduced pH, that inherently induce reversible damage to bacterial cells. As bacteria are subjected to minimal processes or harsh environments, sublethal damage frequently results in the loss of cellular materials including proteins, peptides, amino acids, RNA, enzymes, and various ions (Busta, 1994). Growth responses associated with injured bacteria involve delayed germination of spores, extended lag phase growth, and the inability to multiply and enter log phase growth until repair is facilitated (Busta, 1994).

Bacterial injury, or sub-lethal injury, may impair growth of bacteria on typical enumeration media, but more frequently on selective media (Busta, 1986). Healthy, targeted organisms typically grow well on selective media, but if exposure to environmental stresses induce sublethal injury, growth of the targeted organism can be impaired as well. As a result, foods that have been subjected to minimal processes may yield inaccurately low populations of *E. coli* O157:H7 and other foodborne pathogens when selective enumeration media are used, leading to false conclusions regarding the safety of foods.

Injured microorganisms also may develop increased sensitivity to various food preservatives such as benzoates and sorbates, in addition to octanoic and decanoic acids

(Busta, 1994). The hurdle approach utilizes the concept of introducing multiple stresses for bacteria to overcome, before growing and spoiling foods. Many commercially produced apple ciders take advantage of this concept by the addition of antimicrobials such as potassium sorbate and sodium benzoate. Sodium benzoate has been shown to reduce the ability of *E. coli* O157:H7 to survive in apple cider (Zhao et al., 1993).

Recovery of injured bacteria. Injured organisms possess increased sensitivity to compounds tolerated by the organism prior to injury, and many of these compounds, such as brilliant green, crystal violet, neutral red, antibiotics, or bile salts, are typical constituents in selective bacteriological recovery media (Busta, 1994). Tollison and Johnson (1985) reported that after 30 min of heating at 50°C, *Shigella flexneri* became sensitive to 0.85% bile salt, which is the concentration contained in *Salmonella-Shigella* agar. Abdul-Raouf et al. (1993b) reported that heat- and acid-stressed *E. coli* O157:H7 became sensitive to compounds present in sorbitol macConkey agar supplemented with 4-methylumbelliferyl- β -D-glucuronide (MSMA), as evidenced by enhanced recovery on nonselective trypticase soya agar (TSA).

The addition of peroxide and O₂ degrading compounds to enumeration media in order to improve recovery of injured bacteria has been extensively investigated. When catalase and sodium pyruvate were added to selective culture media, injured *S. aureus*, *Pseudomonas fluorescens*, *Salmonella typhimurium*, and *E. coli* were recovered in greater numbers (Martin et al., 1976). McDonald et al. (1983) reported that the addition of sodium pyruvate and 3,3'-thiodipropionic acid to recovery media significantly improved

detection of sublethally freeze- and heat-injured *E. coli*; catalase added to media tempered to 45°C resulted in only negligible differences in detection. In a similar study, Gram et al. (1984) reported that catalase or 3,3'-thiodipropionic acid added to TSA and violet red bile agar (VRBA) had no influence on the recovery of freeze-injured *E. coli* and other Enterobacteriaceae. Catalase is rather heat sensitive (Whitaker, 1972), and the lack of catalase activity reported in these investigations may have resulted from the temperature of tempered culture medium.

Successful direct plating methods for the detection of healthy and injured *E. coli* O157:H7 for foods has yet to be developed. Although MSMA is commonly used for selective enumeration of *E. coli* O157:H7, Clavero and Beuchat (1995) reported that MSMA was the least efficient medium tested for recovering heat- and freeze- injured *E. coli* O157:H7. Ahmed and Conner (1995) tested eight media, including MSMA, for recovering heat-injured *E. coli* O157:H7 and reported that phenol red sorbitol agar (PRSA) supplemented with 0.005% 4-methylumbelliferyl- β -D-glucuronidase and PRSA supplemented with 1% pyruvic acid consistently recovered higher numbers of organisms. Conner and Hall (1994) evaluated several media for enumerating freeze-injured *E. coli* O157:H7, and reported that enumeration on TSA, TSA supplemented with 1% pyruvic acid, and PRSA resulted in the highest recovery of *E. coli* O157:H7.

Inadequate recovery of injured bacteria on selective media is well documented, and is thought to occur due to changes in the nutritional requirements of stressed cells (Maxy, 1973). Speck et al. (1975) reported higher recovery of freeze-stressed *E. coli* and

coliforms when injured populations were spread plated onto TSA, incubated for 1 to 2 hr at 25°C, then overlaid with violet red bile agar. Similarly, McCleery and Rowe (1995) observed greater recovery of heat-injured *E. coli* O157:H7 when populations were surface plated and allowed to recover for 2 hr at 25°C on TSA, then overlaid with MSMA. It was also reported in their study that better recovery was achieved when catalase was spread onto the surface of TSA plates prior to surface plating injured *E. coli* O157:H7 and overlaying with MSMA.

Factors influencing injury repair. Storage conditions of minimally processed foods are critical. Although growth of injured organisms may be impaired, metabolic and structural repair can occur rapidly if conditions suitable for growth exist. It is therefore important to focus on handling and storage conditions, and properties of the food itself after processing and before consumer purchase.

Environmental conditions such as incubation atmosphere can substantially affect the ability of bacteria to overcome injury. Gram-negative facultative anaerobes often are sensitized to oxygen after sublethal stress, and certain atmospheric conditions, particularly those low in oxygen, may promote repair and growth (Gadzella and Ingram, 1994). Murano and Pierson (1993) reported that anaerobic incubation, increased the ability of *E. coli* O157:H7 to overcome and recover from heat stress. Golden et al. (1989) reported that growth of uninjured and heat-injured *A. hydrophila* at 4°C was enhanced when packaged in N₂, while packaging in CO₂ imparted bacteriocidal effects on populations stored at incubated at 4 and 30°C.

In the United States, retail cuts of beef are packaged in modified gaseous atmospheres containing CO₂ to inhibit microbial growth and O₂ to maintain the desired color of meat (Varnam and Sutherland, 1995). Results presented in these studies suggest evidence for the ability of injured bacteria to undergo repair in atmospheres typically found in commercially packaged meats. Fresh beef contains some carbohydrates and many free amino acids which may supply pathogens such as *E. coli* O157:H7 with necessary nutrients for repair and growth. If adequate storage temperatures persist in foods subjected to minimal processing protocols or harsh environmental stresses, injured bacteria may repair and grow rendering the food potentially dangerous for human consumption.

REFERENCES

- Abdoul-Raouf, U.M., L.R. Beuchat, and M.S Ammar. 1993a. Survival and growth of *Escherichia coli* O157:H7 on salad vegetables. Appl. Environ. Microbiol. 59:1999-2006.
- Abdoul-Raouf, U.M., L.R. Beuchat, and M.S Ammar. 1993b. Survival and growth of *Escherichia coli* O157:H7 in ground roasted beef as affected by pH, acidulants, and temperature. Appl. Environ. Microbiol. 59:2364-2368.
- Ahmed, N.M., and D.E. Conner. 1995. Evaluation of various media for recovery of thermally-injured *Escherichia coli* O157:H7. J. Food Prot. 58:357-360.
- Alexander, E.R., J. Boase, M. Davis, L. Kirchner, C. Osaki, T. Tanino, M. Samadpour, P. Tarr, M. Goldoft, S. Lankford, J. Kobayashi, P. Stehr-Green, P. Bradley, B. Hinton, P. Tghe, B. Pearson, G.R. Flores, R. Bryant, S.B. Werner, and D.J. Vugia. 1995. *Escherichia coli* O157:H7 outbreak linked to commercially distributed dry-cured salami- Washington and California. MMWR. 44(9):157-159.
- Anderson, M.E., and R.T. Marshall. 1989. Interaction of concentration and temperature of acetic acid solution on reduction of various species of microorganisms on beef surfaces. J. Food Prot. 52:312-315.
- Barkate, M.L., G.R. Acuff, L.M. Lucia, and D.S. Hale. 1993. Hot water decontamination of beef carcasses for reduction of initial bacterial numbers. Meat Sci. 35:397-401.
- Berrang, M.E., R.E. Brackett, and L.R. Beuchat. 1989. Growth of *Listeria monocytogenes* on fresh vegetables stored under controlled atmosphere. J. Food Prot. 52:702-705.
- Besser, R.E., S.M. Lett, J.T. Beber, M.P. Doyle, T.J. Barret, J.G. Wells, and P.M. Griffin. 1993. An outbreak of diarrhea and hemorrhagic uremic syndrome from *Escherichia coli* O157:H7 in fresh pressed apple cider. JAMA. 269:2217-2220.
- Beuchat, L.R., and R.E. Brackett. 1990. Survival and growth of *Listeria monocytogenes* on lettuce as influenced by shredding, chlorine treatment, modified atmosphere packaging and temperature. J. Food Sci. 55:755-758,780.
- Blickstad, E., and G. Molin. 1983. The microbial flora of smoked pork loin and frankfurter sausage stored in different gas compositions at 4°C. J. Appl. Bacteriol. 54: 45-56.

- Borczyk, A.A., H. Loir, and B. Ciebin. 1987. False positive identifications of *Escherichia coli* O157 in foods. *Int. J. Food Microbiol.* 4:347-349.
- Brackett, R.E., Y.Y. Hao, and M.P. Doyle. 1994. Ineffectiveness of hot acid sprays to decontaminate *Escherichia coli* O157:H7 on beef. *J. Food Prot.* 57:198-203.
- Busta, F.F. 1986. Introduction to injury and repair of microbial cells. 195-201.
- Busta, F.F. 1994. Importance of injured food-borne microorganisms in minimal processing. *In* Minimal processing of foods and process optimization. Edited by R.P. Singh and F.A.R. Oliveira. CRC Press, Boca Raton. pp 227-231.
- Cannon, M., H. Thomas, W. Sellers, M. Bates, P. Blake, H. Stetler, K. Toomey, J. Fowler, S. Halford, G. Young, S. Hall, P. Erwin, V. Boaz, and G. Swinger. 1996. Outbreak of *Escherichia coli* O157:H7 infection-- Georgia and Tennessee. *MMWR.* 45(12):249-251.
- Carter, A.O., A.A. Borczyk, J.A.K. Carlson, B. Harvey, J.C. Hockin, M.A. Karmali, C. Krishnan, D.A. Korn, and H. Lior. 1987. A severe outbreak of *Escherichia coli* O157:H7-associated hemorrhagic colitis in a nursing home. *New Eng. J. Med.* 317:1496-1500.
- Clavero, M.R.S., and L.R. Beuchat. 1995. Suitability of selective plating media for recovering heat- or freeze-stressed *Escherichia coli* O157:H7 from tryptic soy broth and ground beef. *Appl. Environ. Microbiol.* 61:3268-3273.
- Code of Federal Regulations. 1993. Title 21. Food and drugs. Ch. I, part 184. Office of the Federal Register, National Archives and Records Administration, Washington, DC.
- Conner, D.E., and Hall. 1994. Efficacy of selected media for recovery of *Escherichia coli* O157:H7 from frozen chicken meat containing sodium chloride, sodium lactate or polyphosphate. *Food Microbiol.* 11:337-344.
- Conner D.E., and J.S. Kotrola. 1995. Growth and survival of *Escherichia coli* O157:H7 under acidic conditions. *Appl. Environ. Microbiol.* 61:382-385.
- Cray, W.C., Jr., and H.W. Moon. 1995. Experimental infection of calves and adult cattle with *Escherichia coli* O157:H7. *Appl. Environ. Microbiol.* 61:1586-1590.

- Cutter, C.N., and G.R. Siragusa. 1994. Efficacy of organic acids against *Escherichia coli* O157:H7 attached to beef carcass tissue using a pilot scale model carcass washer. *J. Food Prot.* 57:97-103.
- Davis, M., C. Osaki, D. Gordon, M.W. Hinds, K. Mottram, C. Winegar, E.D. Avner, P.I. Tarr, D. Jardine, M. Goldoft, B. Bartleson, J. Lewis, J.M. Kobayashi, G. Billman, J. Bradley, S. Hunt, P. Tanner, M. Ginsberg, L. Barret, S.B. Werner, G.W. Rutherford, R.W. Jue, H. Root, D. Brothers, R.L. Chehey, R.H. Hudson, F.R. Dixon, D.J. Maxson, L. Empey, O. Ravenholt, V.H. Ueckart, A. DiSalvio, D.S. Kwalick, R. Salcido, and D. Brus. 1993. Update: multistate outbreak of *Escherichia coli* O157:H7 infections from hamburgers -- western United States, 1992, 1993. *MMWR.* 42: 1-4.
- Doores, S. 1983. Organic acids.. *In* Antimicrobials in foods. Edited by A.L. Branen and P.M. Davidson. Marcel Dekker, Inc., New York. pp. 75-108
- Dorn, C.R. 1993. Review of foodborne outbreak of *Escherichia coli* O157:H7 infection in the western united states. *JAVMA.* 203:1583-1587.
- Doyle, M.P., and J.L. Schoeni. 1984. Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. *Appl. Environ. Microbiol.* 48:855-856.
- Doyle, M.P., and J.L. Schoeni. 1987. Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. *Appl. Environ. Microbiol.* 53:2394-2396.
- Duncan, S.E., and C.R. Hackney. 1994. Relevance of *Escherichia coli* O157:H7 to the dairy industry. *Dairy Food Environ. Sanit.* 14:656-660.
- Farber, J.M. 1991. Microbiological aspects of modified-atmosphere packaging technology - a review. *J. Food Prot.* 54:58-70.
- Feresu, S., and H. Nyati. 1990. Fate of pathogenic and non-pathogenic strains in two fermented milk products. *J. Appl. Microbiol.* 69:814-821.
- Food and Drug Administration. 1993. Food Code, Pub. no. PB94-113941AS. U.S. Public Health Service, U.S. Dept. of Commerce, Technology Administration, National Technical Information Service, Springfield, VA.
- Fratamico, P.M., R.L. Buchanan, and P.H. Cooke. 1993. Virulence of an *Escherichia coli* O157:H7 sorbitol-positive mutant. *Appl. Environ. Microbiol.* 59:4245-4254.

- Frost, B., C. Chaos, L. Ladaga, W. Day, M. Tenney, D. McWilliams, E. Barret, L. Branch, S. Jenkins, M. Linn, E. Turf, D. Woolard, G.B. Miller, S. Henderson, B. Campbell, M. Mismas, J. Dvorak, D. Patel, D. Peery, J. Morano, and K. Campbell. 1995. *Escherichia coli* O157:H7 outbreak at a summer camp--Virginia. MMWR. 44(22):419.
- Gadzella, T.A. and S.C. Ingram. 1994. Heat shock, anaerobic jar incubation and fluid thioglycollate medium have contrasting effects on D-values of *Escherichia coli*. J. Food Prot. 57:671-673.
- Gibson, A.M, and T.A. Roberts. 1986. The effect of pH, water activity, sodium nitrite and storage temperature on the growth of enteropathogenic *Escherichia coli* and salmonellae in a laboratory medium. Int. J. Food Microbiol. 3:183-194.
- Glass, K.A., J.M. Loeffelholz, J.P. Ford, and P.M. Doyle. 1992. Fate of *Escherichia coli* O157:H7 as affected by pH or sodium chloride and in fermented, dry sausage. Appl. Environ. Microbiol. 58:2513-2516.
- Golden, D.A., M.J. Eyles, and L.R. Beuchat. 1989. Influence of modified-atmosphere storage on the growth of uninjured and heat injured *Aeromonas hydrophila*. Appl. Environ. Microbiol. 55:3012-3015.
- Gram, L., P. Pedersen, and H. Sogaard. 1984. An evaluation of the effect of catalase and 3,3-thiodipropionic acid on the recovery of freeze-injured coliform bacteria. Int. J. Food Microbiol. 1:155-162.
- Grau, F.H., L.E. Brownlie, and M.G. Smith. 1969. Effects of food intake on numbers of *Salmonella* and *Escherichia coli* in rumen faeces of sheep. J. Appl. Bacteriol. 32:112-117.
- Grecz, N., Y.A. El-Zawahry. 1984. Effect of radiation and freezing on [3H] DNA of *Yersinia enterocolitica*. Appl. Environ. Microbiol. 5:1101-1105.
- Greer, G.G. and B.D. Dilts. 1992. Factors affecting the susceptibility of meatborne pathogens and spoilage bacteria to organic acids. Can. Inst. Food Sci. Technol. 355-364.
- Griffin, P.M., and R.V. Tauxe. 1991. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol. Rev. 13:60-98.

- Gunzer, F., H. Bohm, H. Russmann, S. Aleksic, and H. Karch. 1992. Molecular detection of sorbitol-fermenting *Escherichia coli* O157:H7 in patients with hemolytic-uremic-syndrome. *J. Clin. Microbiol.* 30:1807-1810.
- Hamby, P.L., J.W. Savell, G.R. Acuff, C. Vanderzant, and H.R. Cross. 1987. Spray-chilling and carcass decontamination systems using lactic and acetic acid. *Meat Sci.* 21:1-14.
- Hanna, M.O., J.C. Stewart, Z.L. Carpenter, and C. Vanderzant. 1977. Effect of heating, freezing, and pH on *Yersinia enterocolitica*-like organisms from meat. *J. Food Prot.* 40:689-692.
- Hao, Y.Y., and R.E. Brackett. 1993. Growth of *Escherichia coli* O157:H7 in modified atmosphere. *J. Food Prot.* 56:330-332.
- Harries, D., and A.D. Russell. 1966. Revival of heat-damaged *Escherichia coli*. *Experientia* 22:803-804.
- Hartsell, S.E. 1951. The longevity and behavior of pathogenic bacteria in frozen foods: The influence of plating media. *Amer. J. Publ. Health.* 41:1072-1077.
- Hintlian, C.B., and J.H. Hotchkiss. 1986. The safety of modified atmosphere packaging: a review. *Food Technol.* 12:70-76.
- Hitchins, A.D., P.A. Hartman, and E.C.D. Todd. 1992. Coliforms-*Escherichia coli* and its toxins, ch 24. In C. Vanderzant and D.F. Splittstoesser (ed.), *Compendium of methods for the examination of foods*, 3rd ed. American Public Health Association, Washington, DC.
- Huffman, D.L. 1974. Effect of gas atmospheres on microbial quality of pork. *J Food Sci.* 39:723-725.
- Jay, J.M. 1996. *Modern food microbiology*. 5th ed. Chapman and Hall, New York, NY.
- Kasrazadeh, M., and C. Genigeorgis. 1995. Potential growth and control of *Escherichia coli* O157:H7 in soft Hispanic type cheese. *Int. J. Food Microbiol.* 25:289-300.
- Keene, W.E., J.M. McAnulty, F.C. Hoesly, L.P. Williams, K. Hedberg, G.L. Oxman, T.J. Barrett, M.A. Pfaller, and D.W. Fleming. 1994. A swimming-associated outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7 and *Shigella sonnei*. *New. Engl. J. Med.* 331:379-584.

- Krishnan, C., V.A. Fitzgerald, S.J. Dakin, R.J. Behme. 1987. Laboratory investigation of outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7. J. Clin. Microbiol. 25:1043-1047.
- Levine, M.M. 1987. *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic, and enteroadherent. J. Infect. Dis. 155:377-389.
- Loir, H. 1994a. Classification of *Escherichia coli*, ch 2. In C.L. Gyles (ed.), *Escherichia coli* in domestic animals and humans. Biddles Ltd, Guildford.
- Loir, H. 1994b. *Escherichia coli* O157:H7 and verotoxigenic *Escherichia coli* (VTEC). J. Dairy Food Environ. Sanit. 14:378-382.
- March, S.B., and S. Ratnam. 1986. Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J. Clin. Microbiol. 5:869-872.
- Martin, L.M., L.D. Shipman, J.G. Wells, I.K. Wachsmuth, I.K., R.V. Tauxe, J.P. Davies, J. Arnoldi, and J. Tilleli. 1986. Isolation of *Escherichia coli* O157:H7 from dairy cattle associated with two cases of hemolytic uraemic syndrome. Lancet ii, 1043.
- Martin, S.E., R.S. Flowers, and Z.J. Ordal. 1976. Catalase: its effect on microbial enumeration. Appl. Environ. Microbiol. 32:731-734.
- Maxy, R.B. 1973. Condition of coliform organisms influencing recovery of subcultures on selective media. J. Milk Food Technol. 36:414-416.
- McCleery, D.R., and M.T. Rowe. 1995. Development of a selective plating technique for the recovery of *Escherichia coli* O157:H7 after heat stress. Lett. Appl. Microbiol. 21:252-256.
- McDonald, L.C., C.R. Hackney, and B. Ray. 1983. Enhanced recovery of injured *Escherichia coli* by compound that degrade hydrogen peroxide or block its formation. Appl. Environ. Microbiol. 45:360-365.
- Membrillo-Hernandez, J., A. Nunez-de la Mora, T. del Rio-Albrechtsen, R. Camacho-Carranza, and M.C. Gomez-Eichelmann. 1995. Thermally-induced cell lysis in *Escherichia coli* K12. J. Basic Microbiol. 1:41-46.
- Miller, L.G., and C.W. Kaspar. 1994. *Escherichia coli* O157:H7 acid tolerance and survival in apple cider. J. Food Prot. 57:460-464.

- Molenda, J.R. 1994. *Escherichia coli* (including O157:H7): an environmental health perspective. Dairy Food Environ. Sanit. 14:742-747.
- Morgan, D., C.P. Newman, D.N. Hutchinson, A.M. Walker, B. Rowe, and F. Majid. 1993. Verotoxin producing *Escherichia coli* O157 infections associated with consumption of yogurt. Epidemiol. Infect. 111:181-187.
- Murano, E.A. and M.D. Pierson. 1993. Effect of heat shock and incubation atmosphere on injury and recovery of *Escherichia coli* O157:H7. J. Food Prot. 56:568-572.
- Neidhardt, F.C., J.L. Ingraham, and M. Schaechter. 1990. Physiology of the bacterial cell: A molecular approach. Sinauer Associates, Inc. Sunderland, MA.
- Notenboom, R.H., A. Borczyk, M.A. Karmali, and L.M.C. Duncan. 1986. Clinical relevance of a serological cross-reaction between *Escherichia coli* O157 and *Brucella abortus*. Lancet ii. 275.
- Nutsch, A.L., R.K. Phebus, D.E. Shafer, M.J. Riemann, R.C. Wilson, and J. Leising. 1996. Evaluation of a steam pasteurization process in a commercial beef processing facility. 83rd Annual Meeting of IAMFES. June 30-July 3. Abstract 9.
- O'Brien, A.D., A.R. Melton, C.K. Schmitt, M.L. McKee, M.L. Batts, and D.E. Griffin. 1993. Profile of *Escherichia coli* O157:H7 pathogen responsible for hamburger-borne outbreak of hemorrhagic colitis and hemolytic uremic syndrome in Washington. J. Clin. Microbiol. 10:2799-2801.
- Okrend, A.J.G., B.E. Rose, and C.P. Lattuada. 1990. Use of 5-bromo-4-chloro-3-indoxyl- β -D-glucuronide in macConkey sorbitol agar to aid in the isolation of *Escherichia coli* in ground beef. J. Food Prot. 53:941-943.
- Padhye, N.V., and M.P. Doyle. 1992. *Escherichia coli* O157:H7: epidemiology, pathogenesis, and methods for detection in food. J. Food Prot. 55:555-565.
- Raghubeer, E.V., and J.R. Matches. 1990. Temperature range for growth of *Escherichia coli* serotype O157:H7 and selected coliforms in *E. coli* medium. J. Clin. Microbiol. 28:803-805.
- Rajkowski, K.T., and B.S. Marmer. 1995. Growth of *Escherichia coli* O157:H7 at fluctuating incubation temperatures. J. Food Prot. 58:1307-1313.

- Rasmussen, M.A., W.C. Cray, T.A. Casey, and S.C. Whipp. 1993. Rumen contents as a reservoir of Enterohemorrhagic *Escherichia coli*. FEMS Microbiol. Lett. 114:79-84.
- Ray, B. 1986. Impact of bacterial injury and repair in food microbiology: its past, present, and future. J. Food Prot. 49:651-655.
- Ray, B. 1989. Injured index and pathogenic bacteria: occurrence and detection in food, water and feeds. CRC Press Inc., Boca Raton, FL.
- Reagan, J.O. 1993. Foodborne pathogens. *Escherichia coli* O157:H7: issues and answers. Proc. Recip. Meat Conf. 46:119-123.
- Rice E.W., E.G. Sowers, C.L. Johnson, M.E. Dunnigan, N.A. Strockbrine, and S.C. Edberg. 1992. Serological cross-reactions between *Escherichia coli* O157 and other species of the genus *Escherichia*. J. Clin. Microbiol. 30:1315-1316.
- Riley, L.W., R.S. Remis, S.D. Helgerson, H.B. McGee, J.G. Wells, B.R. Davis, R.J. Hebert, E.S. Olcott, L.M. Johnson, N.T. Hargrett, P.A. Blake, and M.L. Cohen. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. New Engl. J. Med. 308:681-685.
- Riordan, D., G. Duffy, J. Sheridan, R. Whiting, B. Eblen, D. McDowell, and I. Blair. 1996. Studies on the survival of *Escherichia coli* O157:H7 in fermented meat products. 83rd Annual Meeting of IAMFES. June 30-July 3. Abstract 186.
- Rodrigue, D.C., E.E. Mast, K.D. Greene, J.P. Davis, M.A. Hutchinson, J.G. Wells, T.J. Barret, P.M. Griffin. 1995. A University outbreak of *Escherichia coli* O157:H7 infections associated with roast beef and an unusually benign clinical course. J. Infect. Dis. 172:1122-1125.
- Ryan, C.A., R.V. Tauxe, G.W. Hisek, J.G. Wells, P.A. Stoesz, H.W. McFadden, P.W. Smith, G.F. Wright, and P.A. Blake. 1986. *Escherichia coli* O157:H7 diarrhea in a nursing home: clinical, epidemiological, and pathological findings. J. Infect. Dis. 154:631-638.
- Salmon, R.L., I.D. Farrell, J.G.P. Hutchinson, D.J. Coleman R.J. Gross, N.K. Fry, B. Rowe, and S.R. Palmer. 1989. A christening party outbreak of hemorrhagic colitis and haemolytic uraemic syndrome associated with *Escherichia coli* O157:H7. Epidem. Inf. 103:249-254.

- Samadpour, M., J.E. Ongerth, J. Liston, N. Tran, D. Nguyen, T.S. Whittam, R.A. Wilson, P.I. Tarr. 1994. Occurrence of Shiga-like toxin-producing *Escherichia coli* in retail fresh seafood, beef, lamb, pork, and poultry from grocery stores in Seattle, Washington. *Appl. Environ. Microbiol.* 60:1038-1040.
- Seideman, S.C, Z.L. Carpenter, G.C. Smith, C.W. Dill, and C. Vanderzant. 1979. Physical and sensory characteristics of beef packaged in modified gas atmospheres. *J. Food Prot.* 42:233-239.
- Semanchek, J.J., and D.A. Golden. 1996. Survival of *Escherichia coli* O157:H7 during fermentation of apple cider. *J. Food Prot.* 59(12):1-4.
- Smith, M.G. 1995. Survival of *E. coli* and *Salmonella* after chilling and freezing in liquid media. *J. Food Sci.* 60:509-512.
- Speck, M.L., B. Ray, and R.B. Read. 1975. Repair and enumeration of injured coliforms by a plating procedure. *Appl. Microbiol.* 29:549-550.
- Sussman, M. 1985. The virulence of *Escherichia coli*. Academic Press, Inc. Orlando, FL.
- Steele, B.T., N. Murphy, G.S. Arbus, and C.P. Rance. 1982. An outbreak of hemolytic uremic syndrome associated with ingestion of fresh apple juice. *J. Pediatr.* 101:963-965.
- Swerdlow, D.L., B.A. Woodruff, R.C. Brady, P.M. Griffin, S. Tippet, H.D. Donnell, E. Geldreich, B.J. Payne, A. Meyer, J.G. Wells, K.D. Greene, M. Bright, N.H. Bean, and P.A. Blake. 1992. A waterborne outbreak in Missouri of *Escherichia coli* O157:H7 associated with bloody diarrhea and death. *Ann. Intern. Med.* 117:812-819.
- Tarr, P.I. 1993. What do nutrition professionals need to know about *E. coli*. *Food Safety.* 65:34-35.
- Tarr, P.I. 1994. *Escherichia coli* O157:H7: overview of clinical and epidemiological issues. *J. Food Prot.* 57:632-636.
- Tollison, S.B., and M.G. Johnson. 1985. Sensitivity to bile salts of *Shigella flexneri* sublethally heat stressed in buffer or broth. *Appl. Environ. Microbiol.* 50:337-341.
- Tzipori, S., I.K. Wachsmuth, J. Smithers *et al.* 1988. Studies in gnotobiotic piglets on non-O157:H7 *Escherichia coli* serotypes isolated from patients with hemorrhagic colitis. *Gastroenterol.* 94:590-597.

- Varnam, A.H., and J.P. Sutherland. 1995. Meat and meat products. Chapman and Hall. London, UK.
- Warner, M., K. Kuo, L. Williams, B. Adam, C. Langkop, R. Ruden, B. Francis, and T. Haupt. 1996. Lake-associated outbreak of *Escherichia coli* O157:H7--Illinois. MMWR. 45(21):437-444.
- Weagant, S.D., J.L. Bryant, and D.H. Bark. 1994. Survival of *Escherichia coli* O157:H7 in mayonnaise and mayonnaise-based sauces at room and refrigerated temperatures. J. Food Prot. 57:629-631.
- Weeratna, R.D., and M.P. Doyle. 1991. Detection and production of verotoxin 1 of *Escherichia coli* O157:H7 in food. Appl. Environ. Microbiol. 57:2951-2955.
- Wells, J.G., B.R. Davis, I.K. Wachsmuth, L.W. Riley, R.S. Remis, R. Sokolow, and G.K. Morris. 1983. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. J. Clin. Microbiol. 18:512-520.
- Wells, J.G., L.D. Shipman, K.D. Greene, E.G. Sowers, J.H. Green, D.N. Cameron, F.P. Downes, M.L. Martin, P.M. Griffin, S.M. Ostroff, M.E. Potter, R.V. Tauxe, and I.K. Wachsmuth. 1991. Isolation of *Escherichia coli* serotype O157:H7 and other Shiga-like-toxin-producing *E. coli* from dairy cattle. J. Clin. Microbiol. 29:985-989.
- Whitaker, J. 1972. Principles of enzymology for the food sciences. Marcel Dekker, New York.
- Wolfe, S.K. 1980. Use of CO- and CO₂ enriched atmospheres for meats, fish, and produce. Food Technol. 34:55-58,63.
- Woodcock, E., and G.W. Grigg. 1972. Repair of thermally induced DNA breakage in *Escherichia coli*. Nature New Biol. 237:76-79.
- Zepeda C.M., C.L. Kastner, B.L. Willard, R.K. Phebus, J.R. Schwenke, B.A. Fijal, and R.K. Prasai. 1994. Gluconic acid as a fresh beef decontaminant. J. Food Prot. 57:956-962.
- Zhao, T., M.P. Doyle, and R.E. Besser. 1993. Fate of Enterohemorrhagic *Escherichia coli* O157:H7 in apple cider with and without preservatives. Appl. Environ. Microbiol. 8:2526-2530.

Zhao, T., M.P. Doyle, J. Shere, and L. Garber. 1995. Prevalence of enterohemorrhagic *Escherichia coli* O157:H7 in a survey of dairy herds. Appl. Environ. Microbiol. 61:1290-1293.

CHAPTER III
INACTIVATION AND INJURY OF *ESCHERICHIA COLI* O157:H7
CULTURED AT 10 AND 37°C, AS INDUCED BY HEATING,
LACTIC ACID, AND FREEZING

ABSTRACT

The influence of growth temperature on heat-, lactic acid-, and freeze- induced inactivation and injury of *Escherichia coli* O157:H7 in 0.1% peptone water was investigated. Three strains of *E. coli* O157:H7 isolated from salami, apple cider, and ground beef were evaluated. Growth of strains at 10°C as compared with 37°C had a significant impact on reducing ($P<0.01$) D-values obtained for heating, acid exposure, and freezing, with the exception of the cider strain stored in lactic acid. When strains were cultivated at 10 and 37°C and heated at 54 and 56°C, the salami strain possessed the highest ($P<0.01$) D-values. When grown at 10°C, the beef strain had the lowest ($P<0.01$) D-values after heating at 52, 54, and 56°C. The salami strain grown at 10°C had the highest ($P<0.01$) D-values in all concentrations of lactic acid. After growth at 37°C, the salami strain had the highest ($P<0.01$) D-values after storage in 0.1 and 0.25% lactic acid, while D-values for salami and beef strains did not differ ($P>0.05$) when stored in 0.5% lactic acid. The cider strain grown at 37°C had lower ($P<0.01$) D-values than the beef strain after storage in 0.25 and 0.5% lactic acid. Substantial portions of strain populations were sublethally injured after heat and lactic acid treatments, as evidenced by the inability

of injured organisms to form colonies on tryptone soya agar containing 2% NaCl. Strains cultured at 10°C were more susceptible to sublethal injury, as compared with strains cultured at 37°C. Storage of test strains at -20°C for 7 months resulted in a 4 to 6 log CFU/ml reduction in viable population, but induced only minimal sublethal injury. After 5 months at -20°C, strains cultured at 10°C were more sensitive to freeze-inactivation than strains cultured at 37°C. When grown at 10 and 37°C and stored at -20 for 7 months, the cider strain possessed higher ($P<0.01$) D-values than beef and salami strains.

INTRODUCTION

In 1993 a major outbreak of *Escherichia coli* O157:H7 foodborne illness was linked to consumption of improperly cooked hamburger meat (Davis et al., 1993). Several additional *E. coli* O157:H7 outbreaks have occurred with roast beef (Rodrigue et al., 1995), turkey roll (Salmon et al., 1989), yogurt (Morgan et al., 1993), apple cider (Besser et al., 1993), mayonnaise (Erickson et al., 1995) and salami (Alexander et al., 1995) being implicated as causative agents. Survival of *E. coli* O157:H7 in foods with diverse intrinsic characteristics (i.e., acidity, pH, and water activity) presents substantial evidence for variability between strains of *E. coli* O157:H7.

Minimal processes are often applied to foods to extend shelf-life. However, when bacteria are subjected to some of these processes, sublethal injury can occur. Injured bacteria may be present in foods that have been exposed to heat, refrigeration, freezing, acid, low pH, and sanitizers (Ray, 1989). As bacteria are subjected to minimal processes,

sublethal damage frequently results in the loss of cellular materials including peptides, amino acids, RNA, enzymes, and various ions (Busta, 1994). Growth responses associated with injured bacteria involve delayed germination of spores, extended lag phase of growth, and the inability to multiply and enter the log phase of growth until repair is facilitated (Busta, 1994). Sublethal injury is evidenced by the inability of bacteria to form colonies on common selective media that would otherwise support growth of cells not subjected to environmental stresses. The importance of bacterial injury from a food safety standpoint lies in the ability of injured organisms to undergo repair and resume growth, thereby rendering the food potentially dangerous for consumption.

E. coli O157:H7 outbreaks linked to apple cider (Besser et al., 1993) and mayonnaise (Weagant et al., 1994) present substantial evidence for tolerance of this organism to reduced pH environments. In 1992, the United States Department of Agriculture (USDA) approved the application of organic acid rinses to beef carcasses to reduce microbial contamination (Reagan, 1993). Brackett et al. (1994) reported that heated and non-heated concentrations of 0.5, 1.0, and 1.5% acetic, lactic, and citric acids applied to fresh beef had no antagonistic effect on *E. coli* O157:H7. Dickson and Siragusa (1994) reported that washing beef tissue with 1% lactic or acetic acid resulted in sublethal injury to *Salmonella typhimurium*, *Listeria monocytogenes*, and *E. coli* O157:H7.

Storage of food at low temperatures may affect the response of pathogens such as *E. coli* O157:H7 to sublethal stresses. Although refrigeration is normally considered to

provide a useful hurdle in delaying growth or inhibiting survival of bacteria, *E. coli* O157:H7 has been shown to survive better in acidic foods such as apple cider and mayonnaise stored at refrigeration temperatures, as compared to survival at ambient temperature (Weagant et al., 1994; Zhao et al., 1993). Conner and Hall (1996) reported that *E. coli* O157:H7 grew at 4°C in trypticase soy broth containing sodium lactate, while no growth was observed in TSB containing no sodium lactate. *E. coli* O157:H7 also has been reported to be resistant to freezing in ground beef (Padhye and Doyle, 1992) and chicken meat (Conner and Hall, 1996). Jackson et al. (1996) reported that storage of *E. coli* O157:H7 in frozen ground beef patties increased the heat resistance of the organism. Freezing is not an effective means of destroying bacteria. Nevertheless, the loss of viability in cells subjected to freezer storage can differ from strain to strain and depends on the nature and composition of the food, type of freezing employed, freezing temperature, and duration of storage (Jay, 1996).

Recently, the USDA approved a high-temperature vacuum technology for reducing microbial contamination on beef carcasses (Anonymous, 1996). *E. coli* O157:H7 possesses no unusual heat resistance (Doyle and Schoeni, 1984). However, the nature of the heating menstrua can greatly affect survival of microorganisms exposed to heat stress. Reported D-values for *E. coli* O157:H7 are 39.8 and 4.5 min at 54.4 and 57.2°C, respectively, in ground beef (Doyle and Schoeni, 1984) and 5.0 and 1.0 min at 55 and 58°C, respectively, in apple cider (Splittstoesser et al., 1996).

It is possible that the meat used in the preparation of hamburgers implicated in the *E. coli* O157:H7 outbreak in the Pacific Northwestern United States came from beef carcasses that were treated with organic acid rinses or exposed to abusive storage temperature. Furthermore, it is possible that *E. coli* O157:H7 cells on treated carcasses were unaffected or sublethally injured by acid treatment or cold storage, then recovered and grew during subsequent transport, handling, and/or storage. This study was designed to examine the inactivation and development of injury in three strains of *E. coli* O157:H7 grown at 10 and 37°C, as induced by heating, lactic acid, and freezing.

MATERIALS AND METHODS

Test organisms and culture maintenance. Three strains of *E. coli* O157:H7 were evaluated: a human isolate from the 1993 outbreak in the Pacific Northwestern U.S. involving improperly cooked ground beef; strain 7927, isolated from a patient involved in a U.S. apple cider-associated outbreak; and strain 380-94, from an outbreak associated with salami. These strains are hereafter referred to as beef, cider, and salami strains, respectively. Beef and cider strains were obtained from Dr. M.P. Doyle, The University of Georgia, Griffin. The salami strain was obtained from Dr. A.M. McNamara, USDA-FSIS, Washington D.C.

Test strains were grown and maintained at 37°C in tryptone soya broth (TSB; Unipath Oxoid USA, Ogdenburg, NY). Test organisms were cultured substantially below (10°C) and at optimum (37°C) growth temperatures to determine the effects of growth

temperature on the subsequent development of injury. Growth curves were constructed for each test strain to determine early stationary phase in TSB at 10 and 37°C. Test cultures were subjected to a minimum of three successive stationary phase transfers before experimentation.

Determination of sublethal injury. Stationary phase cultures of *E. coli* O157:H7 were plated onto tryptone soya agar (TSA; Unipath Oxoid USA, Ogdenburg, NY) and TSA containing various concentrations of NaCl to determine salt-tolerance of uninjured *E. coli* O157:H7. TSA containing 2% NaCl, the maximum concentration of NaCl in TSA that did not impair colony formation of unstressed cells, was used as the stress medium for recovery of cells subjected to sublethal stress. Sublethal injury was determined on the basis of increased sensitivity of stressed organisms to TSA containing 2% NaCl (TSA-NaCl), as compared to the recovery of stressed organisms on TSA. The purpose of NaCl in the stress medium was to inhibit repair of metabolic and structural injury within sublethally stressed test populations.

Heat inactivation and injury. Heat inactivation and injury was induced using a modification of the method of Golden et al. (1988). Stationary phase cultures of *E. coli* O157:H7 cultured in TSB at 10 and 37°C were subjected to heating temperatures of 52, 54, and 56°C under constant agitation (150 rpm) in an Orbit shaker water bath (Lab Line Instruments, Inc., Melrose Park, IL.). One milliliter of stationary phase cultures of beef, cider, and salami strains were inoculated into 100 ml of 0.1% peptone water (PW) in 250-ml Erlenmeyer flasks (final population of approximately log 7 CFU/ml). Prior to

inoculation, Erlenmeyer flasks were tempered to the desired heating temperature. The water level of the water bath exceeded the level of PW in the Erlenmeyer flasks. Samples were withdrawn at selected intervals for up to 90 min of heating, deposited into sterile test tubes, and immediately placed in an ice-water bath to terminate heat inactivation. Samples were diluted as appropriate in PW and surface plated (0.1 ml) in duplicate onto TSA and TSA-NaCl. Populations of *E. coli* O157:H7 on TSA and TSA-NaCl were determined after incubation for 48 hr at 37°C.

Lactic acid inactivation and injury. Sublethal acid inactivation and injury was induced using a modification of procedures described by Ahamad and Marth (1990) and Blankenship (1981). Stationary phase cultures of *E. coli* O157:H7, cultured as described above for heating studies, were inoculated into 100 ml of PW adjusted to concentrations of 0.1 (pH 3.2), 0.25 (pH 2.8), and 0.5% (pH 2.5) of an 85% lactic acid solution (Sigma Chemical Co., St. Louis, MO) in 250-ml Erlenmeyer flasks. Flasks were incubated at 25°C in an Orbit Environ-shaker incubator (Lab Line Instruments, Inc., Melrose Park, IL.). At selected intervals, samples were removed from Erlenmeyer flasks, neutralized with 0.1 N NaOH, surface plated (0.1 ml) in duplicate on TSA and TSA-NaCl, and plates were incubated for 48 hr at 37°C. Injury was determined as described above. Test strains cultured at 10 and 37°C and inoculated into PW containing no lactic acid served as controls.

Freeze inactivation and injury. Sublethal freeze-inactivation and injury was induced according to a modification of the method of Golden et al. (1988). Stationary

phase cultures of *E. coli* O157:H7, cultured as described above for heating studies, were inoculated (final population of approximately log 7 CFU/ml) into 4 ml of PW in 25x150-mm sterile screw-cap test tubes. Tubes were sealed and stored at -20°C for up to 7 months. At selected intervals throughout the frozen storage period, samples were removed from the freezer and the contents thawed under cool running tap water. Thawed PW cultures were appropriately diluted in PW, surface plated (0.1 ml) in duplicate onto TSA and TSA-NaCl, and plates were incubated for 48 hr at 37°C. Injury was determined as described above.

Statistical analysis. Linear regression analysis was performed on survival curve data for organisms recovered on TSA. Decimal reduction times (D-values) were calculated as the negative reciprocal of the slopes of the linear regression survivor curves for strains exposed to heat, lactic acid and freezing. Statistical analyses (were conducted on D-values for all strains using Proc mixed, SAS version 6.1 (SAS institute; Cary, NC). Data were fit to a completely randomized design with a factorial treatment arrangement. Differences were evaluated noting the effects of growth temperature, treatment level (i.e., heating temperature or acid concentration), and strain on the survival of *E. coli* O157:H7. All procedures were performed in triplicate.

RESULTS

Heat inactivation and injury. Beef, cider, and salami strains reached stationary growth phase within 16 hr at 37°C and 7 days at 10°C. Thermal inactivation curves for

the test strains grown at 10 and 37°C and heated at 52, 54, and 56°C are illustrated in Figures 1 - 3, respectively. The salami strain was the most heat resistant strain tested, whereas the beef strain was the least heat resistant strain tested. When strains were cultured at 37°C, the greatest reduction (3.27 log CFU/ml) in the beef strain occurred after heating for 60 min at 52°C. A maximum reduction of 2.33 log CFU/ml was observed for the cider strain after heating at 56°C for 30 min, whereas maximum reduction of 1.21 log CFU/ml for the salami strain occurred following 60 min at 54°C. Heat induced inactivation was greater for strains grown at 10°C as compared with heat inactivation of strains at 37°C. When grown at 10°C, the beef strain was the least heat resistant strain and was reduced to 0.53 log CFU/ml after heating for 15 min at 56°C.

Decimal reduction times (D-values) for strains grown at 10 and 37°C and heated at 52, 54, and 56°C are listed in Table 1. Growth of strains at 10°C as compared with 37°C had a significant impact on reducing ($P<0.01$) D-values at all heating temperatures. When strains were cultivated at 10 and 37°C and heated at 54 and 56°C, the salami strain possessed the highest ($P<0.01$) D-values. When grown at 10°C, the beef strain had the lowest ($P<0.01$) D-values at all heating temperatures. However, when strains were grown at 37°C, these differences were less apparent.

Substantial portions of test populations grown at 37°C and heated at 52, 54, and 56°C consisted of injured organisms, as illustrated in Figures 1 - 3, respectively. The salami strain, grown at 37°C, was most resistant to heat stress and exhibited only 60.3%

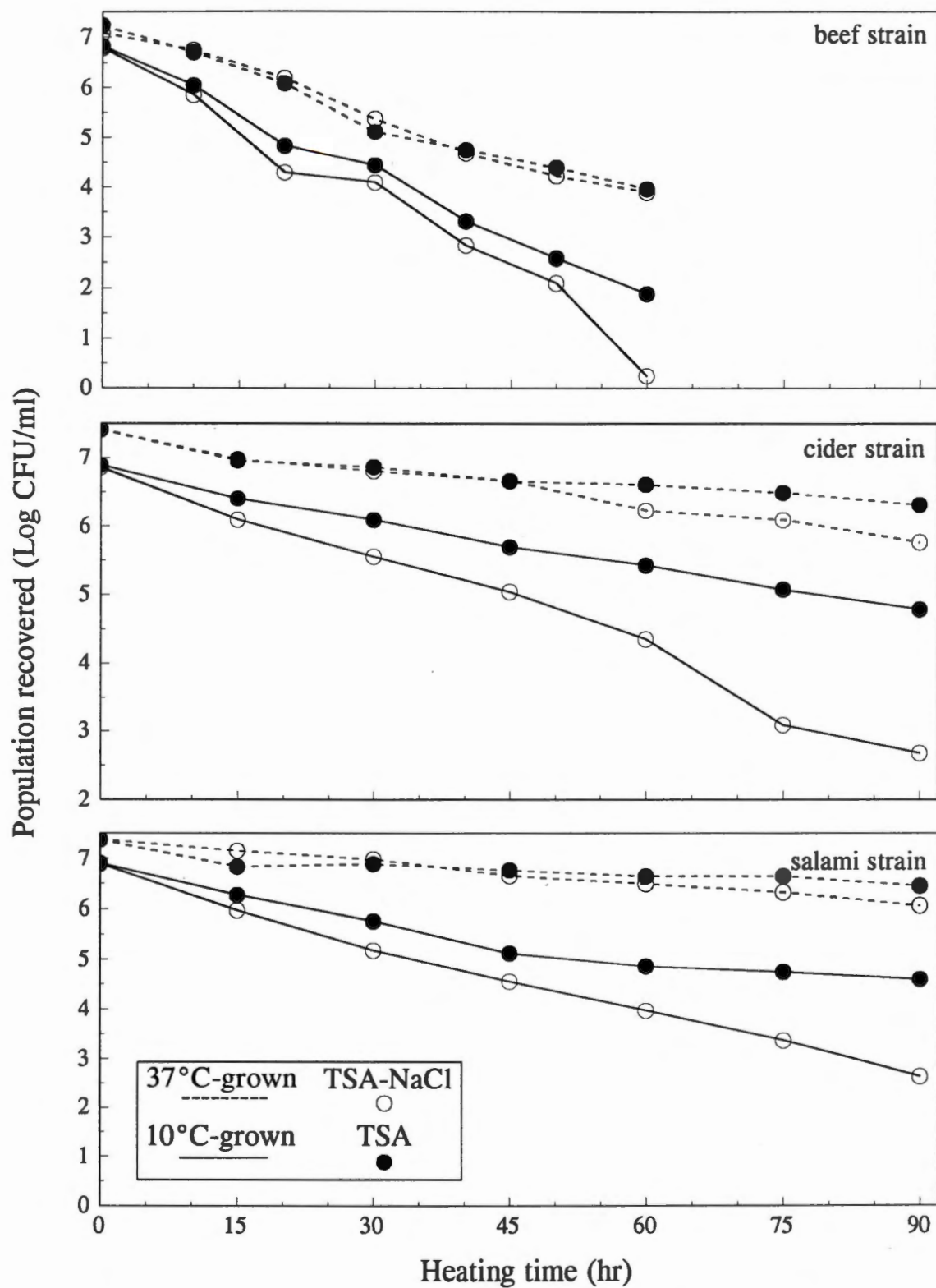


Figure 1. Inactivation and injury of three strains of *E. coli* O157:H7 heated at 52°C in 0.1% peptone water.

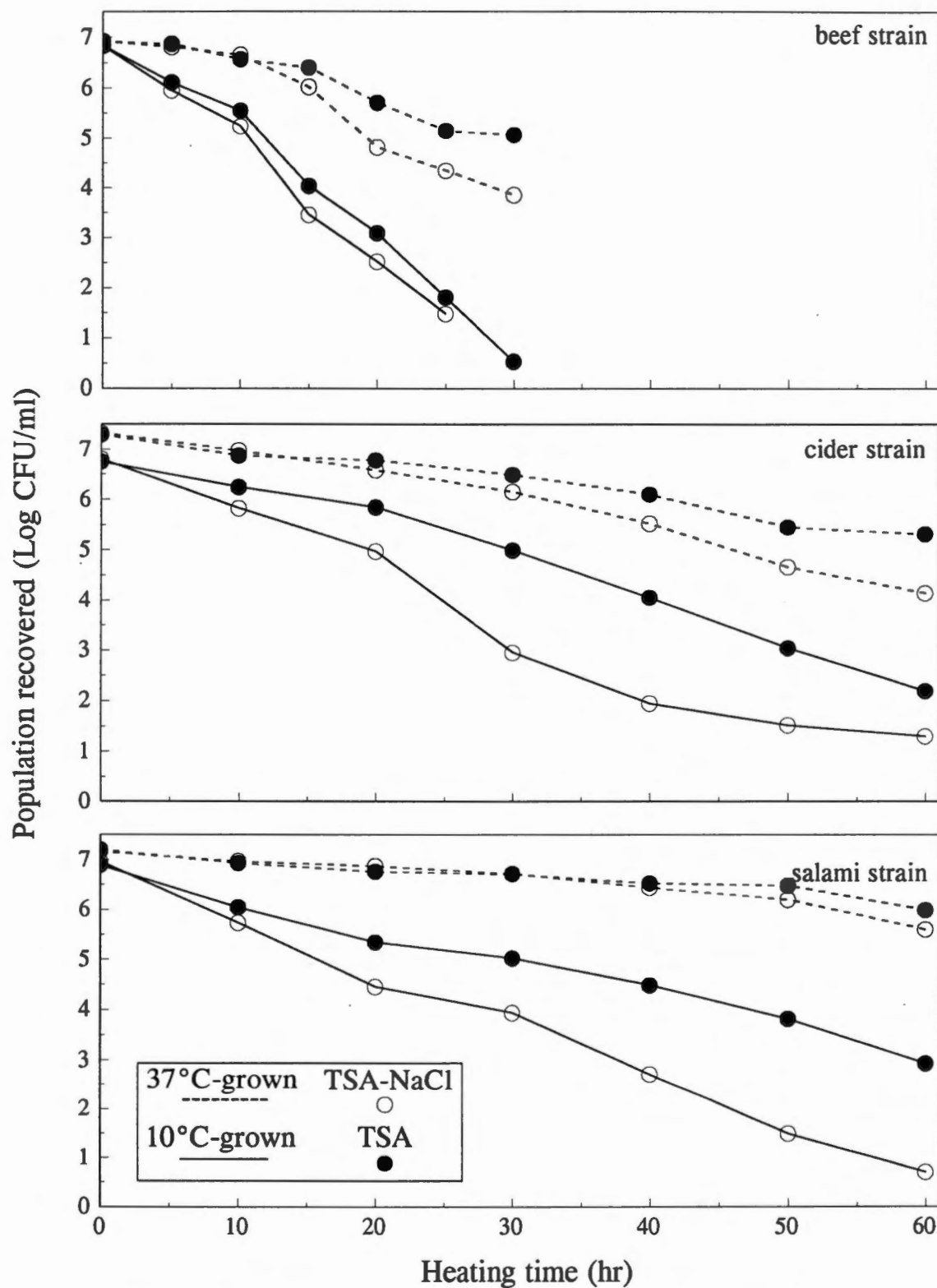


Figure 2. Inactivation and injury of three strains of *E. coli* O157:H7 heated at 54°C in 0.1% peptone water.

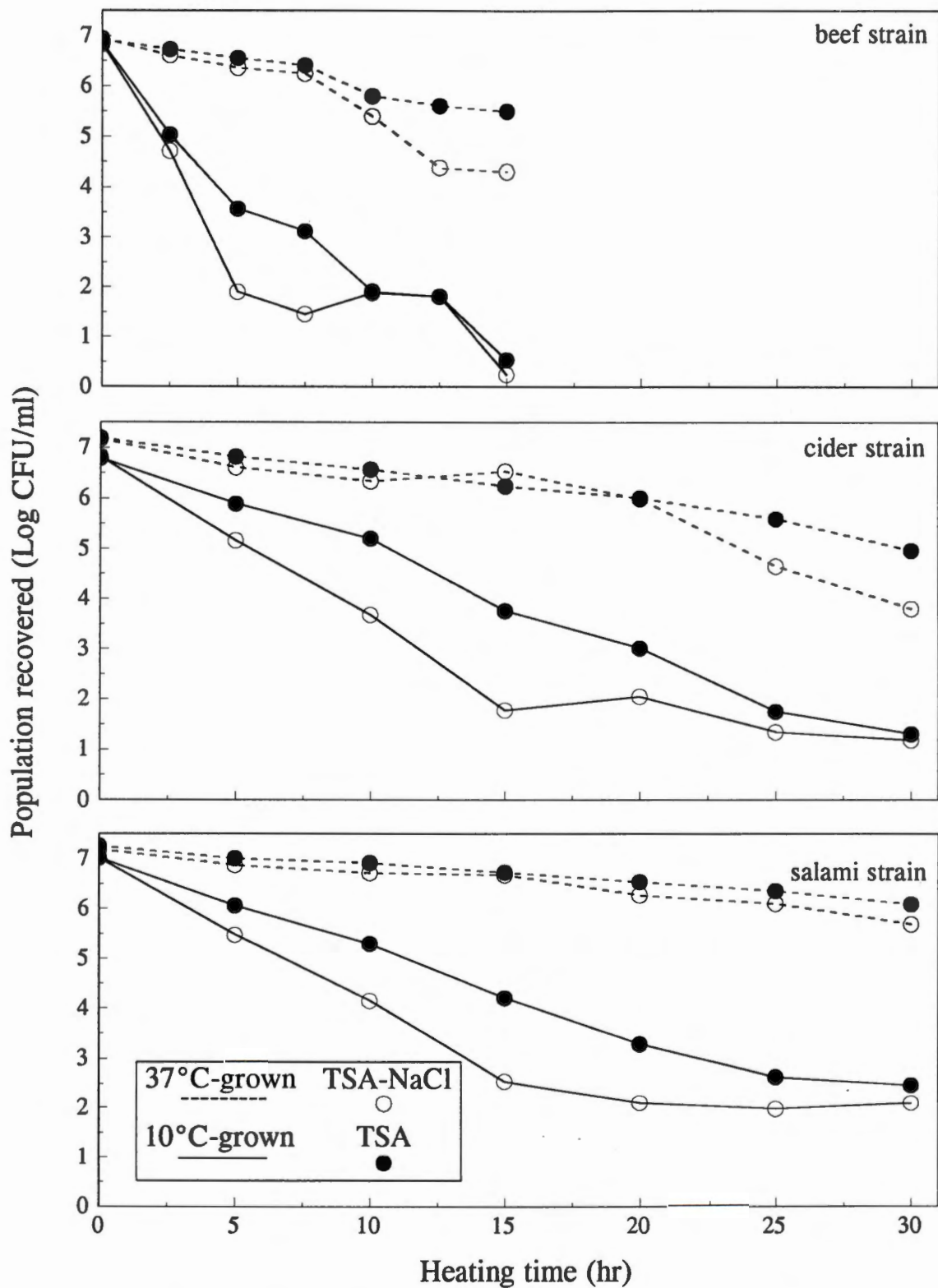


Figure 3. Inactivation and injury of three strains of *E. coli* O157:H7 heated at 56°C in 0.1% peptone water.

Table 1. D-values¹ for *E. coli* O157:H7 strains grown at 10 and 37°C and subjected to heating, lactic acid, and freezing.

Growth temp (°C)		D-value (min) ² in peptone water:						
		heated at:			containing lactic acid (%) ³ :			at -20°C
		52°C	54°C	56°C	0.1%	0.25%	0.5%	
10	beef	11.2 ^a	4.1 ^a	2.5 ^a	3.7 ^a	1.2 ^a	0.35 ^a	1.3 ^a
	cider	40.7 ^b	12.4 ^b	5.1 ^b	4.3 ^a	1.8 ^a	0.44 ^a	1.7 ^b
	salami	37.6 ^b	15.9 ^c	5.9 ^c	9.5 ^b	3.6 ^b	0.76 ^b	1.4 ^a
37	beef	17.7 ^a	12.9 ^a	9.3 ^a	6.4 ^a	4.2 ^b	1.7 ^b	1.6 ^a
	cider	89.4 ^{ab}	29.4 ^b	14.1 ^a	4.8 ^a	2.3 ^a	0.47 ^a	2.2 ^b
	salami	120.5 ^b	59.7 ^c	26.4 ^b	14.8 ^b	8.8 ^c	1.9 ^b	1.7 ^a

¹Means within a column and within the same growth temperature followed by the same superscript(s) are not different (P>0.05).

²Strains grown at 37°C had higher (P<0.01) D-values than for strains grown at 10°C, with the exception of the cider strain stored in lactic acid.

³85% lactic acid solution.

maximum injury at 56°C. Considerably greater portions of cell populations were injured when all strains were grown at 10°C (Table 2). Little difference in susceptibility to heat-injury was observed among strains grown at 10°C.

Lactic acid inactivation and injury. Inactivation was performed in PW held at 25°C for up to 25 hr. Populations of strains (controls) cultured at 10°C did not grow during storage for 10 hr in PW containing no lactic acid, while strains grown at 37°C increased by approximately 1 log CFU/ml after storage for 25 hr. Injury was not observed among any strains grown at 10 or 37°C in PW containing no lactic acid.

Lactic acid inactivation of strains grown to stationary phase at 10 and 37°C in PW containing 0.1, 0.25, and 0.5% of an 85% lactic acid solution are illustrated in Figures 4 - 6, respectively. Greater rates of inactivation in strains grown at 10 and 37°C occurred at higher concentrations of lactic acid. The salami strain was the most lactic acid resistant strain, as was expected due to the environment from which the strain was isolated. When test strains were cultured at 37°C, the cider strain was the least resistant to lactic acid inactivation. Greater lactic acid induced inactivation resulted when strains were grown at 10°C, as compared with strains grown at 37°C. When strains were grown at 10°C, the beef strain was the least lactic acid resistant strain.

Table 1 illustrates D-values for beef, cider, and salami strains subjected to lactic acid treatments. D-values for strains grown at 10°C were lower ($P < 0.01$) than D-values

Table 2. Maximum injury in *E. coli* O157:H7 strains grown at 10 and 37°C and subjected to heating, lactic acid, and freezing.

		Maximum injury (%) in:						
Growth		PW ¹ heated at:			PW containing lactic acid ² solution:			PW at -20°C
temp. (°C)	Strain	52°C	54°C	56°C	0.1%	0.25%	0.5%	
10	beef	97.7	97.1	97.8	99.2	92.3	100	57.5
	cider	99.2	99.2	98.9	93.3	100	100	38.3
	salami	98.9	99.5	97.7	79.4	99.8	99.8	41.6
37	beef	32.8	93.9	94.1	99.6	98.2	100	46.9
	cider	71.8	93.3	93.1	98.8	99.8	100	52.9
	salami	59.3	59.7	60.3	94.8	96.5	99.8	36.5

¹0.1% peptone water.²85% lactic acid stock solution.

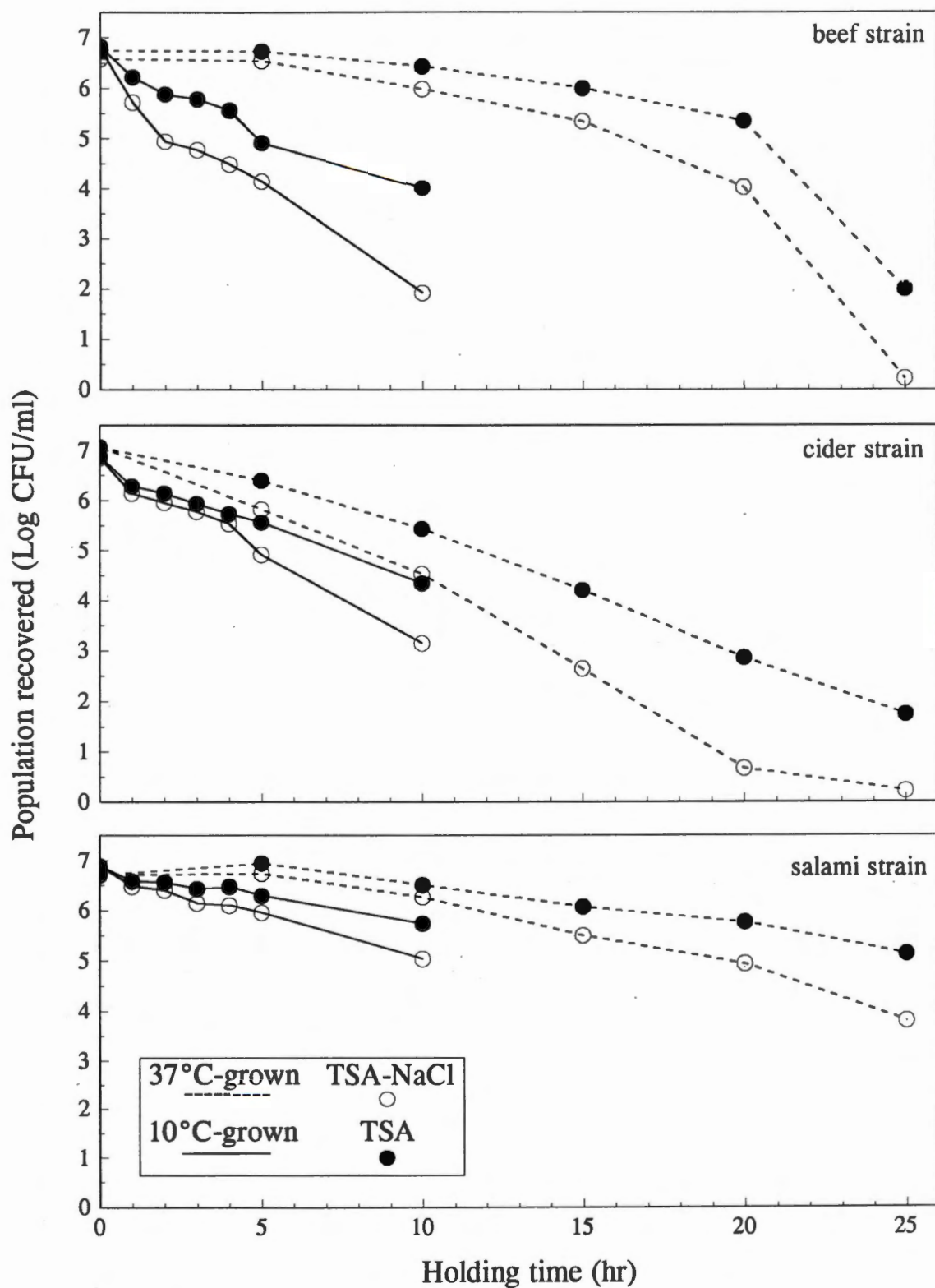


Figure 4. Inactivation and injury of three strains of *E. coli* O157:H7 in 0.1% peptone water containing 0.1% of an 85% lactic acid solution.

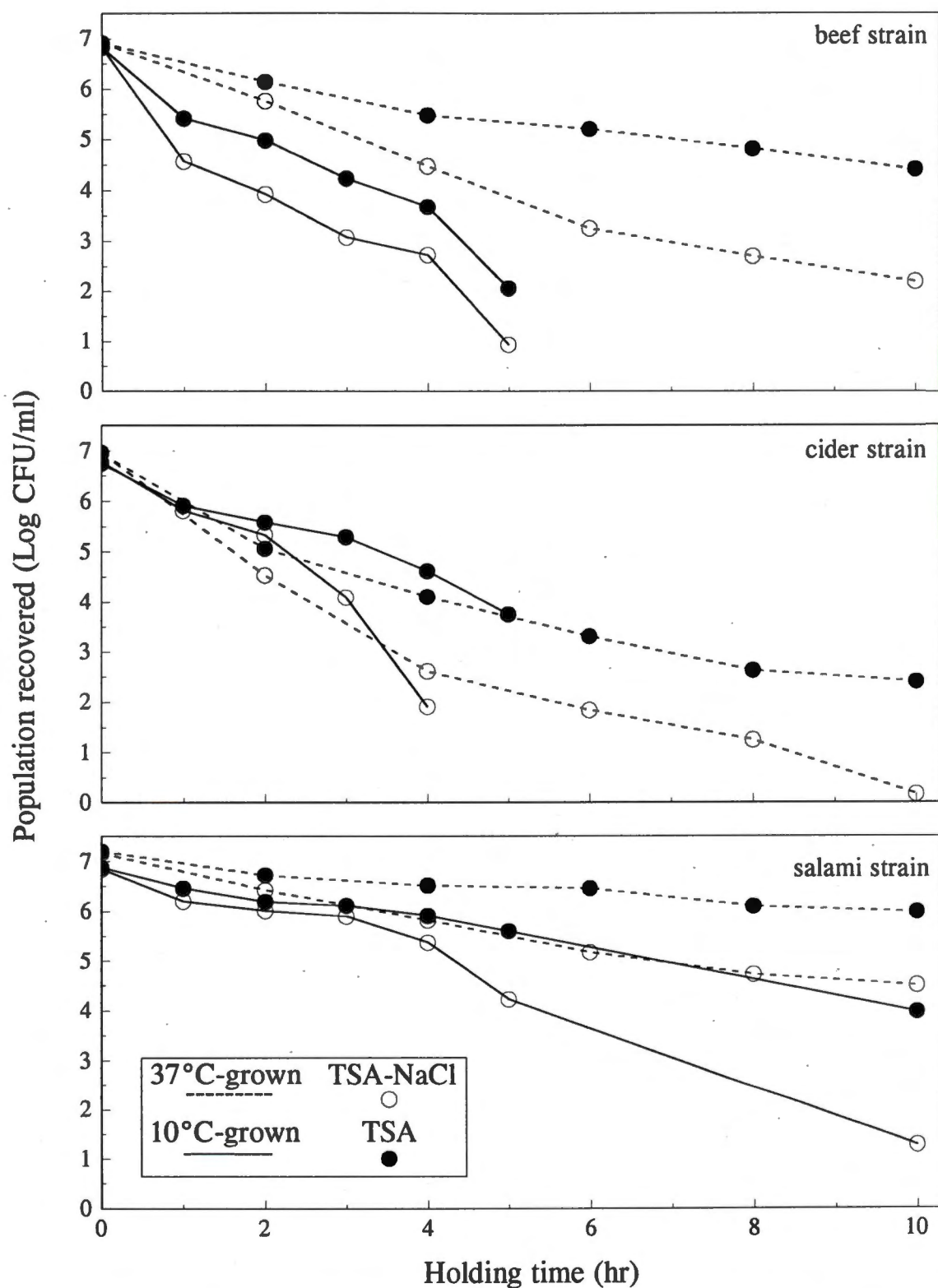


Figure 5. Inactivation and injury of three strains of *E. coli* O157:H7 in 0.1% peptone water containing 0.25% of an 85% lactic acid solution.

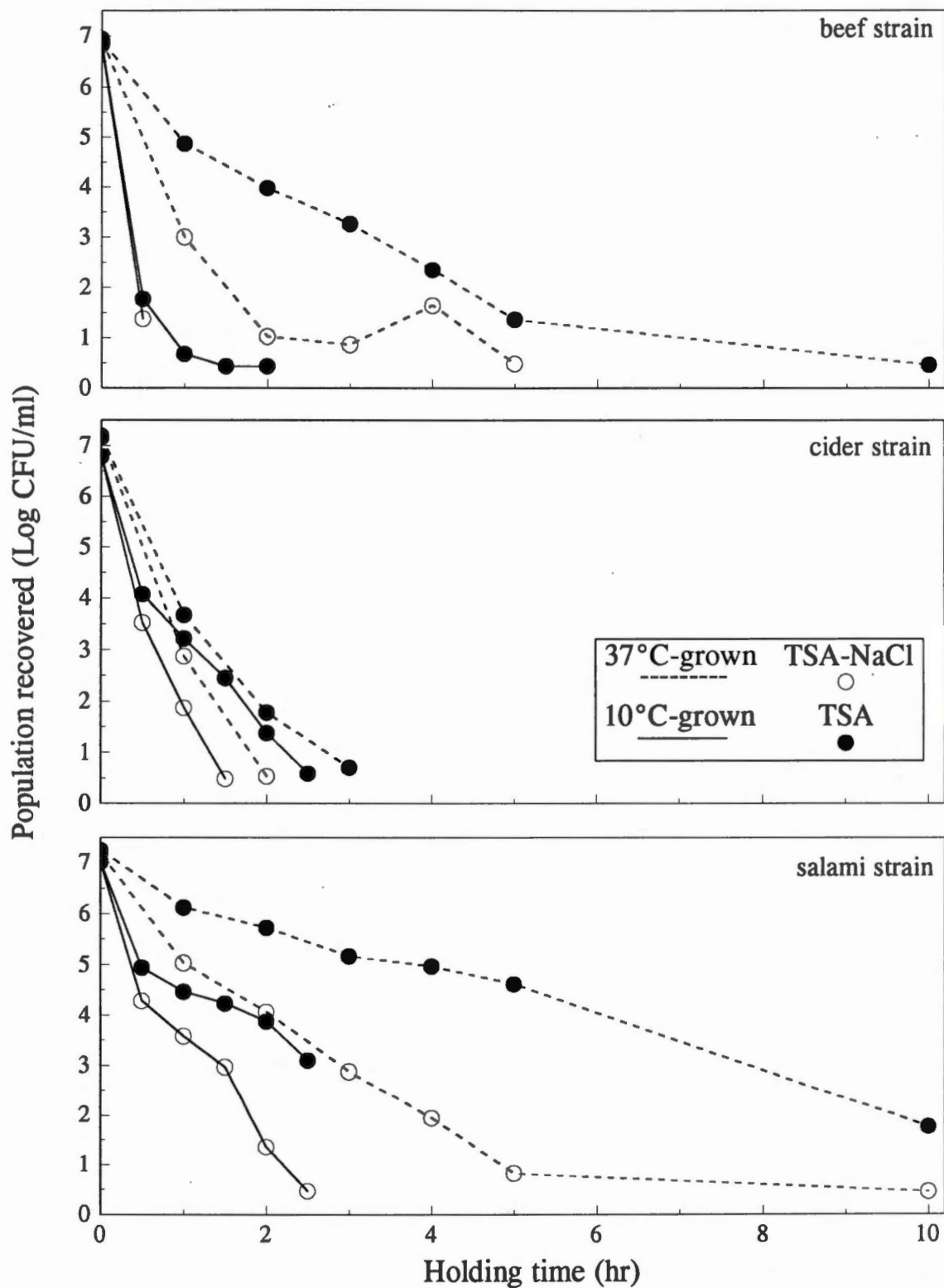


Figure 6. Inactivation and injury of three strains of *E. coli* O157:H7 in 0.1% peptone water containing 0.5% of an 85% lactic acid solution.

for strains grown at 37°C, with the exception of the cider strain stored in lactic acid. When grown at 10°C, the salami strain had the highest ($P<0.01$) D-values in all concentrations of lactic acid. When grown at 37°C, the salami strain possessed higher ($P<0.01$) D-values after storage in 0.1 and 0.25% lactic acid, while D-values for salami and beef strains did not differ ($P>0.05$) when stored in 0.5% lactic acid. D-values for beef and cider strains grown at 10°C and stored in 0.25 and 0.5% lactic acid did not differ ($P>0.05$). However, when strains were grown at 37°C the cider strain stored in 0.25 and 0.5% lactic acid had lower ($P<0.01$) D-values than the beef strain.

Maximum percentages of lactic acid induced injury in test strains grown at 10 and 37°C are shown in Table 2. For strains grown at 37°C, lactic acid induced injury was minimal in 0.1% lactic acid but became more pronounced in 0.25 and 0.5% lactic acid (Figures 4 - 6). When test strains were held in 0.5% lactic acid, all surviving cells of the beef and cider strains were sublethally injured after 10 and 3 hr, respectively. When strains were grown at 10°C, greater injury was apparent at all test concentrations of lactic acid (Figures 4 - 6). After storage for 1 and 2 hr in 0.5% lactic acid, all survivors of beef and cider strains, respectively, were injured.

Freeze inactivation and injury. The effects of storage at -20°C in PW on injury and survival of strains grown at 10 and 37°C are illustrated in Figure 7. All strains exhibited an initial reduction of 1.5 to 2.0 log CFU/ml after storage for 0.5 months. Viable populations of all test strains were recovered throughout 7 months of storage at -20°C. Tolerance to freezing was similar for all test strains. Survival of test strains was

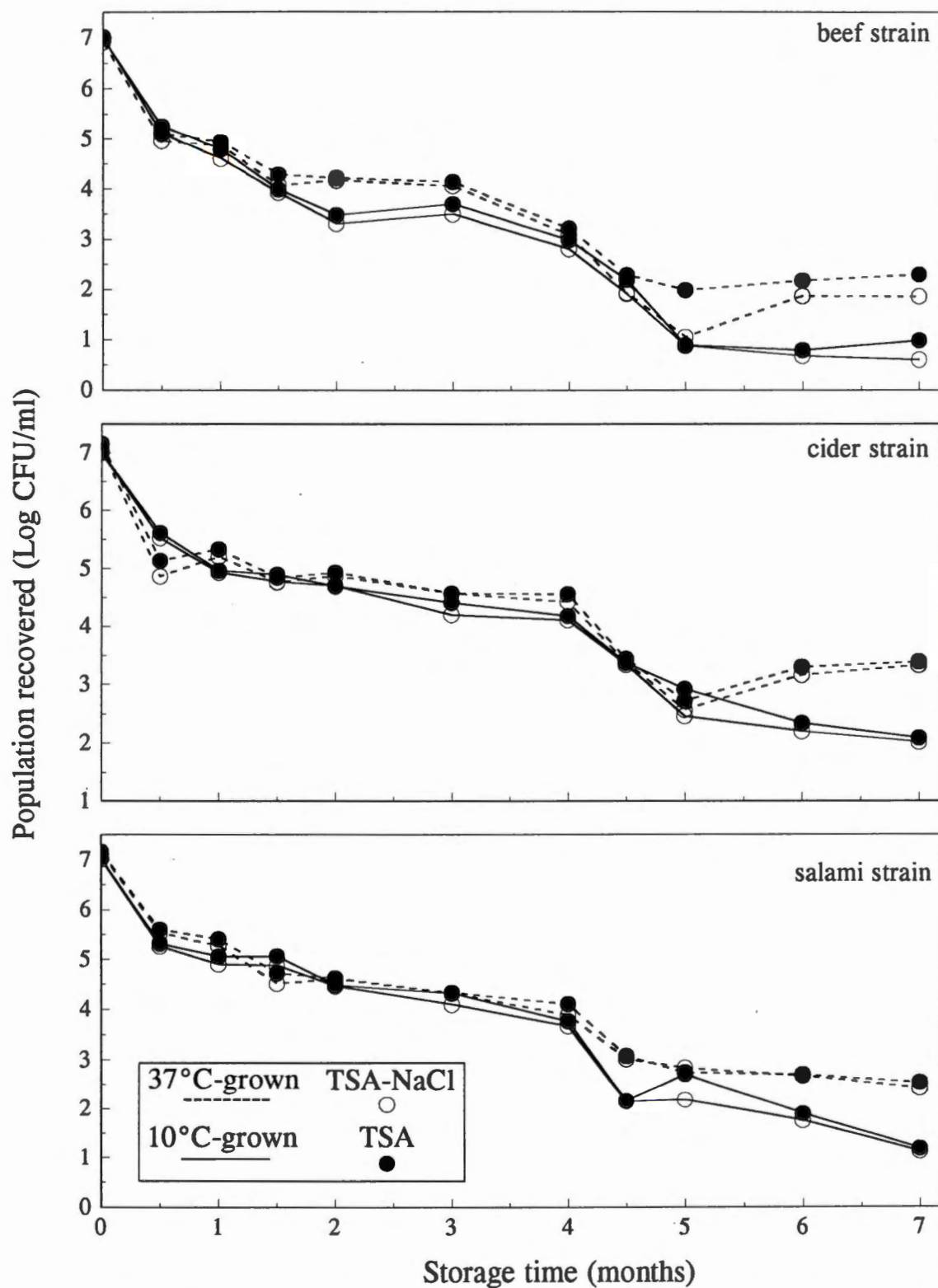


Figure 7. Inactivation and injury of three strains of *E. coli* O157:H7 stored at -20°C in 0.1% peptone water.

not appreciably influenced by cultivation temperature until after 5 months of storage, when strains cultivated at 37°C exhibited greater resistance to inactivation by freezing.

Table 1 illustrates D-values for strains subjected to storage at -20°C for 7 months. D-values for strains grown at 10°C were lower ($P<0.01$) than D-values for strains grown at 37°C. When grown at 10 and 37°C and stored at -20 for 7 months, the cider strain possessed higher ($P<0.01$) D-values than beef and salami strains, while D-values for beef and salami strains did not differ ($P>0.05$). Storage at -20°C had only a minor impact on the development of injury within all strains. Only minor differences were observed among strains within or between growth temperatures of 10 and 37°C. Maximum levels of injury observed during the 7 month storage period ranged from 36.5% (salami strain) to 57.5% (cider strain).

DISCUSSION

Since the emergence of beef carcass decontamination practices such as hot water rinses, steam pasteurization, and high vacuum technology, heat resistance of *E. coli* O157:H7 has been of particular interest to the food industry. Results of this investigation indicate that strains of *E. coli* O157:H7 are relatively resistant to heating at 52°C, but become increasingly sensitive with increased temperature. Reportedly, *E. coli* O157:H7 possesses no unusual heat resistance and is easily killed at pasteurization temperatures (Doyle and Schoeni, 1984). However, the nature of the heating medium can dramatically influence tolerance of microorganisms to heat (Rama Raju and Nambudripad, 1987).

Constituents in food such as fat, carbohydrates, and proteins can increase heat resistance, whereas deviation from neutral pH, certain salts, heat-resistant antibiotics, and preservatives in food may promote heat sensitivity (Jay, 1996). Line et al. (1991) reported *E. coli* O157:H7 D-values at 52°C of 79 and 118 min, in lean and fatty ground beef, respectively. In our study, D-values for *E. coli* O157:H7 heated at 52°C in PW were 17.7, 89.4, and 120.5 min for beef, cider, and salami strains, respectively.

Results of this and other investigations indicate that *E. coli* O157:H7 strains vary tremendously in their tolerance to heat inactivation. The salami strain was more heat resistant than beef and cider strains when grown at 10 and 37°C. Variability in the heat resistance exhibited among these strains of *E. coli* O157:H7 indicate that exposure to different environments may select for resistance to sub-optimum conditions or subsequent stress. It has been reported that certain sub-optimum environmental conditions may result in protection from subsequent stresses (Jenkins et al., 1988). Jackson et al. (1996) reported that *E. coli* O157:H7 populations frozen in ground beef demonstrated increased resistance to subsequent heat when compared with populations not frozen.

In the present study, strains cultivated at 10°C were considerably less resistant to heating than strains grown at 37°C. Similarly, Jackson et al. (1996) reported that stationary phase populations of *E. coli* O157:H7 grown at 23 and 30°C were more sensitive to heat than populations grown at 37°C. Katsui et al. (1981) concluded that increased heat resistance associated with changes in growth temperature were attributable

to alteration of fatty acid composition in bacterial membranes. Bacteria grown at low temperatures may incorporate more unsaturated fatty acids into their cell membranes in order to maintain functional membrane fluidity (Beuchat, 1978). As a result, decreased heat resistance may occur due to the reduced boiling point of unsaturated fatty acids within the cell membrane.

E. coli O157:H7 is fairly resistant to $\leq 1.5\%$ acetic, citric, or lactic acids applied to beef carcasses (Brackett et al., 1994). Results obtained in our study indicate that, although some variability exists among strains of *E. coli* O157:H7, the organism is tolerant to concentrations up to 0.5% of an 85% (i.e., 0.425% lactic acid) lactic acid solution in a non-nutritional medium. Miller and Kaspar (1994) reported that strains of *E. coli* O157:H7 survived in TSB adjusted to pH 2 with hydrochloric acid. Glass et al. (1992) reported that reducing pH with lactic acid, as compared to hydrochloric acid, results in an increased inhibitory response from *E. coli* O157:H7.

The salami strain was the most lactic acid resistant strain tested, as was expected due to the nature of the food from which the strain was isolated. The beef strain grown at 37°C was more resistant to lactic acid than the cider strain, possibly due to exposure of the beef strain to lactic acid produced during rigor mortis of beef. Conner and Kotrola (1995) reported that survival of *E. coli* O157:H7 in reduced pH culture medium is greatly affected by the type of organic acid acidulant. Although the cider strain may be tolerant to acidity associated with apple cider (Miller and Kaspar, 1994; Semanchek and Golden,

1996; Zhao et al., 1993), it should not be assumed that this strain is tolerant to acidic conditions in general.

Sub-optimum growth temperature had a considerable impact on reducing lactic acid tolerance of the beef and salami strains. Although information concerning the effect of growth temperature on acid resistance of *E. coli* O157:H7 has not been reported, several investigators have reported increased tolerance of *E. coli* O157:H7 to acidic conditions when apple cider was stored at higher near-refrigeration temperatures, compared with storage at higher temperatures (Besser et al., 1993; Miller and Kaspar, 1994; Zhao et al., 1993). Furthermore, Conner and Kotrola (1995) concluded that at 4°C, the presence of organic acids results in enhanced survival of *E. coli* O157:H7. In another investigation, addition of sodium lactate to TSB stored at 4°C enhanced *E. coli* O157:H7 survival for the initial 3 weeks of storage, and stimulated growth after 4 to 5 weeks of storage (Conner and Hall, 1996).

Although populations of *E. coli* O157:H7 strains declined steadily throughout storage at -20°C in PW, substantial numbers were recovered after 7 months of storage. Conner and Hall (1996) reported that *E. coli* O157:H7 decreased only 1 log CFU/g after storage for 18 months at -20°C in chicken meat. It has been reported that freezing may alter nutritional requirements of Gram-negative pathogens (Hartsell, 1951), and the loss of viability in cells subjected to freezer storage can differ from strain to strain and depend on the nature and composition of the food (Jay, 1996).

Although freezing had minimal impact on the development of injury, substantial portions of strain populations subjected to heat and lactic acid were injured. Growth of strains at 10°C resulted in considerably greater injury within all strains subjected to heat and lactic acid. Storage at 10°C could be considered as an abusive temperature situation to which foods may be exposed during improper refrigeration. The presence of sublethally injured *E. coli* O157:H7 in improperly refrigerated foods could be overlooked due to inadequate recovery of injured cells on selective recovery media.

Results of this investigation indicate the need to account for injured organisms when assessing the microbial load of processed foods and foods which inherently possess characteristics conducive to sublethal bacterial injury. With technological advances in food processing on the rise, the possibility exists that increasing numbers of injured bacteria may be present in food but not detected. Development of injury, as well as inactivation, may vary from strain to strain, possibly due to environmental adaptations. Therefore the need to test multiple strains for responses to such processes will become increasingly important.

REFERENCES

- Alexander, E.R., J. Boase, M. Davis, L. Kirchner, C. Osaki, T. Tanino, M. Samadpour, P. Tarr, M. Goldoft, S. Lankford, J. Kobayashi, P. Stehr-Green, P. Bradley, B. Hinton, P. Tghe, B. Pearson, G.R. Flores, R. Bryant, S.B. Werner, and D.J. Vugia. 1995. *Escherichia coli* O157:H7 outbreak linked to commercially distributed dry-cured salami- Washington and California. MMWR. 44: 157-159.
- Ahamad, N. and E.H. Marth. 1990. Behavior of *Listeria monocytogenes* at 7, 13, 21 and 35°C in tryptose broth acidified with acetic, citric, or lactic acid. J. Food Prot. 52: 688-695.
- Anonymous. 1996. USDA approves new method for removing contamination from beef carcasses. USDA Press Release No. 0151.96.
- Besser, R.E., S.M. Lett, J.T. Beber, M.P. Doyle, T.J. Barret, J.G. Wells, and P.M. Griffin. 1993. An outbreak of diarrhea and hemorrhagic uremic syndrome from *Escherichia coli* O157:H7 in fresh pressed apple cider. JAMA. 269: 2217-2220.
- Beuchat, L.R. 1978. Injury and repair of Gram-negative bacteria, with special consideration of the involvement of the cytoplasmic membrane. Adv. Appl. Microbiol. 23: 219-243.
- Blankenship, L.C. 1981. Some characteristics of acid injury and recovery of *Salmonella bareilly* in a model system. J. Food Prot. 44: 73-77.
- Brackett, R.E., Y.Y. Hao, and M.P. Doyle. 1994. Ineffectiveness of hot acid sprays to decontaminate *Escherichia coli* O157:H7 on beef. J. Food Prot. 57: 198-203.
- Busta, F.F. 1994. Importance of injured food-borne microorganisms in minimal processing. In Minimal processing of foods and process optimization. Edited by R.P. Singh and F.A.R. Oliveira. CRC Press, Boca Raton. pp 227-231.
- Conner, D.E., and G.S. Hall. 1996. Temperature and food additives affect growth and survival of *Escherichia coli* O157:H7 in poultry meat. Dairy Food Environ. Sanit. 16: 150-153.
- Conner D.E., and J.S. Kotrola. 1995. Growth and survival of *Escherichia coli* O157:H7 under acidic conditions. Appl. Environ. Microbiol. 61: 382-385.

- Davis, M., C. Osaki, D. Gordon, M.W. Hinds, K. Mottram, C. Winegar, E.D. Avner, P.I. Tarr, D. Jardine, M. Goldoft, B. Bartleson, J. Lewis, J.M. Kobayashi, G. Billman, J. Bradley, S. Hunt, P. Tanner, M. Ginsberg, L. Barret, S.B. Werner, G.W. Rutherford, R.W. Jue, H. Root, D. Brothers, R.L. Chehey, R.H. Hudson, F.R. Dixon, D.J. Maxson, L. Empey, O. Ravenholt, V.H. Ueckart, A. DiSalvio, D.S. Kwalick, R. Salcido, and D. Brus. 1993. Update: multistate outbreak of *Escherichia coli* O157:H7 infections from hamburgers -- western United States, 1992, 1993. MMWR. 42(14): 258-63.
- Dickson, J.S., and G.R. Siragusa. 1994. Survival of *Salmonella typhimurium*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* during storage on beef sanitized with organic acids. J. Food Safety. 14: 313-327.
- Doyle, M.P., and J.L. Schoeni. 1984. Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. Appl. Environ. Microbiol. 48: 855-856.
- Erickson, J.P., J.W. Stamer, M. Hayes, D.N. McKenna, and L.A. Van Alstine. 1995. An assessment of *Escherichia coli* O157:H7 contamination risks in commercial mayonnaise from pasteurized eggs and environmental sources, and behavior in low-pH dressings. J. Food Prot. 58: 1059-1064.
- Glass, K.A., J.M. Loeffelholz, J.P. Ford, and M.P. Doyle. 1992. Fate of *Escherichia coli* O157:H7 as affected by pH or sodium chloride and in fermented, dry sausage. Appl. Environ. Microbiol. 58: 2513-2516.
- Golden, D.A., L.R. Beuchat, and R.E. Brackett. 1988. Inactivation and injury of *Listeria monocytogenes* as affected by heating and freezing. Food Microbiol. 5: 17-23.
- Hartsell, S.E. 1951. The longevity and behavior of pathogenic bacteria in frozen foods: The influence of plating media. Amer. J. Publ. Health. 41: 1072-1077.
- Jackson, T.C., M.D. Hardin, and G.R. Acuff. 1996. Heat resistance of *Escherichia coli* O157:H7 in a nutrient medium and in ground beef patties as influenced by storage and holding temperatures. J. Food Prot. 59: 230-237.
- Jay, J.M. 1996. Modern food microbiology. 5th ed. Chapman and Hall, New York, NY.
- Jenkins, D.E., J.E. Schultz, and A. Matin. 1988. Starvation-induced cross protection against heat or H₂O₂ challenge in *Escherichia coli*. J. Bacteriol. 170: 3910-3914.

- Katsui, N., T. Tsuchido, M. Takano, and I. Shibasaki. 1981. Effect of preincubation temperature on the heat resistance of *Escherichia coli* having different fatty acid compositions. J. Gen. Microbiol. 122: 357-361.
- Line, J.E., A.R. Fain Jr., A.B. Moran, L.M. Martin, R.V. Lechowich, J.M. Carosella, and W.L. Brown. 1991. Lethality of heat to *Escherichia coli* O157:H7: D-value and z-value determinations in ground beef. J. Food Prot. 54: 762-766.
- Miller, L.G., and C.W. Kaspar. 1994. *Escherichia coli* O157:H7 acid tolerance and survival in apple cider. J. Food Prot. 57: 460-464.
- Morgan, D., C.P. Newman, D.N. Hutchinson, A.M. Walker, B. Rowe, and F. Majid. 1993. Verotoxin producing *Escherichia coli* O157 infections associated with consumption of yogurt. Epidemiol. Infect. 111: 181-187.
- Padhye, N.V. and M.P. Doyle. 1992. *Escherichia coli* O157:H7: epidemiology, pathogenesis, and methods for detection in food. J. Food Prot. 55: 555-565.
- Rama Raju, V.V. and V.K.N. Nambudripad. 1987. Effect of heat injury and death of coliform bacteria in different media. Indian J. Dairy. Sci. 40: 359-363.
- Ray, B. 1989. Injured index and pathogenic bacteria: occurrence and detection in food, water and feeds. CRC Press Inc., Boca Raton, FL.
- Rodrigue, D.C., E.E. Mast, K.D. Greene, J.P. Davis, M.A. Hutchinson, J.G. Wells, T. J. Barret, and P.M. Griffin. 1995. A University outbreak of *Escherichia coli* O157:H7 infections associated with roast beef and an unusually benign clinical course. J. Infect. Dis. 172: 122-1125.
- Salmon, R.L., I.D. Farrell, J.G.P. Hutchinson, D.J. Coleman, R.J. Gross, N.K. Fry, B. Rowe, and S.R. Palmer. 1989. A christening party outbreak of hemorrhagic colitis and haemolytic uraemic syndrome associated with *Escherichia coli* O157:H7. Epidemiol. Inf. 103: 49-254.
- Semanchek, J.J. and D.A. Golden. 1996. Survival of *Escherichia coli* O157:H7 during fermentation of apple cider. J. Food Prot. 59(12): 1-4.
- Splittstoesser, D.F., M.R. Mclellan, and J.J. Churey. 1996. Heat resistance of *Escherichia coli* O157:H7 in apple cider. J. Food Prot. 59: 226-229.

Weagant, S.D., J.L. Bryant, and D.H. Bark. 1994. Survival of *Escherichia coli* O157:H7 in mayonnaise and mayonnaise-based sauces at room and refrigerated temperatures. J. Food Prot. 57: 29-631.

Zhao, T., M.P. Doyle, and R.E. Besser. 1993. Fate of enterohemorrhagic *Escherichia coli* O157:H7 in apple cider with and without preservatives. Appl. Environ. Microbiol. 8: 526-2530.

CHAPTER IV
EFFECT OF PACKAGE ATMOSPHERE ON GROWTH AND SURVIVAL
OF UNINJURED AND SUBLETHALLY HEAT-INJURED
***ESCHERICHIA COLI* O157:H7**

ABSTRACT

The effect of atmospheric composition on growth and survival of uninjured and sublethally heat-injured *E. coli* O157:H7, inoculated onto brain heart infusion agar containing 0.3% beef extract, was determined. Media were packaged in air, 100% CO₂, 100% N₂, 20% CO₂/80 %N₂ and vacuum stored at 4, 10, and 37°C for up to 20 days. Package atmosphere and inoculum status (i.e., uninjured or heat-injured) influenced (P<0.01) growth and survival of *E. coli* O157:H7 stored at all test temperatures. Growth of heat-injured *E. coli* O157:H7 was slower (P<0.01) than uninjured *E. coli* O157:H7 stored at 37°C. At 37°C, uninjured *E. coli* O157:H7 reached stationary phase growth earlier than heat-injured populations. Uninjured *E. coli* O157:H7 grew during 10 days of storage at 10°C, while heat-injured populations declined during 20 days of storage at 10°C. Uninjured *E. coli* O157:H7 stored at 10°C reached stationary phase growth within approximately 10 days in all packaging atmospheres except CO₂. Populations of uninjured and heat-injured *E. coli* O157:H7 declined throughout storage for 20 days at 4°C. Survival of uninjured populations stored at 4°C, as well as heat-injured populations stored at 4 and 10°C, was enhanced in CO₂ atmosphere. Survival of heat-injured *E. coli*

O157:H7 at 4 and 10°C was not different ($P>0.05$). Results of this investigation indicate that uninjured and heat-injured *E. coli* O157:H7 are able to survive at low temperatures in the modified atmospheres used in this study. Therefore, packaging treatments commonly applied to fresh beef may be ineffective at inhibiting growth and survival of this pathogen.

INTRODUCTION

Escherichia coli O157:H7 was identified as a foodborne pathogen in 1982, when two outbreaks characterized by bloody diarrhea were linked to consumption of contaminated hamburgers (Riley et al., 1983; Wells et al., 1983). The largest reported outbreak of *E. coli* O157:H7 foodborne illness occurred between in 1993 in the Western United States (Davis et al., 1993). Over 600 patients were treated for hemorrhagic colitis and/or hemolytic uremic syndrome; 75% of reported infections were linked to consumption of hamburger patties (Tarr, 1994). O'Brien et al. (1993) reported that five strains isolated from the Pacific Northwestern U.S. outbreak were indistinguishable from each other and were consistent with the *E. coli* O157:H7 strain EDL993 first isolated in 1982.

Domestic cattle have been suggested to be important reservoirs of *E. coli* O157:H7. Several methods of reducing visual and microbiological contaminants on beef carcasses have been reported. These include hand trimming/spray washing techniques (Gorman et al., 1996), hot water rinses (Barkate et al., 1993), rinsing solutions containing

trisodium phosphate (Dickson et al., 1994), steam-vacuum sanitizing (Dorsa et al., 1996), and organic acid rinses (Cutter and Siragusa, 1994). Organic acid rinses have been shown to result in sublethal injury of *E. coli* O157:H7 on beef carcasses (Dickson and Siragusa, 1994). Sublethal injury in bacteria can also result from exposure to low temperature, heat, reduced pH, drying, and sanitizers (Ray, 1986; 1989).

Modified atmosphere packaging (MAP) is an effective means of extending the shelf-life of fresh, refrigerated foods (Farber, 1991). In the U.S., approximately 80% of fresh beef is vacuum packaged before leaving the packaging facility (Jay, 1996), primarily due to the effectiveness of vacuum packaging at reducing bacterial growth on beef. Hao and Brackett (1993) observed minimal growth by *E. coli* O157:H7 during refrigerated storage. Packaging procedures commonly used by the food industry are ineffective against reducing populations of *E. coli* O157:H7 and *Listeria monocytogenes* (Beuchat and Brackett, 1990).

Most beef in the U.S. is packaged within atmospheres different from that consistent with air. The presence of oxygen in packaged foods selects for growth of aerobic microflora indigenous to the food itself. Although excluding oxygen from the package often extends the shelf-life, typical signs of spoilage, such as off-odors and off-flavors, can be absent. As a result, the microbiological safety of foods can be misjudged due to growth of pathogenic bacteria. Several investigators have reported the ability of foodborne pathogens such as *L. monocytogenes* and *Aeromonas hydrophila* to survive in atmospheres consisting of little or no oxygen (Beuchat and Brackett, 1990; Golden et al.,

1989). Several investigators have also reported that anaerobic atmospheres can enhance survival or growth of pathogens such as *L. monocytogenes* and *A. hydrophila* after exposure to sublethal stress (Gadzella and Ingram, 1994; Golden et al., 1989). For example, *L. monocytogenes* can survive the longtime pasteurization treatment required by the U.S. Food and Drug Administration for milk when cells are grown at 43°C and enumerated by strictly anaerobic Hungate techniques (Knabel et al., 1990). Murano and Pierson (1992) reported that when *E. coli* O157:H7 are grown anaerobically, heat shock proteins are synthesized, resulting in an increase in the number of cells capable of recovery after subsequent heat treatment.

Results of previous investigations suggest that certain gaseous atmospheres may increase the likelihood of *E. coli* O157:H7 recovering from injury or increase its tolerance to subsequent stresses. This investigation was undertaken to determine the effects of package atmosphere on the growth and survival of uninjured and heat-injured *E. coli* O157:H7 stored at 4, 10, and 37°C.

MATERIALS AND METHODS

Test culture and maintenance. *E. coli* O157:H7 strain 380-94, isolated from an outbreak associated with salami (380-94), was used for study, and was obtained from Dr. A.M. McNamara USDA-FSIS, Washington D.C. The test culture was grown to the stationary phase at 10°C in tryptone soya broth (TSB; Unipath Oxoid USA, Ogdenburg, NY). This test strain reaches stationary growth phase after 7 days at 10°C in TSB, and

has been shown to possess substantial resistance to inactivation by heating, freezing, and exposure to lactic acid (Semanchek and Golden, 1996). Test cultures were subjected to a minimum of three successive stationary phase transfers before experimentation.

Preparation of inocula. Sublethal heat-injury (approximately 90%) was induced according to the method of Semanchek and Golden (1996). A stationary phase culture of *E. coli* O157:H7 strain 380-94 was inoculated into 100 ml of 0.1% peptone water (PW) in a 250-ml Erlenmeyer flask (final population of approx. 1×10^7 CFU/ml). The culture suspension was heated for 10 min at 56°C under constant agitation (150 rpm), in an orbital shaking water bath (Lab Line Instruments, Inc., Melrose Park, IL). The water level of the bath was adjusted such that flask contents were below the water level throughout heat treatment. After heating, 10 ml of the heated suspension were removed, placed into sterile test tubes, and placed in ice to terminate heat treatment. Cooled cultures were serially diluted in PW and surface plated (0.1 ml) in duplicate onto tryptone soya agar (TSA; Unipath Oxoid USA, Ogdenburg, NY) and TSA containing 2% sodium chloride to confirm percentages of viable and injured organisms.

Inoculation and gas packaging. Brain heart infusion agar (Oxoid) supplemented with 0.3% beef extract (Oxoid) (beef extract medium, BEM, pH 7.0) in 60 x 15 mm Petri dishes was inoculated with 0.1 ml of heat-injured or uninjured *E. coli* O157:H7. An unheated stationary phase culture, serially diluted in PW to obtain approximately the same population of viable organisms in the heated culture, served as the uninjured control inoculum. Final populations of about 10^3 or 10^5 CFU/agar surface were obtained,

depending on injury status and the subsequent storage temperature that was used. Inocula were distributed over the BEM surface (28.3 cm²) using sterile bent glass rods and were allowed to be absorbed for 15 min before packaging. Three holes (approx. 0.4 cm in diameter) were melted into the lids of BEM plates using a Creative Woodburner Jr. (Walnut Hollow, Dodgeville, WI) to facilitate gas exchange between the agar surface and the atmosphere within each package.

Since *E. coli* O157:H7 was not expected to grow at 4°C, and preliminary work revealed that populations of heat-injured *E. coli* O157:H7 are reduced during storage at 10°C, BEM "samples" were inoculated with higher populations of heat-injured and uninjured *E. coli* O157:H7 (approx. 10⁵ CFU) for monitoring survival. BEM samples containing uninjured *E. coli* O157:H7 at 10°C, as well as samples containing injured and uninjured *E. coli* O157:H7 stored at 37°C, were inoculated with low populations (approx. 10³ CFU) for monitoring growth. Packaged BEM samples were stored at 4 and 10°C for up to 20 days and at 37°C for up to 48 hr.

Gas packaging and analysis. BEM plates were packaged in high barrier bags (Cryovac North America, Duncan, SC) in air, modified atmospheres consisting of 100% CO₂, 100% N₂, a mixture of 20%CO₂/80%N₂, and vacuum, using a multivac packaging system (Koch Multivac, Kansas City, MO). These gas concentrations are hereafter referred as air, CO₂, N₂, CO₂/N₂, and vacuum. Permeability for oxygen in barrier bags is 3 to 6 cm³/m²/24 h/@ 1 atm., 4.4°C, 0% RH. Permeability rates for N₂, and CO₂ were not provided by the manufacturer.

At each sampling interval, headspace gas in packages, except those packaged under Vacuum, was measured prior to microbiological analysis. Septum tape (Modern Controls; Minneapolis, MN) was placed on each sample package, through which a syringe needle was inserted to remove gas samples. Gas samples were removed with packaged samples submerged under water to eliminate the possibility of introducing ambient atmospheres into the syringe. Head-space gas analysis of samples was performed using a Hewlett-Packard 5890 Series II gas chromatograph equipped with a CTR 1 packed column (Alltech; Deerfield, IL). Thermal conductivity detector and injector port were held at ambient temperature. Helium was used as the carrier gas at a rate of 64 ml/min.

Microbial analysis. At selected sampling intervals, *E. coli* O157:H7 populations were determined as follows: the entire agar block from BEM samples was aseptically removed from Petri dishes and placed into sterile stomacher bags with 50 ml of PW. Samples were pummeled for 2 min in a Stomacher 400 Lab Blender (Seward Medical, London, UK), serially diluted in PW, and surface plated (0.1 ml) in duplicate onto TSA. TSA plates were incubated at 37°C for 24 hr before colonies of *E. coli* O157:H7 were enumerated. In addition, to compensate for instances in which surviving populations were not sufficiently high to be enumerated by this dilution scheme, a second BEM sample was removed from storage, unpackaged, and incubated at 37°C for 24 hr. In these instances, survival was determined by the presence or absence of colony formation on

BEM agar. The pH of pummeled samples was determined at the initial and terminal sampling intervals with a Corning model 350 pH meter.

Statistical analysis. Statistical analyses were conducted using SAS version 6.1 (SAS Institute; Cary, NC). Data were fit to a completely randomized design with a factorial treatment arrangement. Differences were evaluated noting the effects of package atmosphere, inoculum status (i.e, uninjured or heat-injured), and storage temperature on the survival and growth of uninjured and sublethally heat-injured *E. coli* O157:H7.

RESULTS

Minimal or no change in pH of BEM was observed during storage, regardless of treatment (Table 1). The initial pH of inoculated BEM samples was 7.22 prior to packaging. As expected, the greatest change in pH occurred in media packaged in elevated CO₂ atmospheres (i.e., CO₂ and CO₂/N₂).

Headspace gas analysis was measured in sample packages to assure bags did not possess leaks. Gas composition of packaged samples throughout storage at 37, 10, and 4°C did not change appreciably during storage. However, small amounts of CO₂ were detected in air packages stored at 37°C and in air packages containing uninjured *E. coli* O157:H7 stored at 10°C. Oxygen was not detected in CO₂, N₂, and CO₂/N₂ packaged samples stored at 4, 10, and 37°C. However, concentrations of up to 6% N₂ and 5% CO₂ were detected in CO₂ and N₂ packages, respectively, during storage at 37, 10, and 4°C.

Table 1. pH of packaged BEM samples stored at 37, 10, and 4°C.

Growth temp. (°C)	Inoculum	Storage time	pH ^a of samples packaged in:				
			air	CO ₂	CO ₂ /N ₂	N ₂	vacuum
37	uninjured	24 hr	7.48	6.49	6.59	6.79	6.81
		48 hr	7.79	6.48	6.65	6.82	7.25
	injured	24 hr	6.82	6.54	6.61	7.32	6.76
		48 hr	7.17	6.62	6.44	7.09	6.88
	uninoculated	48 hr	7.18	6.95	7.06	7.19	7.21
10	uninjured	1 day	7.05	6.78	6.96	7.29	7.33
		20 days	7.34	6.69	6.54	6.74	6.97
	injured	1 day	7.24	6.85	7.04	7.26	7.29
		20 days	7.09	6.73	6.97	7.34	7.32
	uninoculated	20 days	7.34	6.73	6.97	7.34	7.32
4	uninjured	1 day	7.25	6.78	6.97	7.27	7.26
		20 days	7.23	6.59	6.88	7.29	7.28
	injured	1 day	7.26	6.81	6.87	7.22	7.28
		20 days	7.23	6.61	6.9	7.27	7.20
	uninoculated	20 day	7.27	6.85	6.93	7.34	7.30

^a pH of BEM immediately after inoculation with test organisms was 7.22.

Survival and growth of uninjured and heat-injured *E. coli* O157:H7 stored at 4, 10, and 37°C were influenced ($P > 0.01$) by packaging in air, CO₂, N₂, and CO₂/N₂, and vacuum. Survival and growth rate were also affected ($P < 0.01$) by the inoculum status (i.e., uninjured or heat-injured).

Growth of uninjured *E. coli* O157:H7 packaged in air, CO₂/N₂, and vacuum stored at 37°C (Figure 1) was statistically different ($P < 0.01$). Growth rates during the initial 8 hr of storage were greatest for samples packaged in air, CO₂/N₂, and vacuum. *E. coli* O157:H7 packaged in CO₂ and N₂ exhibited extended lag phase growth for up to 4 hr, after which rapid growth occurred until the stationary phase was reached at around 16 h. Populations packaged in vacuum and CO₂/N₂ reached the stationary phase after about 12 hr. *E. coli* O157:H7 packaged in air reached the stationary phase within 16 h, but grew to higher populations than uninjured *E. coli* O157:H7 packaged in all other atmospheres including vacuum.

Heat-injured *E. coli* O157:H7 stored at 37°C (Figure 2) exhibited slower ($P < 0.01$) growth and longer lag phases than uninjured populations stored at 37°C. Heat-injured *E. coli* O157:H7 packaged in air, CO₂, and vacuum remained in the lag phase for 8 hr of storage at 37°C. Slightly better growth was observed during the first 8 hr of storage for heat-injured *E. coli* O157:H7 packaged in CO₂/N₂, while heat-injured populations stored in N₂ were not enumerated until 12 hr. Heat-injured *E. coli* O157:H7 packaged in air, CO₂, CO₂/N₂, and vacuum reached the stationary phase after 32 hr into storage at 37°C, whereas, heat-injured populations packaged in N₂ did not reach the stationary phase

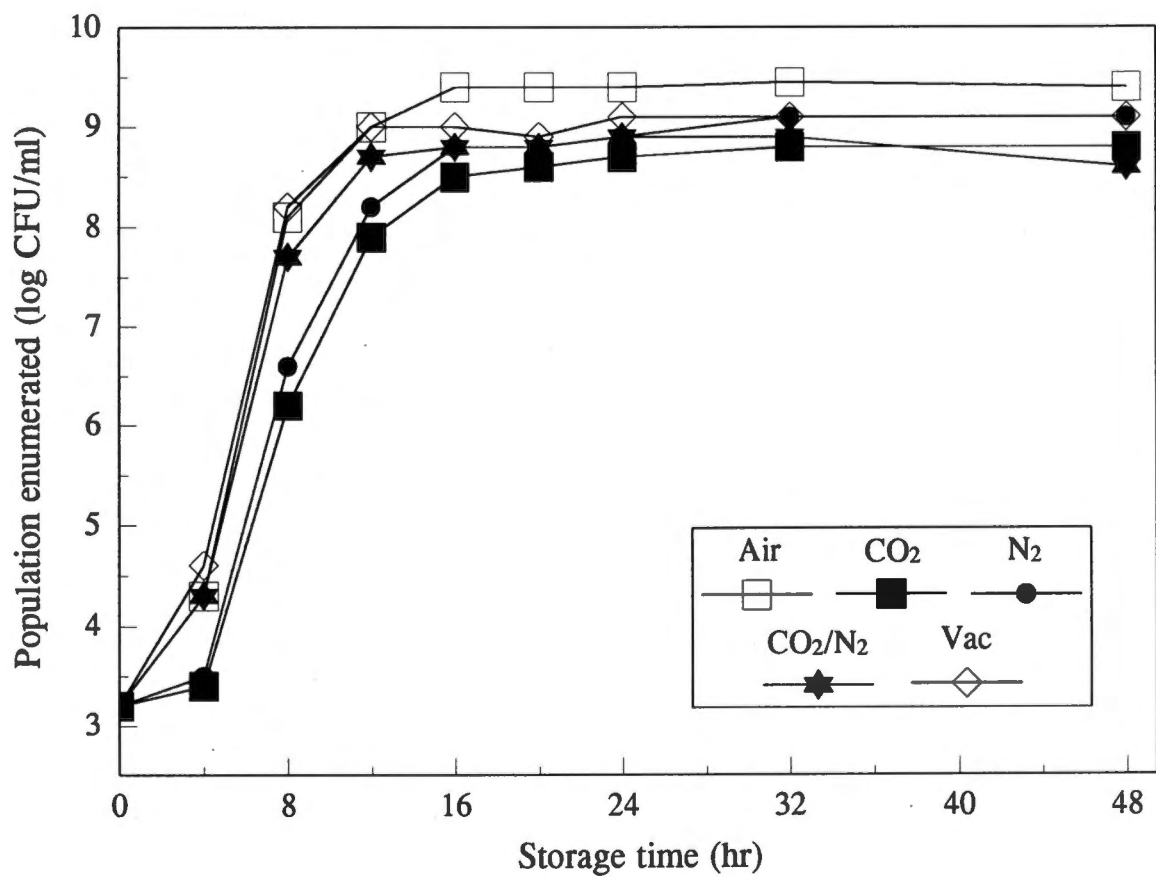


Figure 1. Growth of uninjured *E. coli* O157:H7 at 37°C on beef extract medium packaged in air, 100% CO₂, 100% N₂, 20% CO₂/80% N₂, and vacuum.

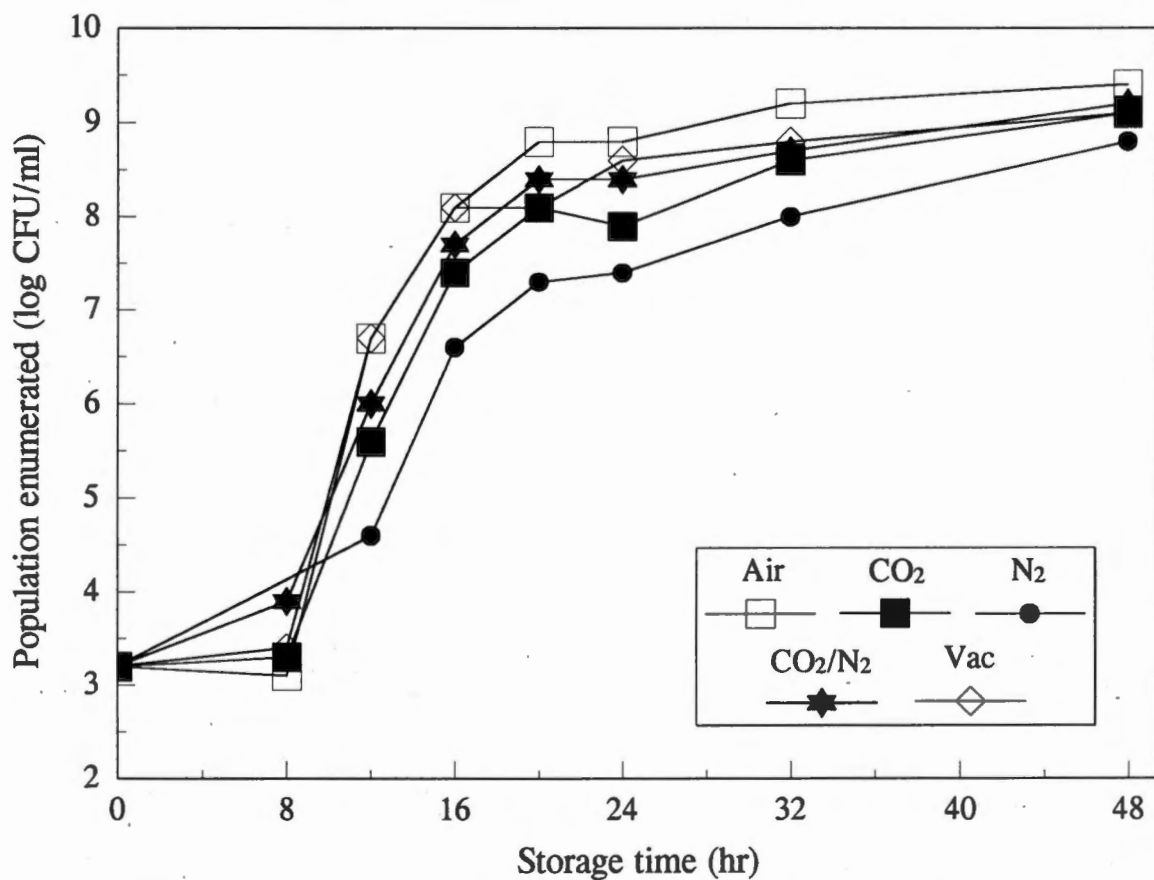


Figure 2. Growth of sublethally heat-injured *E. coli* O157:H7 at 37°C on beef extract medium packaged in air, 100% CO₂, 100% N₂, 20% CO₂/ 80% N₂, and vacuum.

during the 48-hr storage period. Growth of heat-injured populations packaged in air, CO₂, CO₂/N₂, and vacuum was faster than heat-injured populations packaged in N₂.

Growth of uninjured *E. coli* O157:H7 stored at 10°C (Figure 3) were slower ($P<0.01$) than populations stored at 37°C. In addition, all packaging treatments influenced ($P<0.01$) growth of samples stored at 10°C. Uninjured populations remained in the lag phase for up to 1 day, with the exception of those packaged in air. Growth of uninjured populations packaged in air, N₂, and vacuum reached the stationary phase within approximately 8 days of storage at 10°C. Populations of uninjured *E. coli* O157:H7 packaged in CO₂/N₂ continued to increase throughout the 10-day storage period. Uninjured *E. coli* O157:H7 grew slowest in packages containing CO₂, and reached a maximum population of 5.2 log CFU/ml, as compared with uninjured populations of >8 log CFU/ml in all other packaging atmospheres at 10°C.

Heat-injured *E. coli* O157:H7 stored at 10°C were more markedly affected by package atmosphere than uninjured populations ($P<0.01$) and decreased over the 20-day storage period in all packaging treatments (Table 2). Packaging heat-injured *E. coli* O157:H7 in CO₂ resulted in the greatest survival. Heat-injured populations were enumerated for up to 4 days in air, N₂, and vacuum and up to 8 in CO₂/N₂ packaged samples. Heat-injured populations packaged in CO₂/N₂ and N₂ were detected for up to 20 days. Survivors packaged in air and vacuum were detected for up to 4 and 8 days, respectively.

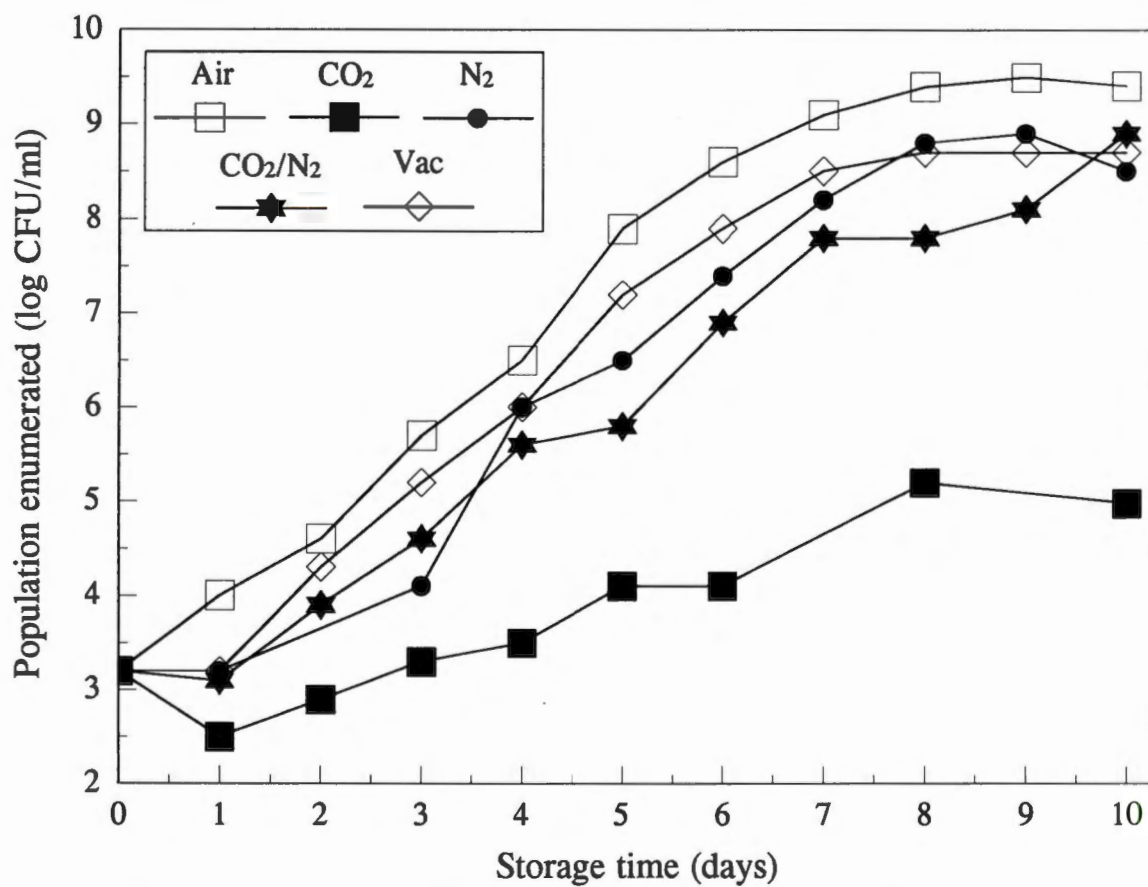


Figure 3. Growth of uninjured *E. coli* O157:H7 at 10°C on beef extract medium packaged in air, 100% CO₂, 100% N₂, 20% CO₂/ 80% N₂, and vacuum.

Table 2. Survival of sublethally heat-injured *E. coli* O157:H7 stored at 10 and 4°C in different gaseous atmospheres.

Growth temperature	Storage time (days)	*Surviving population (log CFU/ml) packaged in:				
		air	CO ₂	CO ₂ /N ₂	N ₂	vacuum
10°C	1	1.5	2.6	2.5	1.9	1.3
	4	1.3	2.7	2.0	0.9	0.5
	8	++ ^b	2.3	1.1	++	nd ^c
	12	nd	2.1	+++	++	nd
	16	nd	1.3	+++	++	nd
	20	nd	0.6	+++	++	nd
4°C	1	1.4	2.4	2.3	2.0	0.8
	4	0.1	2.3	2.2	1.5	0.5
	8	nd	2.2	1.3	0.2	0.1
	12	nd	2.1	1.2	+++	nd
	16	nd	2.1	1.0	++	nd
	20	nd	0.8	0.2	nd	nd

^aInitial heat-injured population was 4.2 log CFU/ml.

^bDetected by incubating BEM plate at 37°C for 24 hr and observing colony formation; each "+" indicates the number of replications in which survivors were detected but not enumerated.

^cNot detected

Package atmosphere affected ($P < 0.01$) the survival of uninjured *E. coli* O157:H7 stored at 4°C, with populations in all packaging treatments decreasing throughout the 20-day storage period (Figure 4). Approximately a 1.8 log CFU/ml decrease was observed after 1 day of storage at 4°C, after which, steady declines in population were observed for all packaging treatments. Uninjured *E. coli* O157:H7 survived best in CO₂. After 20 days at 4°C, survival of uninjured *E. coli* O157:H7 was enhanced by package atmosphere in the order CO₂ > CO₂/N₂ > air > N₂ > vacuum. Survival of heat-injured *E. coli* O157:H7 at 4°C was poorer than uninjured populations ($P < 0.01$). Heat-injured populations packaged in air or N₂ and vacuum were enumerated for up to 4 or 8 days, respectively, when stored at 4°C (Table 2). Samples packaged in CO₂ and CO₂/N₂ were enumerated at populations of 0.8 and 0.2 log CFU/ml, after storage for 20 days at 4°C. Survival of heat-injured *E. coli* O157:H7 stored at 4°C was enhanced by package atmosphere in the order CO₂ > CO₂/N₂ > N₂ > vacuum > air. Interestingly, survival of heat-injured *E. coli* O157:H7 stored at 4°C was not different ($P > 0.05$) than survival at 10°C.

DISCUSSION

BEM was used as the test medium to simulate a fresh beef substrate, with the exception that pH was favorably adjusted to minimize adverse growth affects other than those influenced by packaging atmosphere or temperature. Hao and Brackett (1993) reported that *E. coli* O157:H7 was not affected by modified atmospheres consisting of 0 to 10% CO₂, 5 to 21% O₂, and 70 to 95% N₂, when stored at 5, 10, and 20°C. Growth of

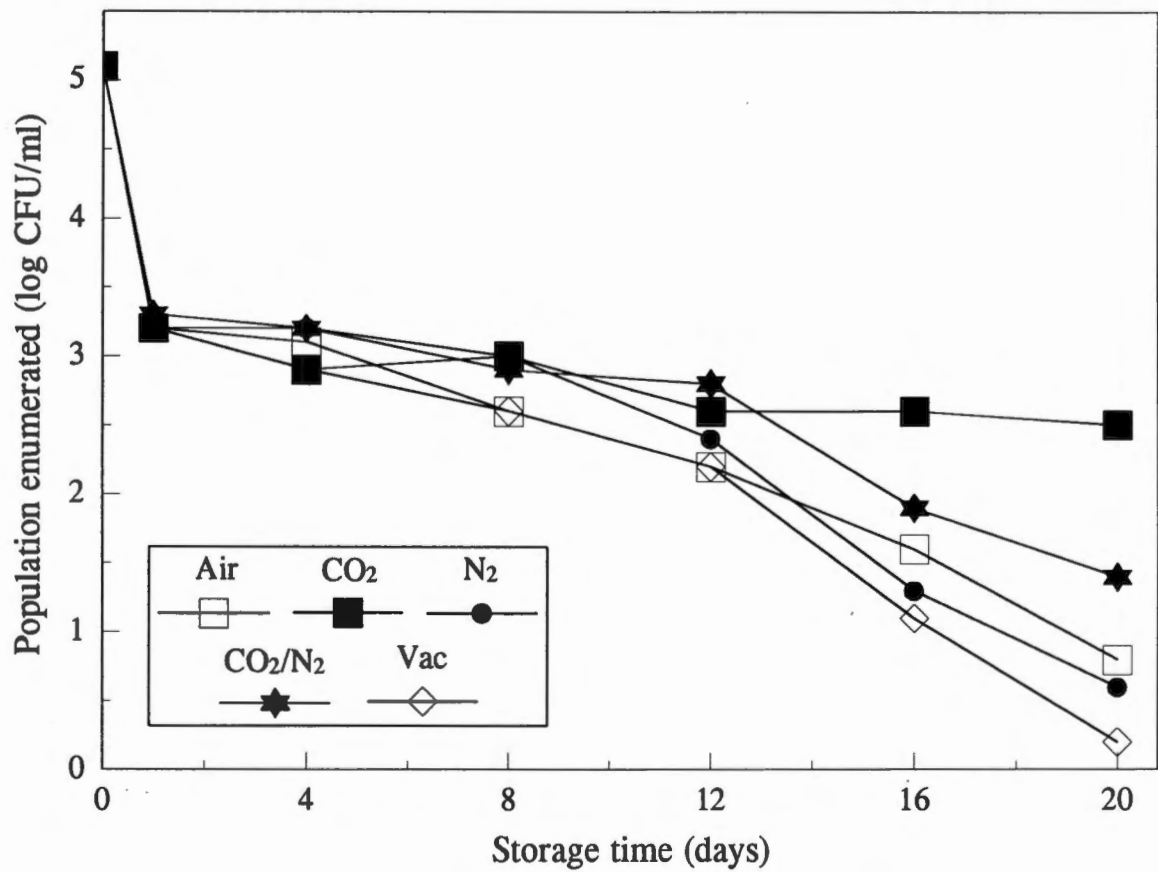


Figure 4. Survival of uninjured *E. coli* O157:H7 at 4°C on beef extract medium packaged in air, 100% CO₂, 100% N₂, 20% CO₂/80% N₂, and vacuum.

E. coli O157:H7 is also not affected by modified atmospheres commonly used for packaging vegetables (Abdul-Raouf et al., 1993).

Phebus et al. (1991) reported that survival of *Campylobacter jejuni* on turkey roll, packaged in air was significantly lower at 4°C, as compared to other gas mixtures tested, but storage in air allowed for substantial increases in aerobic spoilage microflora. In the present study, uninjured *E. coli* O157:H7 survived poorly at 4°C in air packaged samples, while heat injured populations of *E. coli* O157:H7 stored at 4 and 10°C in air packaged samples survived shorter than in sample packages not containing O₂. It is well documented that some microorganisms possess catalase enzyme systems that provide protection against lethally high levels of oxygen. Martin et al. (1976) reported that stress due to heat and NaCl resulted in decreased catalase activity in *Staphylococcus aureus*. It is likely that heat-injured *E. coli* O157:H7 in our study may have possessed dysfunctional catalase enzyme systems. Storage at 4 and 10°C was likely inadequate to facilitate repair of catalase enzymes in oxygen containing packages, whereas storage at 37°C possibly provided cells adequate conditions to initiate repair, thus allowing for subsequent growth in the presence of O₂.

Vacuum packaging is effective in extending shelf-life of fresh meats by reducing bacterial growth (Jay, 1996). Heat-injured and uninjured *E. coli* O157:H7 grew well on vacuum packaged BEM at 37°C, compared to other package atmospheres. However, when stored in vacuum at 4 and 10°C, populations were reduced more so than samples packaged in other atmospheres, suggesting that storage temperature plays an important

role in the effectiveness of vacuum packaging. Nissen et al. (1996) reported that significant differences were not noted in coliform populations present on beef packaged in 100% CO₂ or vacuum at 2 and 6°C. It is important to note that the concentration of O₂ was likely higher in vacuum packaged BEM, than that in vacuum packaged beef, due to the lack of respiring tissue or oxidative metabolism by indigenous microflora.

Although Blickstad and Molin (1983) reported that packaging pork loins and frankfurters in 100% N₂ extended shelf-life longer than vacuum packaging, beef packaged in 100% N₂ enhanced survival of *C. jejuni* at 4°C (Stern et al., 1986). In the present investigation uninjured *E. coli* O157:H7 grew well in N₂ packages stored at 10°C, but survival of injured and uninjured *E. coli* O157:H7 at 4°C, and injured populations stored at 10°C, were quickly reduced to undetectable levels in N₂ packaged samples. Interestingly, survival of uninjured and heat injured *A. hydrophila* stored at 4°C on brain heart infusion agar is enhanced when packaged in 100% N₂ (Golden et al., 1989).

CO₂ is believed to affect nutrient uptake, enzymatic activity, cell membrane integrity, and cause adverse changes on the physio-chemical properties of proteins in bacterial cells (Farber, 1991). Huffman et al. (1975) reported that steaks stored at 1.1°C in 100% CO₂ had lower populations of aerobic microorganisms after 27 days than steaks packaged in 100% N₂, 100% O₂, or air. In the present study, uninjured *E. coli* O157:H7 in CO₂ packages exhibited restricted growth at 10°C, grew well at 37°C, and survived longer at 4°C. It has been reported that *Yersinia enterocolitica* was reduced by 100, 98, and 43% in TSB packaged in 100% CO₂ stored at 2, 6, and 20°C (Eklund and Jarmond,

1983). However, the organism can grow on high-pH beef packaged in 100% CO₂ at 5 and 10°C, while *L. monocytogenes* and *A. hydrophila* grew only at 10°C (Gill and Reichel, 1989).

The enhanced lethal effect of CO₂ at lower temperatures is well documented, and probably results from the increased solubility of CO₂ and subsequent pH reduction at lower temperatures (Wolfe, 1980). Hudson et al. (1994) reported that populations of *L. monocytogenes* declined on roast beef packaged in saturated CO₂ at 1.5°C, but grew at 3°C. In the present investigation, heat-injured *E. coli* O157:H7 populations stored at 10°C survived better when packaged in CO₂ and CO₂/N₂ than other package atmospheres. Nissen et al. (1996) reported that coliform populations on beef packaged in 20% CO₂/80% N₂ increased over 10 weeks of storage at 2°C, while packaging beef in 100% CO₂ decreased coliform populations over 5 weeks. Our results indicate that uninjured and heat-injured populations packaged in CO₂ and CO₂/N₂ at 4°C were recovered in the highest populations, and there was no significant difference between storage of heat-injured *E. coli* O157:H7 at 4 and 10°C. Nissen et al. (1996) reported that reducing storage temperature from 2 to -1°C enhanced the bacteriostatic effects of elevated levels of CO₂ in packaged samples.

"Hurdle technology" has been used in food preservation for over a century (Jay, 1996), and is based on the concept of introducing multiple stresses for bacterial populations to overcome before proliferating in food. In the present investigation, the overall result of sublethal heat-treatment was an increased inhibitory effect of storage

temperature and package atmosphere on growth and survival of *E. coli* O157:H7. Other investigators have reported that packaging inoculated chicken thighs in air, 100% CO₂, and vacuum after exposure to increasing levels of potassium sorbate, resulted in a more inhibitory effect on growth of *S. aureus* and *Salmonella enteritidis* (Gray et al., 1984). Similarly, growth of *L. monocytogenes* was inhibited on cooked tenderloin pork, when samples were treated with nisin before packaging in atmospheres containing elevated levels of CO₂ (Fang and Lin, 1994)

Results of this investigation indicate that heat-injured *E. coli* O157:H7 are more susceptible to MAP and low storage temperatures. Nevertheless, uninjured and heat-injured *E. coli* O157:H7 are able to survive at low temperatures in the modified atmospheres used in this study. Also, packaging treatments commonly applied to fresh beef, may be ineffective at inhibiting growth and survival of this pathogen. Therefore, there is need to test the survival of uninjured and injured *E. coli* O157:H7 in fresh beef, while stored at low temperatures in atmospheres containing elevated levels of CO₂.

REFERENCES

- Abdoul-Raouf, U.M., L.R. Beuchat, and M.S. Ammar. 1993. Survival and growth of *Escherichia coli* O157:H7 on salad vegetables. *Appl. Environ. Microbiol.* 59:1999-2006.
- Barkate, M.L., G.R. Acuff, L.M. Lucia, and D.S. Hale. 1993. Hot water decontamination of beef carcasses for reduction of initial bacterial numbers. *Meat Sci.* 35:397-401.
- Beuchat, L.R., and R.E. Brackett. 1990. Survival and growth of *Listeria monocytogenes* on lettuce as influenced by shredding, chlorine treatment, modified atmosphere packaging and temperature. *J. Food Sci.* 55:755-758,780.
- Blickstad, E., and G. Molin. 1983. The microbial flora of smoked pork loin and frankfurter sausage stored in different gas compositions at 4°C. *J. Appl. Bacteriol.* 54: 45-56.
- Cutter, C.N., and G.R. Siragusa. 1994. Efficacy of organic acids against *Escherichia coli* O157:H7 attached to beef carcass tissue using a pilot scale model carcass washer. *J. Food Prot.* 57:97-103.
- Davis, M., C. Osaki, D. Gordon, M.W. Hinds, K. Mottram, C. Winegar, E.D. Avner, P.I. Tarr, D. Jardine, M. Goldoft, B. Bartleson, J. Lewis, J.M. Kobayashi, G. Billman, J. Bradley, S. Hunt, P. Tanner, M. Ginsberg, L. Barret, S.B. Werner, G.W. Rutherford, R.W. Jue, H. Root, D. Brothers, R.L. Chehey, R.H. Hudson, F.R. Dixon, D.J. Maxson, L. Empey, O. Ravenholt, V.H. Ueckart, A. DiSalvio, D.S. Kwalick, R. Salcido, and D. Brus. 1993. Update: multistate outbreak of *Escherichia coli* O157:H7 infections from hamburgers -- western United States, 1992, 1993. *MMWR.* 42(14): 258-263.
- Dickson, J.S., and G.R. Siragusa. 1994. Survival of *Salmonella typhimurium*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* during storage on beef sanitized with organic acids. *J. Food Safety.* 14: 313-327.
- Dickson, J.S., C.G. Nettles Cutter, and G.R. Siragusa. 1994. Antimicrobial effects of trisodium phosphate against bacteria attached to beef tissue. *J. Food Prot.* 57:952-955.
- Dorsa, W.J., C.N. Cutter, and G.R. Siragusa. 1996. Effectiveness of a steam-vacuum sanitizer for reducing *Escherichia coli* O157:H7 inoculated to beef carcass surface tissue. *Lett. Appl. Microbiol.* 23:61-63.

- Eklund, T. and T. Jarmund. 1983. Microculture model studies on the effect of various gas atmospheres on microbial growth at different temperatures. *J. Appl. Bacteriol.* 55:119-125.
- Fang, T.J., and L.W. Lin. 1994. Growth of *Listeria monocytogenes* and *Pseudomonas fragi* on cooked pork in a modified atmosphere packaging/nisin combination system. *J. Food Prot.* 57:479-485.
- Farber, J.M. 1991. Microbiological aspects of modified-atmosphere packaging technology - a review. *J. Food Prot.* 54:58-70.
- Gadzella, T.A. and S.C. Ingram. 1994. Heat shock, anaerobic jar incubation and fluid thioglycollate medium have contrasting effects on D-values of *Escherichia coli*. *J. Food Prot.* 57:671-673.
- Gill, C.O., and M.P. Reichel. 1989. Growth of cold-tolerant pathogens *Yersinia enterocolitica*, *Aeromonas hydrophila*, and *Listeria monocytogenes* on high-pH beef packaged under vacuum or carbon dioxide. *Food Microbiol.* 6:223-230.
- Golden, D.A., M.J. Eyles, and L.R. Beuchat. 1989. Influence of modified-atmosphere storage on the growth of uninjured and heat injured *Aeromonas hydrophila*. *Appl. Environ. Microbiol.* 55:3012-3015.
- Gorman, B.M., J.N. Sofos, J.B. Morgan, G.R. Schmidt, and G.C. Smith. 1996. Evaluation of hand-trimming, various sanitizing agents, and hot water spray washing as decontamination interventions for beef brisket adipose tissue. *J. Food Prot.* 58:899-907.
- Gray, R.J.H., P.H. Elliott, and R.I. Tomlins. 1984. Control of two major pathogens on fresh poultry using a combination potassium sorbate/carbon dioxide packaging system. *J. Food Sci.* 49:142-145,179.
- Hao, Y.Y., and R.E. Brackett. 1993. Growth of *Escherichia coli* O157:H7 in modified atmosphere. *J. Food Prot.* 56:330-332.
- Hudson, J.A., S.J. Mott, and N. Penney. 1994. Growth of *Listeria monocytogenes*, *Aeromonas hydrophila*, and *Yersinia enterocolitica* on vacuum and saturated carbon dioxide controlled atmosphere-packaged slice roast beef. *J. Food Prot.* 57:204-203.
- Huffman, D.L., K.A. Davis, D.N. Marple, J.A. McGuire. 1975. Effect of gas atmospheres on microbial growth, color and pH of beef. *J. Food Sci.* 40:1229-1231.

- Jay, J.M. 1996. Modern food microbiology. 5th ed. Chapman and Hall, New York, NY.
- Knabel, S.J., H.W. Walker, P.A. Hartman, and A.F. Mendonca. 1990. Effects of growth temperature and strictly anaerobic recovery on the survival of *Listeria monocytogenes* during pasteurization. Appl. Environ. Microbiol. 56:370-376.
- Martin, S.E., R.S. Flowers, and Z.J. Ordal. 1976. Catalase: its effect on microbial enumeration. Appl. Environ. Microbiol. 32:731-734.
- Murano, E.A. and M.D. Pierson. 1992. Effect of heat shock and incubation atmosphere on injury and recovery of *Escherichia coli* O157:H7. J. Food Prot. 56:568-572.
- Nissen, H., O. Sorheim, and R. Dainty. 1996. Effects of vacuum, modified atmospheres and storage temperature on the microbial flora of packaged beef. Food Microbiol. 13:183-191.
- O'Brien, A.D., A.R. Melton, C.K. Schmitt, M.L. McKee, M.L. Batts, and D.E. Griffin. 1993. Profile of *Escherichia coli* O157:H7 pathogen responsible for hamburger-borne outbreak of hemorrhagic colitis and hemolytic uremic syndrome in Washington. J. Clin. Microbiol. 10:2799-2801.
- Phebus, R.K., F.A. Draughon, and J.R. Mount. 1991. Survival of *Campylobacter jejuni* in modified atmosphere packaged turkey roll. J. Food. Prot. 54:194-199.
- Ray, B. 1986. Impact of bacterial injury and repair in food microbiology: its past, present, and future. J. Food Prot. 49:651-655.
- Ray, B. 1989. Injured index and pathogenic bacteria: occurrence and detection in food, water and feeds. CRC Press Inc., Boca Raton, FL.
- Riley, L.W., R.S. Remis, S.D. Helgerson, H.B. McGee, J.G. Wells, B.R. Davis, R.J. Hebert, E.S. Olcott, L.M. Johnson, N.T. Hargrett, P.A. Blake, and M.L. Cohen. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. New Engl. J. Med. 308:681-685.
- Semanchek, J.J. and D.A. Golden. 1996. Inactivation and injury of *Escherichia coli* O157:H7 cultured at 10 and 37°C, as induced by heating, lactic acid, and freezing. Can. J. Microbiol. (in preparation).
- Stern, N.J., M.D. Greenburg, and D.M. Kinsman. 1986. Survival of *Campylobacter jejuni* in selected gaseous environments. J. Food Sci. 51:652-654.

- Tarr, P.I. 1994. *Escherichia coli* O157:H7: overview of clinical and epidemiological issues. J. Food Prot. 57:632-636.
- Wells, J.G., B.R. Davis, I.K. Wachsmuth, L.W. Riley, R.S. Remis, R. Sokolow, and G.K. Morris. 1983. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. J. Clin. Microbiol. 18:512-520.
- Wolfe, S.K. 1980. Use of CO- and CO₂ enriched atmospheres for meats, fish, and produce. Food Technol. 34:55-58,63.

CHAPTER V

SUMMARY AND CONCLUSIONS

Results of this investigation indicate that the environment from which *E. coli* O157:H7 is isolated, as well as growth temperature, can influence resistance of the organism to heat- lactic acid-, and freeze-stress. Uninjured and sublethally heat-injured are able to survive at low temperatures in the modified atmospheres used in this study. Therefore, packaging treatments commonly applied to fresh beef may be ineffective at inhibiting growth and survival of this pathogen. Also, beef carcass steam pasteurization can cause sublethal injury in bacterial populations thereby resulting in slower microbial growth. However, enumeration media should accommodate the fastidious growth requirements of injured bacteria for adequate detection. These conclusions were formulated based on the following important observations:

- (1) Growth of strains at 10°C compared to 37°C, dramatically reduced resistance to heat-, lactic acid-, and freeze-stress, with the exception of the cider strain stored in lactic acid.
- (2) Substantial portions of strain populations were sublethally injured after exposure to heat and lactic acid stress.
- (3) Greater portions of strain populations were sublethally injured when strains were grown at 10°C, as compared to 37°C.
- (4) Difference were noted among the resistance of strains to heat and lactic acid stress.

- (5) Only minimal differences in survival among strains were observed during storage at -20°C, and as affected by growth temperature.
- (6) Package atmosphere influenced growth and survival of uninjured and heat-injured *E. coli* O157:H7 stored at 37, 10, and 4°C.
- (7) Growth of heat-injured *E. coli* O157:H7 was slower at 37°C than uninjured populations.
- (8) Uninjured *E. coli* O157:H7 grew during storage at 10°C for 10 days, while heat-injured populations declined during storage for 20 days at 10°C.
- (9) Uninjured and heat-injured *E. coli* O157:H7 were reduced throughout storage for 20 days at 4°C.
- (10) There was no difference in survival of heat-injured populations stored at 10 and 4°C.
- (11) Elevated levels of CO₂ in packages enhanced survival of heat-injured organisms stored at 4 and 10°C, as well as uninjured populations stored at 4°C.

VITA

Jeffrey John Semanchek was born in New Alexandria, Pennsylvania on May 4, 1971. He graduated from Greensburg Salem High School in June, 1989. Several months later he moved to Rock Hill, SC to attend Winthrop University. In December of 1994, he officially received his Bachelor's of Science Degree in Biology, with a minor in general chemistry. In August 1994, he entered a Master's Degree program in Food Science and Technology at The University of Tennessee (Knoxville, TN). He graduated with his Master's Degree in May, 1996.

8278 4836 13
12/01/97 60 MAB