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DISSERTATION

EVALUATION OF PROCESSES TO DESTROY *ESCHERICHIA COLI* O157:H7 IN WHOLE MUSCLE HOME DRIED BEEF JERKY

Submitted by

Susan Nesbitt Duncan Albright

Department of Food Science and Human Nutrition

In partial fulfillment of the requirement

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Summer 2000

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May 2, 2000

WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY SUSAN NESBITT DUNCAN ALBRIGHT ENTITLED EVALUATION OF PROCESSES TO DESTROY *ESCHERICHIA COLI* O157:H7 IN WHOLE MUSCLE HOME DRIED BEEF JERKY BE ACCEPTED AS FULFILLING IN PART REQUIREMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

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ABSTRACT OF DISSERTATION

EVALUATION OF PROCESSES TO DESTROY *ESCHERICHIA COLI* O157:H7 IN WHOLE MUSCLE HOME DRIED BEEF JERKY

The United States Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) recommends a 5-log reduction in *Escherichia coli* O157:H7 in processed fermented and semidry foods to be considered safe. Discrepancies exist as to whether current practices for home dried jerky preparation can achieve such destruction. This project was designed to investigate, in three phases, the survival of inoculated *E. coli* O157:H7 in dried beef jerky, methods to enhance destruction of the pathogen, and sensory properties of jerky prepared by selected procedures.

In phase one, the survival of inoculated *E. coli* O157:H7 cells during preparation of whole muscle beef jerky with and without marinade and followed by drying at two temperatures (62.5 or 68.3°C) was determined. Survival of bacterial cells was determined by plating on two culture media. Significant ($P < 0.05$) bacterial destruction occurred during the first 4 h of drying (2.1-4.1 log CFU/cm^2), but little destruction occurred between 4 and 10 h of drying and a 5-log reduction was not obtained (2.2-4.6 log CFU/cm^2).

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In phase two, the survival of inoculated *E. coli* O157:H7 populations in beef jerky prepared by each of four pre-drying treatments and followed by drying at 62.5°C was evaluated. Four pre-drying treatments were developed to enhance destruction of *E. coli* O157:H7 before and during drying; survival of bacterial cells was evaluated using two agar media. After 10 h of drying, significant ($P < 0.05$) bacterial destruction (4.3-5.8 log CFU/cm^2) was achieved; however, only one pre-drying treatment achieved a $\geq$ 5-log reduction (5.7-5.8 log CFU/cm^2) with both media.

In phase three, consumer responses to uninoculated beef jerky prepared using the four pre-drying treatments was evaluated. A taste panel (n=120) was conducted at three central locations to evaluate five sensory characteristics typical of beef jerky for the treatment methods, plus one commercial jerky product. Mean scores for the commercial jerky were significantly ($P < 0.05$) higher than mean scores for the experimental products. Consumer results indicated moderate acceptance of the experimental products.

Overall, this research provided information on survival of inoculated *E. coli* O157:H7 in beef jerky, and sensory characteristics typical to beef jerky.

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ACKNOWLEDGMENTS

"And whatever you do, whether in word or deed, do it all in the name of the Lord Jesus, giving thanks to God the Father through him." Colossians 3:17

I would like to express my deep appreciation to those individuals who contributed to this research effort:

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To Woody, my wonderful and awesome Man of God---you have truly helped me "find my place in this world" and "Go get 'um, Tiger!";

Finally, "Then choose for yourselves this day whom you will serve, but for me and my house, we will serve the Lord." Joshua 24:15

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CHAPTER I

INTRODUCTION

Escherichia coli O157:H7 is a unique challenge to food processors, as well as the food consumer. The pathogen has a very low infectious dose and is unevenly distributed, making spot testing of the pathogen in raw ingredients unreliable (Tilden et al., 1996). In addition, *E. coli* O157:H7 has been found to survive many weeks at pH levels between 3.4 to 4.3 (Leyer et al., 1995; Besser et al., 1993; Miller and Kaspar, 1994) and at refrigeration (8 °C) for up to 31 days (Zhao et al., 1993). There is also a strong indication for the potential risk of *E. coli* O157:H7 to survive in certain dried foods. Dried meats (jerky) may cause a greater threat for contamination than fermented foods, since dried jerky is easily prepared at home by consumers and does not require much equipment or precision (Faith et al., 1998a).

Dried jerky is a favorite food among hikers and athletes, as well as others who enjoy its flavor and texture (CNN, 1999a, b, c). In addition, many hunters and their families enjoy homemade game jerky throughout the long winter and spring months until hunting season arrives the following year. Hunters and other consumers need to understand that wild game should be handled and cooked with the same caution as other meats, since investigations established deer jerky as the vehicle of *E. coli* O157:H7 transmission (Keene et al., 1997). Thus, it is crucial that consumers and hunters follow jerky preparation methods that minimize the risk of *E. coli* O157:H7.

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The United States Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) recommends a 5-log reduction of *E. coli* O157:H7 for processed fermented and semidry foods to be considered safe (Billy, 1997; Reed, 1995). However, it is not established that current practices for home dried jerky preparation can achieve such reduction. The USDA-FSIS Meat and Poultry Hotline (USDA-FSIS, 1998) has suggested pre-heating meat to 71.1°C (160°F) before drying jerky. While safe, many consumers may object to the heated flavor of jerky prepared in this manner.

Harrison and Harrison (1996) determined the survival of *E. coli* O157:H7 in a whole muscle beef product made with either a commonly used jerky preparation method (Reynolds and Williams, 1993) or the USDA suggested method of pre-heating meat to 71.1°C before drying jerky (USDA-FSIS, 1998). Inoculated samples were either marinated at 4°C (39°F) for 1 h or placed in a shallow pan, covered with jerky marinade and pre-heated on a hot plate to 71.1°C, for an unspecified time. All jerky samples were dried at 60°C (140°F) for 10 h in home-type dehydrators. After 10 h of drying, populations of *E. coli* O157:H7 on the inoculated, unheated meat decreased by 5.5 to 6.0 log units. Populations on the inoculated, pre-heated meat decreased by 5 log units during pre-heating, prior to drying, and after 10 h of drying populations decreased to undetectable levels (detection limit not stated) (Harrison and Harrison, 1996). The authors concluded that using a marinade in jerky preparation and drying at 60°C for 10 h were sufficient to reduce levels of *E. coli* O157:H7 by at least 5 log units on unheated and pre-heated meat (Harrison and Harrison, 1996).

Keene et al. (1997) assessed the fate of *E. coli* O157:H7 during jerky preparation using marinated, whole muscle venison dried in home-type dehydrators at 51.7°C

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(125°F) or 62.8°C (145°F) and inoculated with 10^3 or 10^5 CFU/g of *E. coli* O157:H7, respectively. Results indicated that *E. coli* O157:H7 counts declined slowly in marinated meat when dried at 51.7°C for 10 h, while counts on marinated product dried at 62.8°C declined over the first 6-8 h to levels undetectable by direct plating (<10 CFU/g). However, upon enrichment, *E. coli* O157:H7 could be cultured from samples dried under both conditions. Keene et al. (1997) concluded that low-temperature (≤ 62.8°C) dehydration was an unreliable means of eradicating *E. coli* O157:H7 from contaminated meat.

Harrison et al. (1998) investigated ground beef jerky inoculated with *E. coli* O157:H7 and made with a spice and cure mix or a spice mix only. Before drying in home-type dehydrators, half of the jerky was pre-heated to 71.1°C (160°F) in a convection oven (time unspecified). Half of these pre-heated strips contained a spice (ingredients not listed) and cure mix (salt and sodium nitrite), while the other half contained a spice mix only. The remaining jerky was not pre-heated and half contained a spice and cure mix, while half contained a spice mix only. All jerky strips were dried at 60°C (140°F) for 6 h (heated strips) or 8 h (unheated strips). Populations of inoculated *E. coli* O157:H7 were reduced by 5.2 logs CFU/g in both pre-heated and unheated strips containing the spice and cure mix (Harrison et al., 1998). In contrast, log reductions of *E. coli* O157:H7 populations were 4.8 and 4.3 logs CFU/g in pre-heated and unheated jerky strips containing a spice mix only, respectively. The authors concluded that inactivation of *E. coli* O157:H7 cells was enhanced through the addition of a cure mix to the spice mix (Harrison et al., 1998).

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In addition, the survival of inoculated *E. coli* O157:H7 was evaluated in beef with various levels of fat (5 and 20%) that was ground and formed into jerky (Faith et al., 1998a). The researchers concluded that jerky should be prepared from leaner cuts of meat and dried at temperatures ≥ 63°C for at least 8 h in order to achieve an appreciable reduction in *E. coli* O157:H7 (Faith et al., 1998a).

There are inconsistencies in the results of research conducted with dried jerky, resulting in consumers and the public receiving mixed messages on food safety. As homemade jerky gains popularity among hikers, athletes, and families, the probability of future outbreaks increases. In the past, the information distributed to consumers contained recommendations for preparing and drying jerky using traditional methods, drying marinated raw strips at temperatures between 52 and 63°C (125 and 145°F) for 8 to 24 h (Reynolds and Williams, 1993). These methods (Reynolds and Williams, 1993) are now being reconsidered as foodborne pathogens become more resistant to antibiotics and chemicals (Levy, 1992). Home drying procedures must work to eliminate *E. coli* O157:H7 risk in dried food products.

In addition, there are many home type dehydrators available for consumer use. However, Faith et al. (1998a) found that the temperature settings on home-type dehydrators and the equilibrated air temperature within the dehydrator were "not in good agreement" and "the actual temperatures were 3 to 22°C higher" than the temperature indicated on the control dial of the dehydrator. These results emphasize the importance of accurately monitoring dehydrator temperature, as well as the internal temperature or the jerky temperature (Faith et al., 1998a).

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Considering the results of previous work, the objectives of the studies reported in this dissertation were threefold:

1. To determine survival of inoculated *E. coli* O157:H7 populations during preparation of whole muscle beef jerky with and without marinade and drying at two temperatures.
2. To determine survival of inoculated *E. coli* O157:H7 populations during preparation of whole muscle beef jerky following four pre-drying treatments.
3. To evaluate consumer responses to uninoculated beef jerky prepared following four pre-drying treatments found to result in a 4.3 to 5.8 log reduction of inoculated *E. coli* O157:H7 populations.

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CHAPTER II

REVIEW OF LITERATURE

INTRODUCTION

In July 1996, the United States Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS) adopted Pathogen Reduction, Hazard Analysis and Critical Control Point Systems (HACCP) to improve the food safety of meat and poultry (USDA-FSIS, 1996; Hogue et al., 1998). One element of this rule is the mandatory testing of *Escherichia coli* since the bacterium is the best indicator of fecal contamination. Operations are also required to verify procedures that will prevent and reduce fecal contamination. Part of the reason for the adoption of the Pathogen Reduction, HACCP rule were reported findings that bacteria were emerging as causative agents for significant health problems that were not previously associated with foodborne illnesses (Hogue et al., 1998). With increased international travel and an increase in global economy, bacteria are readily transported from one area to another and become adapted to various food processes and conditions. One pathogenic bacterium known to be partially resistant to numerous changes in a food's physical and chemical situation is *Escherichia coli* O157:H7.

In the past decade, news releases of outbreaks of foodborne illnesses caused by *E. coli* O157:H7 have become common and seem to be increasing each year (CDC, 1999).

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The first documented occurrence of human illness associated with *E. coli* O157:H7 occurred in 1982 when two outbreaks due to undercooked hamburger meat were reported (Riley et al., 1983). Through further investigation, ground meat was identified as the carrier of the food pathogen, later identified as *E. coli* O157:H7 (Riley et al., 1983). It is currently estimated that there are 73,480 total illnesses of *E. coli* O157:H7 each year with 62,458 of these being foodborne (Mead et al., 1999). This pathogen makes up 0.2% of the total estimated annual foodborne illnesses in the United States; however, it accounts for 3% of all hospitalizations and deaths due to foodborne pathogens (Mead et al., 1999). Originally, outbreaks of *E. coli* O157:H7 seemed to be limited to undercooked ground meat products (Riley et al., 1983). However, as investigators continue to study *E. coli* O157:H7, other food sources have been found as carriers of the pathogen or involved in foodborne illness.

Concerns are raised for food products consumed raw, or with inadequate cooking, such as unpasteurized juices (Besser et al., 1993), radish and alfalfa sprouts (Taormina et al., 1999; Hara-Kudo et al., 1997), salad vegetables (Abdul-Raouf et al., 1993b), as well as fermented meats (Hinkens et al., 1996; Faith et al., 1997, 1998a, b, c). Besides these foods, *E. coli* O157:H7 outbreaks have also been associated with dry cured salami (CDC, 1995b), deer jerky (Keene et al., 1997), produce from manure-fertilized soil, and yogurt (Buchanan and Doyle, 1997; Morgan et al., 1993). Outbreaks in the mid 1990s associated with foodborne pathogens (*Salmonella* and *E. coli* O157:H7) in dried and fermented meats (CDC, 1995b, c) prompted federal action mandating that manufacturers of dry and semi-dry fermented meats demonstrate a 5-log reduction of *E. coli* O157:H7 during processing (Billy, 1997; Reed, 1995). According to USDA-FSIS, a validated step

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in processing to obtain a 5-log reduction for processed fermented meat food systems or a 7-log reduction for roasted beef, in *E. coli* O157:H7 populations, is required to be considered safe. Thus, previous food processing and preparation steps are being evaluated to verify the prevention and reduction of contamination.

There is great public concern about foodborne pathogens and research indicates that preservation methods previously thought to be safe, may not minimize the risk of *E. coli* O157:H7; thus, there was a strong indication for the potential risk of *E. coli* O157:H7 surviving certain dried foods. Dried meats may cause a greater threat for contamination than fermented foods, since dried jerky is easily prepared at home by consumers and does not require much equipment or precision (Faith et al., 1998a). The cell populations of *E. coli* O157:H7 that exist in the dried product pose a concern for individuals who prepare jerky in their homes because there are severe consequences of infection that can affect all age groups (Buchanan and Doyle, 1997). *Escherichia coli* O157:H7 is dangerous in part because of a very low infectious dose (2-2000 cells) (Monnens et al., 1998; Buchanan and Doyle, 1997). In addition, the pathogen is unevenly distributed in foods and this makes spot testing of raw ingredients unreliable (Tilden et al., 1996). Furthermore, *E. coli* O157:H7 has been found to survive many weeks at pH levels between 3.4 to 4.3 (Leyer et al., 1995; Besser et al., 1993; Miller and Kaspar, 1994); at refrigeration (8°C) for up to 31 days (Zhao et al., 1993) and after freezing (-20°C) (Sage and Ingham, 1998).

Making jerky is a popular past time with the increase in commercial home dehydrators available to consumers and those active in local 4-H groups across the country. Beef jerky continues to be sold at convenience stores, supermarkets, and roadside stands across the county. Even entire Internet web sites are devoted to beef

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jerky recipes: www.beefjerky.net; www.greatjerky.com; www.beefjerkyoutlet.com; www.thejerkyguy.com (www.iwon.com, 2000). Beef jerky is a convenient, inexpensive way to preserve red meat when prices are low and the beef jerky can be stored for long periods. In addition, many hunters and their families enjoy homemade game jerky throughout the long winter and spring months until hunting season arrives the following year. Hunters and other consumers need to understand that wild game should be handled and cooked with the same caution as other meats since investigations established deer jerky as the vehicle of *E. coli* O157:H7 transmission (Keene et al., 1997). Thus, it is crucial that consumers and hunters follow jerky preparation methods that minimize the risk of *E. coli* O157:H7.

There are inconsistencies in results of research conducted with dried jerky, resulting in consumers and the public receiving mixed messages about its safety. Dried jerky is a favorite snack and food among hikers and athletes, as well as others who enjoy its flavor (CNN, 1999a, b, c). As homemade jerky gains popularity among hikers, athletes, and families, the probability of future outbreaks increases. Throughout the nation, many consumers obtain information concerning drying jerky from local and State Cooperative Extension agents. Cooperative Extension is a primary source of home drying information (Cooperative Extension Service, Colorado State University, 1994); thus, it is necessary and pertinent that Extension agents are able to distribute acceptable home drying procedures. In the past, the information distributed contained recommendations for preparing and drying jerky using traditional methods drying marinated raw strips at temperatures between 52 and 63°C (125 and 145°F) for 8 to 24 h (Reynolds and Williams, 1993). These methods (Reynolds and Williams, 1993) are now

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being reconsidered as foodborne pathogens become more resistant to antibiotics and chemicals (Levy, 1992). With foodborne pathogens increasing in number and strength every year (Mead et al., 1999), consumers must realize that previous methods of cooking must be revised to continue to fight the potential bacteria that may exist in their foods. Home drying procedures must work to eliminate the risk of *E. coli* O157:H7 in dried food products.

FOODBORNE ILLNESS

As the United States continues to build food safety programs, it is estimated that foodborne diseases cause 76 million cases of gastrointestinal illness, 325,000 hospitalizations, and 5,000 deaths annually (Mead et al, 1999). Research must be done to provide consumers with the knowledge and resources to prevent foodborne illness within their own homes. A foodborne illness results from eating food containing harmful parasites, viruses or bacteria (NRA, 1995). However, in order to make a person ill, the pathogen must reach the food and survive until it is ingested. When ingested, the microorganism grows in the intestine and multiplies to reach infectious levels, as well as to produce toxins. In addition, the individual ingesting the pathogen must be susceptible to the subsequent illness.

There are three disease classifications associated with food contaminated with foodborne microorganisms: infection, intoxication and toxin-mediated infections. A foodborne infection is defined as a disease that results by ingesting food containing infectious, toxigenic microorganisms (Todd, 1994). In a foodborne infection, microorganisms or pathogenic bacteria invade and multiply in the intestinal mucosa or in other tissues. Next, a foodborne intoxication or poisoning is the result from ingesting

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poisonous chemicals or toxins produced by the pathogenic microorganisms (Todd, 1994). Finally, a toxin-mediated infection occurs when certain pathogenic bacteria produce enterotoxins, during the colonization and growth in the intestinal tract, which can affect the transfer of water, glucose, and electrolytes.

The threat of a foodborne illness is a concern among many of today's households and consumer education should be a priority among food safety educators (Gravani, 1997). The American food supply may be the safest in the world, but the food could be safer (Daniels, 1998). Surveys have shown that many consumers lack the knowledge or skills to practice food safety techniques. Gravani (1997) reported that 50% of the public eat raw or undercooked eggs, 23% eat undercooked hamburger, 17% eat raw clams and oysters and 26% do not wash cutting boards after using them for raw meat or poultry. These findings suggest educational messages must be developed to target issues important for consumer information.

In addition, there are new challenges arising in the food service industry with changing demographics and lifestyles (Hollingsworth, 1999). Demographic changes across the world have resulted in a section of the population being susceptible to a variety of severe foodborne illnesses (Alterkruse et al, 1998). This section contains two segments of the population which have continued to increase steadily in recent years, the elderly and those with autoimmune disorders. There has been an increase in the elderly population as the "baby boomer" generation ages, as well as with the number of individuals infected with HIV or those who have compromised immune function (Alterkruse et al, 1998).

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With changing lifestyles, there has been a decrease in the knowledge associated with safe food practices and handling (Katz, 1999). Some of these lifestyle changes are due to the change in family structure and the increased use of convenience foods (Katz, 1999). The number of women working has made it necessary for more children to prepare the food for the entire family, making quick and easy foods more desirable than foods that take longer to prepare and are well done (Hollingsworth, 1999). Therefore, industries produce foods that are minimally processed and/or require a decreased amount of heat. Also, as food safety continues to be eliminated from health education, due to education focusing on the prevention of obesity, AIDS and substance abuse, opportunities for food safety instruction are reduced (Alterkruse et al., 1998). The average person is unaware of the contamination associated with raw foods and pathogens in the kitchen. In addition, as nutrition professionals continue to promote the intake of fruits and vegetables in the diet, the increased consumption of fresh produce may be a cause of disease outbreaks (Alterkruse et al., 1998). Some factors that often lead to outbreaks associated with contaminated product may be attributed to contaminated foods consumed raw or contaminated foods being prepared with inadequate heat (Daniels, 1998).

In January 1996, the Centers for Disease Control and Prevention (CDC), the USDA, and the Food and Drug Administration (FDA) designed a network surveillance system capable of determining foodborne illnesses in the United States (Angulo et al., 1998). Through the CDC's Emerging Infections Program (EIP), the collaborative project was called FoodNet, standing for Foodborne Diseases Active Surveillance Network. FoodNet monitors the number of cases of foodborne diseases that occur each year in

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selected areas of the United States (Angulo et al., 1998). However, even if a foodborne illness does occur, various problems may exist in order to accurately report a foodborne illness. The individual experiencing the illness must first seek medical attention and even if medical professionals were contacted, proper diagnostics tests may not be used to determine if it was a foodborne illness. In addition, a laboratory analysis must be able to show that a specific food was the source of the foodborne illness (Todd, 1994). As medical technology continues to try to improve testing methods, misdiagnoses occur due to an inability to determine the contaminating agents, late /incomplete laboratory investigations, lack of recognition of the pathogen as a disease agent, and/or the inability to identify pathogens with available laboratory techniques (Patterson and Haas, 1999; Strockbine et al., 1998).

Several approaches have been employed to help reduce the risk of contamination and minimize foodborne outbreaks. The Hazard Analysis Critical Control Point (HACCP) system enables the highest level of food safety assurance available for food processing facilities (National Advisory Committee on Microbiological Criteria for Foods, 1997). Good Manufacturing Practices (GMPs), sanitation, and hygiene are prerequisites when considering a HACCP plan. The HACCP system allows a thorough plan to be developed that considers all points of production that could be hazardous to the food safety and quality. HACCP is based on seven principles that effectively control the prevention or inhibition of potential risks of microorganisms (National Advisory Committee on Microbiological Criteria for Foods, 1997).

As part of a HACCP plan, food industries test for the presence of indicator organisms. A common type of indicator organism used are bacteria called coliforms

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(National Advisory Committee on Microbiological Criteria for Foods, 1997; Jay, 1994). When coliforms, such as *Escherichia coli* or *Enterobacteriae*, are found in heat-processed foods, it usually indicates post-process contamination (Marth, 1998; Jay, 1994). Contaminants are not uniformly distributed; thus, the absence of coliforms gives a false sense of security, while no positive samples means only that contaminants were not detected in samples collected and tested (Jay, 1994). It has become common practice to see news stories reporting the latest foodborne outbreak and major food recall. Particularly, throughout the past few years, outbreaks of foodborne pathogens have been numerous and wide spread (Mead et al., 1999). However, *E. coli* O157:H7 stands out because of its severe consequences. The importance of *E. coli* O157:H7 affects businesses continually and this one small pathogen has had a tremendous influence on the government, industry, and public (Wachsmuth, 1997).

ESCHERICHIA COLI O157:H7

Escherichia coli has been used since 1890 as a non-pathogenic indicator of enteric pathogens such as *Salmonella* (Doyle and Padhye, 1989). As enteric diseases increased, investigators began isolating strains of *E. coli* that had acquired virulence characteristics causing pathogenicity to humans or animals (Buchanan and Doyle, 1997; Neill et al., 1994). *Escherichia coli* is a bacterium that displays coliform and fecal coliform characteristics (Todd, 1994). Fecal coliforms, such as *E. coli*, grow in the gastrointestinal tract of warm-blooded animals and they are enumerated in food as an index of fecal contamination. Fecal coliforms are differentiated from nonfecal coliforms by subculturing of suspect organisms at 45°C (Doyle and Padhye, 1989; Jay, 1994). Coliform bacteria include aerobic and facultative anaerobic, Gram negative,

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nonsporeforming rods that ferment lactose, forming acid and gas within 48 hours, at 35°C (coliforms) and 45.5°C (fecal coliforms and *E. coli*) (Jay, 1994.)

Discovery

Escherichia coli O157:H7 was initially discovered following two hemorrhagic colitis (HC) outbreaks in 1982 (Riley et al., 1983). One outbreak occurred in Oregon with 26 cases infected, of which, 19 were hospitalized. The other outbreak occurred in Michigan, three months later, with 21 cases, of which, 14 were hospitalized. Undercooked hamburgers from the same fast food restaurant chain were identified as the vehicle through which the pathogen was transmitted. *Escherichia coli* O157:H7 was isolated from patients and a frozen ground beef patty (Riley et al., 1983). This finding was significant because it was the first recognition of this *E. coli* serotype as a human pathogen (Karmali et al., 1983). Shortly after *E. coli* O157:H7 was determined to be a human pathogen, Karmali et al. (1983) observed that stool samples, from children with hemolytic uremic syndrome (HUS) contained a substance that was toxic to Vero (African green monkey kidney) tissue culture cells. Verocytotoxin was produced by *E. coli* isolates, with *E. coli* O157:H7 the prominent serotype causing infection.

Pathogenicity

There are six recognized classes of diarrheagenic *E. coli* (Doyle et al., 1997; Neill et al., 1994; Doyle and Padhye, 1989). Within each class, strain characteristics are used to determine placement into the various classes, which include distinct, plasmid encoded virulence traits, epidemiological and clinical features, as well as a similar pathophysiologic mechanism of action (Neill et al., 1994). One class is enterohemorrhagic (EHEC). The class received this distinction, since the class included

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the serotype O157:H7 and hemorrhagic colitis (HC), i.e. bloody diarrhea was used to describe the illness associated with this class. However, not all EHEC infections produce apparent blood in stool. It has been documented that nonbloody diarrhea was seen with *E. coli* O157:H7 infection, as well as with other serotypes. However, the majority of illnesses associated with EHEC infection report bloody diarrhea (Neill et al., 1994). It is often seen after the individual's initial onset of symptoms, which includes mild, nonbloody diarrhea.

All EHEC strains produce a Shiga-like toxin 1 (Stx1) and/or Shiga-like toxin 2 (Stx2). The toxins are also referred to as verotoxin 1 (VT1) and verotoxin 2 (VT2) (Doyle et al., 1997; Neill et al., 1994; Doyle and Padhye, 1989). It is reported the toxin binds to the globotriaosylceramide (Gb_3) receptors found on eucaryotic cell surfaces (Doyle et al., 1997; Neill et al., 1994; Doyle and Padhye, 1989). The toxin proceeds to inactivate a ribosomal subunit of the cell, blocking protein synthesis. The primary targets of the toxins are endothelial cells high in Gb_3 receptors; thus, explaining the toxin's affinity for colon and renal glomeruli, tissues associated with HC and HUS. Besides attaching to the primary targets, the toxins can indirectly damage cells by releasing cytokines, such as the tumor necrosis factor (Doyle et al., 1997; Neill et al., 1994; Doyle and Padhye, 1989). Toxins alone are not enough to make *E. coli* pathogenic. Nonpathogenic, Stx-positive isolates have been isolated from humans frequently. However, to be fully pathogenic, EHEC requires the presence of the virulence markers (Doyle et al., 1997; Neill et al., 1994; Doyle and Padhye, 1989). In addition, the chromosomal gene, *eae*, is found universally among EHEC strains. The gene is responsible for the encoding of an outer membrane protein associated with attachment to

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tissue. The presence of a plasmid-encoded enterohemolysin is characteristic of strains of EHEC (Doyle et al., 1997; Doyle and Padhye, 1989).

The second class of *E. coli* is enterotoxigenic (ETEC) (Neill et al., 1994; Doyle and Padhye, 1989). These strains produce heat changeable and heat stable toxins. ETEC is generally responsible for traveler's diarrhea. A third *E. coli* class is enteroinvasive (EIEC). This class causes diarrhea by attacking the large intestine's epithelial cells and symptoms are similar to those of shigellosis (Neill et al., 1994; Doyle and Padhye, 1989). The fourth class is called enteroaggregative (EaggEC) (Doyle and Padhye, 1989). The fifth class of *E. coli* strains is enteropathogenic (EPEC) (Neill et al., 1994; Doyle and Padhye, 1989) and this class also produces a Shiga-like toxin, the causative organism of infantile diarrhea. The final class is diffusely adherent (DAEC). Of the six classes, the class of most significance currently is the EHEC, since it contains the dangerous strain *E. coli* O157:H7 (Doyle et al., 1997; Doyle and Padhye, 1989; Doyle and Schoeni, 1984).

Biochemical and growth characteristics

Escherichia coli O157:H7 is an unusually virulent strain and is designated by its somatic, O, and flagellar, H, antigens (Neill et al., 1994; Buchanan and Doyle, 1997; Doyle and Padhye, 1989; Doyle and Schoeni, 1984). The organism generally fails to ferment sorbitol, raffinose, and dulcitol after overnight incubation, as well as fails to produce lysine and ornithine decarboxylases (Neill et al., 1994). In addition, the pathogen does not produce Beta-glucuronidase (Buchanan and Doyle, 1997) and thus cannot hydrolyze 4-methylumbelliferone glucoronide (MUG) to a fluorogenic product (Neill et al., 1994). Ideal growth temperatures for *E. coli* O157:H7 are between 30 and 42°C; at temperatures of 44 or 45°C, the pathogen grows poorly. These temperatures

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often are used for the detection of *E. coli* in food samples and probably would have a negative impact on the recovery of this pathogen from food (Neill et al., 1994; Doyle and Schoeni, 1984). Like other *E. coli*, it is presumed that the ultimate source of *E. coli* O157:H7 on carcasses is fecal contamination during animal production and slaughter operations (Dickson and Anderson, 1992). Fecal contamination results in the presence of the organism on the carcass during hide removal and spreading of contamination to other carcasses by equipment and workers hands (Dickson and Anderson, 1992).

Escherichia coli O157:H7 is dangerous because there are severe consequences of infection that can affect all age groups (Buchanan and Doyle, 1997). The organism accounted for at least 8 (73%) of 11 cases of HUS in children from western Washington countries admitted to the Children's Hospital and Medical Center in 1994 and 1995 (Tarr et al., 1997). Also, it is evident that *E. coli* O157:H7 has an apparent special, but inexplicable association with ruminants that are used for food (Buchanan and Doyle, 1997). Benjamin and Datta (1995) reported that the very low infectious dose of *E. coli* O157:H7 is probably associated with the organism's acid tolerance.

The organism's low infectious dose, in combination with the extreme disease severity, requires successful preventive strategies (Riordan et al., 1998; Hinkens et al., 1996; Billy, 1997; Reed, 1995). There is a need to focus on reducing and eliminating the presence of the microorganism, rather than preventing pathogen growth. This is important for raw products that may not be thoroughly cooked before consumption (i.e., sprouts), and ready-to-eat products that do not receive a precise treatment to assure *E. coli* elimination (i.e., fermented sausages, apple cider) (Buchanan and Doyle, 1997). By giving instruction and guidelines, the spread of *E. coli* O157:H7 can be prevented in

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homes, food service and processing establishments, as well as on farms, slaughterhouses, and food production sites. Buchanan and Doyle (1997) stated that the foodborne pathogen, *E. coli* O157:H7, presents a problem to current preventive strategies for food safety protocols and the most desirable process that would control *E. coli* O157:H7 contamination would be a process that includes a step lethal to the pathogen. Public health problems associated with enterohemorrhagic *E. coli* O157:H7 are being recognized throughout the world (Wachsmuth, 1997).

Nature of the disease

Often, there is a delay from the time ingested to when *E. coli* O157:H7 can cause illness. The illness can vary from mild diarrhea that may last from 2 to 9 days up to severe consequences that may be life-threatening (Riemann and Cliver, 1998). The incubation period of the pathogen varies with severity and susceptibility of the infected. Symptoms start with mild, nonbloody diarrhea and may be followed by a period of "crampy" abdominal pain and/or a short-lived fever (Monnens et al., 1998; Buchanan and Doyle, 1997). During the next 24-48 hours to a 4- to 10-day phase, initial diarrhea increases in intensity. In some cases, the illness can progress to symptoms leading to hemorrhagic colitis (HC) that generally occur 1-2 days after eating contaminated food, although longer periods have been reported, up to 3-5 days (Seigler et al., 1993; Monnens et al., 1998), even 9 days (Riemann and Cliver, 1998) following consumption. There is a rapid onset of severe abdominal pain, which is followed by diarrhea, which becomes grossly bloody accompanied with moderate dehydration (Riemann and Cliver, 1998). Severe life-threatening complications may occur in HC patients (Seigler et al., 1993). One complication is hemolytic uremic syndrome (HUS). The onset of HUS occurs

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approximately one week after onset of gastrointestinal symptoms. Characteristics of HUS include pallor, intravascular destruction of red blood cells (microangiopathic hemolytic anemia), depressed platelet counts (thrombocytopenia), lack of urine formation (oligo-anuria), swelling (edema), and acute renal failure (Monnens et al., 1998; Seigler et al., 1993).

HUS occurs most often in children under the age of 10. Half of HUS patients require dialysis, with approximately 15% of the cases leading to the early development of chronic kidney failure. The mortality rate is 5% (Neill et al., 1994) and HUS is the leading cause of renal failure in children (Riemann and Cliver, 1998). HUS-associated complications may include seizures, coma, strokes, colonic perforation, pancreatitis, and/or hypertension and insulin-dependent diabetes may develop (Monnens et al., 1998; Seigler et al., 1993). A small number of HUS cases can reoccur. A second life-threatening complication in HC patients is thrombotic thrombocytopenic purpura (TTP) (Seigler et al., 1993), which has been reported in a few cases. TTP resembles HUS except it generally causes less renal damage and has significant neurological involvement, including central nervous system deterioration, seizures, and strokes (Monnens et al., 1998). Another difference from HUS is that TTP is restricted primarily to adults. A significant portion of HC infections occur sporadically, i.e. not associated with outbreaks (Buchanan and Doyle, 1997).

Foods associated with E. coli *O157:H7*

Since 1982 when it was first recognized as a foodborne pathogen (Riley et al., 1983), numerous outbreaks of *E. coli* O157:H7 have been reported (Buchanan and Doyle, 1997; Neill et al., 1994). Cross-contamination and poor food handling practices are

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potential opportunities to spread the pathogen and cause illness (Abdul-Raouf et al., 1993a). The pathogen itself enhances its capabilities to insure its survival. Researchers indicate that *E. coli* O157:H7 cells, in the stationary phase, have a higher acid tolerance enhancing their survival during fermentation, drying and refrigeration (Palumbo et al., 1997; Brudzinski and Harrison, 1998). Numerous studies have been done to enhance knowledge about *E. coli* O157:H7 and its interactions with temperature (Gill and Phillips, 1985; Guraya et al., 1998; Sutherland et al., 1995; Veeramuthu et al., 1998; Williams and Ingham, 1998, 1997), other bacteria (Brashears, et al., 1998; Siragusa et al., 1998; Ng et al., 1997), and chemicals such as hydrogen peroxide and chlorhexidine (Delazari et al., 1998), organic acids (Uljas and Ingham, 1998; Splittstoesser et al., 1996) and sodium lactate/chloride (Garren et al., 1998). In addition, Fratamico and Strobaugh (1998) evaluated *E. coli* O157:H7 with several methods of detection, such as an enzyme-linked immunosorbent assay (ELISA), direct immunofluorescent filter techniques and a polymerase chain reaction (PCR).

Cattle can be the primary vehicle of transmission (Brown et al., 1997; Cray and Moon, 1995) and proper cooking of ground meat is the current recommendation to properly destroy *E. coli* O157:H7. Ahmed et al. (1995) reported that cooking a product to an internal temperature of 60°C for 2-3 min would provide sufficient kill of any *E. coli* O157:H7 that could exist. Jackson et al. (1996) reported that the heat resistance of *E. coli* O157:H7 strain 43895 in ground beef patties was influenced by storage and holding temperatures. The authors stated that cultures were most heat resistant in frozen patties and least heat resistant in patties stored at 15°C. In addition, Murano and Pierson (1993) reported that heat-shocked cells were better able to survive, or repair themselves, after

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exposure to heat and hydrogen peroxide. Williams and Ingham (1997) investigated changes in heat resistance of *E. coli* O157:H7 following a heat shock. Their results suggested that the refrigeration of ground beef following temperature abuse and prior to cooking will negate any heat-shock effect that may have occurred.

Often foods associated with *E. coli* O157:H7 have come from raw ingredients associated with foods of animal origin, but not only beef (Doyle and Schoeni, 1987). It has been reported that the organism has been isolated from sources such as unpasteurized milk from the bulk tank of a farm at which *E. coli* O157:H7 was isolated from the feces of a heifer (Martin et al., 1986). Another specimen was a frozen chicken nugget obtained from a box opened by a childcare provider for children with the *E. coli* O157:H7 infection. In addition, the pathogen has been isolated from additional ground beef found in a restaurant that was epidemiologically linked to an outbreak of HC (Doyle and Schoeni, 1987).

Doyle and Schoeni (1987) investigated the occurrence of *E. coli* O157:H7 in a variety of retail meats found in the United States and Canada. Various types of meat and poultry specimens were studied (ground beef, pork chop, pork loin, pork hock, ground pork, chicken leg, turkey drumstick, lamb riblet, lamb loin chop, lamb shoulder blade chop, and lamb leg). In their study, *E. coli* O157:H7 was isolated from 3.7% of ground beef samples in the USA and Canada (6 out of 164) (Doyle and Schoeni, 1987). Besides ground beef, the organism was isolated from 1.5% of retail poultry products samples (4 out of 263), 1.5 % pork samples (4 out of 264) and 2% lamb samples (4 out of 205) (Doyle and Schoeni, 1987).

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The investigators isolated the organism from a large percentage of Calgary retail meats sampled (Doyle and Schoeni, 1987). It was isolated from 31% of beef samples (5 out of 17). In addition, the pathogen was isolated from 7.1% of pork samples (1 out of 14). These results suggested the high prevalence of the organism in Calgary meats was responsible for the high incidence of *E. coli* O157:H7 infections in the Calgary area (Doyle and Schoeni, 1987).

In 1991, Griffin and Tauxe reported that ground beef was the food most associated with *E. coli* O157:H7 outbreaks, since outbreaks of *E. coli* O157:H7 had often been linked to undercooked ground meat products. Raw milk was the second food product associated with outbreaks of *E. coli* O157:H7 (MacDonald et al., 1988; Martin et al., 1986). It was first linked to the pathogen in 1986 with the development of HUS in two patients in Minnesota. Besides raw milk, pasteurized milk (Upton and Coia, 1994) has also been associated as a vehicle for *E. coli* O157:H7 infections.

In the 1990s, a number of other food products also became associated with *E. coli* O157:H7. One of these products was unpasteurized apple juice/cider (Besser et al., 1993). In 1991, 28 people were infected with *E. coli* O157:H7 in an outbreak that was epidemiologically linked to fresh apple cider produced in Southeastern Massachusetts (Besser et al., 1993). Again, in 1992, fresh apple cider was a vehicle of infection in 18 individuals in Massachusetts (Besser et al., 1993). It is considered that deer droppings were a possible source of contamination, since deer ranged freely in the orchard. However, testing did not recover the pathogen from the deer feces (Besser et al., 1993). Then in October 1996, two outbreaks of *E. coli* O157:H7 occurred again and were associated with unpasteurized apple juice (Silk et al., 1997). The first outbreak involved

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66 cases of illness, in the Western U.S., and resulted in one death. The second outbreak occurred in Connecticut and involved eight individuals. In addition, a 1980 outbreak of 13 children diagnosed with HUS is now suspected of being caused by EHEC strains in apple juice (Steele et al., 1982).

Escherichia coli O157:H7 can survive in apple juice, with minimal death occurring during most of the expected shelf life of cider (Zhao et al., 1993; Besser et al., 1993; Miller and Kaspar, 1994). Since most apple cider receives no treatment to eliminate food-borne pathogens, cider processors are at considerable risk if soil- or manure-borne pathogens are present on fallen apples (Zhao et al., 1993). Zhao et al. (1993) indicated that *E. coli* O157:H7, when present at 10^5 CFU/ml, can survive in apple cider (pH 3.7) stored at 8°C (ca 46°F) for up to 31 days. Considering that the expected refrigerated shelf life of apple cider is typically less than 2 to 3 weeks, the pathogen is likely to survive during most of the time that the cider would be consumed. In addition to survival, results showed that the pathogen would remain at numbers close to the size of the initial population that was introduced into the product. Ingham and Uljas (1998) reported that short-term ($\leq$ 6 h) storage at room temperature (21°C) may enhance the lethality of pasteurization of apple cider and juice (pH = 3.4).

Miller and Kaspar (1994) found that certain strains of *E. coli* O157:H7 were significantly ($P < 0.05$) more acid-tolerant than other strains. In addition, investigators reported that lower storage temperatures at 4°C (39°F) enhanced the survival at adverse pH levels. Certain *E. coli* O157:H7 strains can reportedly survive 21 days in cider that contains 0.1% sodium benzoate as a preservative (Miller and Kaspar, 1994). This is of concern since generally foods with pH $\leq$ 4.6 are not considered to be potentially

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hazardous (USDHHS-FDA, 1999); typically, cider has a pH of 3.5 to 4.0, and is considered a high-acid food.

In 1994, dry cured salami was associated with outbreaks of the pathogen (CDC, 1995b) in the states of Washington and California. It was the first outbreak in a dried fermented ready-to-eat meat product. *Escherichia coli* O157:H7 was isolated from samples of presliced dry-cured salami from three delicatessens (CDC, 1995b). This outbreak was the first indication that the pathogen could survive in such food products. Before this outbreak, it was generally believed that conditions in such products would preclude pathogen survival (Riordan et al., 1998). The dry cured salami had a low final pH (4.8), water activity (a_w) of < 0.80, 2.5 % added sodium chloride, and 100 ppm added sodium nitrite (Glass et al., 1992; Clavero and Beuchat, 1996). In 1995, a large outbreak was associated with semi-dry fermented sausage involving *E. coli* O111:NM (CDC, 1995a). The outbreak occurred in South Australia and HUS affected approximately 23 children, ranging from 4 months of age up to 16 years old. Additional individuals suffering from bloody diarrhea and gastrointestinal illnesses were reported, but the pathogen could not be isolated. The implicated product was Mettwurst, a raw fermented meat product, from a local manufacturer and it was unclear whether the raw meat was contaminated or the finished product was contaminated through cross contamination (CDC, 1995a).

In addition, radish sprouts were associated with an outbreak (Hara-Kudo et al., 1997) in Japan in 1996. That outbreak was the largest *E. coli* O157:H7 outbreak reported and diarrheic, enterohemorrhagic and/or HUS was seen in approximately 9, 500 individuals throughout Japan, particularly children (Bell and Kyriakides, 1998).

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Hydroponically grown radish sprouts, present in school lunch, were implicated as a vehicle for transmitting *E. coli* O157:H7; however, the contamination route remains uncertain (Hara-Kudo et al., 1997). Possible explanations for the contamination indicate the likelihood of contaminated seeds used for the sprouted vegetables, either seed washing or germination (Bell and Kyriakides, 1998). Due to the agricultural practices associated with sprouted vegetables, research has been done investigating ways to minimize the hazard from *E. coli* O157:H7. In addition, Bari et al. (1999) studied survival of *E. coli* O157:H7 in fresh radish sprouts and found calcinated calcium inhibited growth of the pathogen.

Reported survival of E. coli *O157:H7*

Bell and Kyriakides (1998) reported that foods meeting certain criteria were more likely to be associated with outbreaks of *E. coli* O157:H7. Meeting these criteria are foods that contain raw ingredients of bovine origin or raw ingredients of bovine origin that could come into direct/indirect contamination from other ingredients, as well as foods that contain raw ingredients that are not subjected to a bacterial destruction process, and finally, foods that are subjected to a post-process contamination or a conditions that allow growth of the pathogen (Bell and Kyriakides, 1998). These criteria coincide with reported outbreaks associated with *E. coli* O157:H7. Palumbo et al. (1997) suggested that a food which becomes contaminated with *E. coli* O157:H7 will remain a hazard even if the food is held at 5°C (41°F). Under ideal conditions, *E. coli* O157:H7 cells remain viable. This is significant considering the very low infectious dose of *E. coli* O157:H7 necessary for infection (Palumbo et al., 1997).

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Escherichia coli O157:H7 has an apparent ability to survive acidic conditions (Conner and Kotrola, 1995). Certain *E. coli* O157:H7 strains have been investigated for growth and survival in acidic conditions. These strains have been found to be acid resistant (Fisher and Golden, 1998; Clavero and Beuchat, 1996; Conner and Kotrola, 1995; Leyer et al., 1995; Miller and Kaspar, 1994); however, the growth and survival of *E. coli* O157:H7 is dependent on the type of acid used, as well as the storage temperature.

Enteric bacterial pathogens must survive the acidity of the stomach before they reach the intestine and cause illness. Acid adaptation occurs when a bacterial species is exposed to a moderately acidic environment, enabling cells to survive longer when transferred to a more acidic environment. Tsai and Ingham (1997) studied the effect of acid adaptation on ground beef. The authors investigated the effects of storage temperature and acid adaptation on the survival of inoculated *E. coli* O157:H7 in ketchup, mustard and sweet relish. The authors concluded that small numbers of *E. coli* O157:H7 survived in ketchup for a period of several hours to several days (Tsai and Ingham, 1997). In addition, the survival of the pathogen was greater at refrigeration at 4°C (39°F) than at room temperature (25°C) and survival was enhanced if the cells were acid adapted.

In addition, the stationary-phase *E. coli* O157:H7 cells have been shown to be acid tolerant or able to survive at extremely low pH values (Leyer et al., 1995; Conner and Kotrola, 1995; Benjamin and Datta, 1995). At lower storage temperatures, such as 4°C (39°F), it was found that the inhibitory action of acids on *E. coli* O157:H7 is temperature dependent (Miller and Kaspar, 1994; Abdul-Raouf, et al., 1993a). Considering this characteristic of acid tolerance, in this study, the *E. coli* O157:H7 ATCC

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strain 43895 was investigated, since this particular strain exhibited the greatest survival among pH values tested ranging from 2 to 11 (Miller and Kaspar, 1994).

Studies show that *E. coli* O157 can survive on vegetables (Weeratna and Doyle, 1991; Abdoul-Raouf et al., 1993b; Seo and Frank, 1999) and is capable of growing at 8°C (ca 46°F) (Weeratna and Doyle, 1991). These findings are significant since this temperature (8°C) is below the temperature at which ready-to-eat meals and lightly processed salad vegetables may be exposed for several hours during marketing and on restaurant buffet counters (Abdoul-Raouf et al., 1993b). Abdoul-Raouf et al. (1993b) demonstrated the ability of *E. coli* O157:H7 to grow on raw salad vegetables. Shredded lettuce, sliced cucumber, and shredded carrots were inoculated with *E. coli* O157:H7 and results indicated populations of viable *E. coli* O157:H7 declined on vegetables stored at 5°C (41°F), but growth increased on vegetables stored at 12°C (ca 54°F) and 21°C (ca 70°F) for up to 14 days (Abdoul-Raouf et al., 1993b). Conditions of packaging, storage, and handling in the study were not unlike those conditions typically used in commercial practice. Investigators saw that the most rapid increases in populations of *E. coli* O157:H7 occurred on lettuce and cucumbers stored at 21°C and the ability of *E. coli* O157:H7 to grow at 12 and 21°C on three types of salad vegetables was demonstrated. It was noted that precautions should be taken to prevent contamination of salad vegetables with *E. coli* O157:H7 during production, harvesting, processing, marketing, and preparation in restaurants, as well as homes (Abdoul-Raouf et al., 1993b).

Besides vegetables, *E. coli* O157:H7 can survive on fruits. Del Rosario and Beuchat (1995) studied watermelons and cantaloupes to determine the survival and growth of *E. coli* O157:H7 on freshly cut cubes of cantaloupe and watermelon, as well as

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the rind surface of these fruits. Results found that the pathogen populations remained constant at 5°C (41°F), but increased on fruit cubes stored at 25°C (77°F), over a 34-hour storage period (Del Rosario and Beuchat, 1995). Growth was observed on the rind of melons stored under high relative humidity at 25°C for 14 to 22 days, but the pathogen died rapidly on the rind surface of melons stored at 5°C (Del Rosario and Beuchat, 1995).

Escherichia coli O157:H7 has been shown to survive in mayonnaise (salad dressing) at refrigerated conditions (Hathcox et al., 1995; Zhao and Doyle, 1994). Studies indicated that with good manufacturing practices (GMPs) *E. coli* O157:H7 will not survive in the mayonnaise-based dressings. However, pathogen may survive at 5°C (41°F) for several weeks if the dressing is cross-contaminated by foods such as raw beef, through unclean utensils, or if *E. coli* O157:H7-infected food handlers contaminate opened containers (Hathcox et al., 1995; Weagant et al., 1994).

Reitsma and Henning (1996) showed that *E. coli* O157:H7 can survive the standard cheese-manufacturing process when cheese milk is inoculated with the pathogen. Current standards for the manufacturing of Cheddar cheese require that the cheese can be made two ways. One method of Cheddar cheese manufacture uses pasteurized cheese milk and the second method requires cheese to be held for a minimum of 60 days ≥ 2°C (ca 36°F) when produced from raw or heat-treated milk. Their results pointed toward potential foodborne illness cases with cheese as a vehicle, and the risks would be increased if the percent salt in the moisture phase of the cheese product was low. Thus, their results indicated survival of *E. coli* O157:H7 during the manufacture of cheese, as well as survival of the pathogen for more than 60 days of curing when the product was at 2.75 to 3.76 % salt in the moisture phase (Reitsma and Henning, 1996).

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Sources and transmission

Escherichia coli O157:H7 is most often carried in the intestinal tract of cattle (Neill et al., 1994; Doyle et al., 1997; Doyle and Padhye, 1989). Additional known reservoirs and/or sources of *E. coli* O157:H7 include deer, sheep, and water (Keene at l., 1997; Kudva et al., 1996; Keene et al., 1994; Swerdlow et al., 1992; Doyle et al., 1997; Buchanan and Doyle, 1997). Incidents of person-to-person transmission have been reported and may be increasing (Doyle et al., 1997). In addition, as the number of livestock, living in confined areas is increased with a more rapid turnover of animals, the risk of cross-infections is enhanced. The effect of migratory animals continues to enhance transmission. However, originally, outbreaks were associated primarily with undercooked ground beef. From the association of *E. coli* O157:H7 infections with undercooked ground beef, as well as raw milk, the pathogen was linked to the identification of cattle as reservoirs for the pathogen (Karmali et al., 1983). Zhao et al. (1995) found young animals, calves and heifers, carry *E. coli* O157:H7 more frequently than do adult cattle or weaned calves. In addition, the prevalence of *E. coli* O157:H7 varies among cattle, with levels varying from $<10^2$ CFU/g to 10^5 CFU/g (Zhao et al., 1995). In addition, methods used for isolating *E. coli* O157:H7 influence the isolation rates. As better detection procedures are developed, the prevalence of *E. coli* O157:H7 will be greater (Doyle, 1991). Recently, the USDA-FSIS (1999) began using a more sensitive method for detecting *E. coli* 0157:H7 in raw meat products. This method is approximately four times more sensitive than previous methods, thus, increasing the probability of detecting very low levels of the potentially dangerous pathogen.

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Keene et al. (1997) concluded that deer were the source of a 1995 *E. coli* O157:H7 outbreak in Oregon. Members of an extended household and friends were confirmed with *E. coli* O157:H7 infections and homemade venison jerky was implicated as the source of pathogen transmission. Investigations established that deer were a source of pathogen and the transmission of the pathogen may have occurred between deer and cattle. Researchers isolated *E. coli* O157:H7 from the human cases, leftover jerky, uncooked meat from the same deer, the saw used to cut up the carcass, as well as fragments of the deer hide (Keene et al., 1997). In addition, Kudva et al. (1996) reported sheep to be another source for *E. coli* infection, as well as water, both drinking (Swerdlow et al., 1992) and lake (Keene et al., 1994). Contaminated water has been implicated in several outbreaks of *E. coli* O157:H7. in 1991, water mains were broken and newly installed in-ground water meters may have contributed to a drinking water outbreak which involved 21 cases (Swerdlow et al., 1992). In addition, it is suspected that bathers and toddlers, not yet toilet trained, ingested fecally contaminated lake water (Keene et al., 1994).

As researchers continue to investigate the pathogen, outbreaks other than ground beef have been reported. There are abundant opportunities for cross-contamination of meats and other types of foods with *E. coli* O157:H7 during processing, handling, and marketing (Abdoul-Raouf et al., 1993b). For example, contamination of raw salad vegetables with *E. coli* O157:H7 would be most likely to occur during the assembly of ready-to-eat meals, which may include beef or other potential carriers of the organism, as well as contamination during preparation in kitchens of food service establishments (Abdoul-Raouf et al., 1993b). Besides food processing facilities, there is the possibility

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of *E. coli* being present on raw produce originating from agronomic systems irrigated with contaminated water. This contamination method should not be disregarded (Buchanan and Doyle, 1997).

Hara-Kudo et al. (1997) studied the contamination of *E. coli* O157:H7 with radish sprouts grown in or exposed to *E. coli* 0157:H7-inoculated water. The authors concluded that *E. coli* O157:H7 contamination, in the edible parts of radish sprouts, could pose a serious hazard if the seeds or hydroponic water were contaminated with the bacterium. Thus, sanitation control of the radish seeds and hydroponic water may be critical for prevention of transmission of pathogenic bacteria by raw hydroponically grown vegetables (Hara-Kudo et al., 1997). In addition, water inoculated with *E. coli* O157:H7 was studied (Warburton et al., 1998; Wang and Doyle, 1998) and results indicated that the pathogen can survive for a long period of time in water, especially at cold temperatures. Findings indicated the pathogen could survive > 300 days in inoculated bottled water or pure water and also, *E. coli* O157:H7 survived better in pure water, than in lake or reservoir water. However, in naturally occurring conditions, factors such as sunlight or organic matter also may effect the pathogen's survival. Thus, precautions should be taken when using lake or river water for drinking or recreational purposes due to the apparent hardiness of the pathogen surviving long periods of time in contaminated water (Wang and Doyle, 1998).

Survival of E. coli *O157:H7 in fermented and dried meat*

There are disagreements in reported research results of proper home-drying methods, as well as methods associated with dry and fermented meats. Hinkens et al. (1996) found that when pepperoni was inoculated with *E. coli* O157:H7, fermented at

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36°C (96°F) to a pH ≤ 5.0, and dried at 13°C (ca 55°F) with 65% relative humidity, there was only a 1 log reduction in pathogen numbers. However, a 5-log reduction was obtained when pepperoni sticks were heated after fermentation to an internal temperature of 63°C (ca 145°F) instantaneously or 53°C (ca 128°F) for 60 min (Hinkens et al., 1996). Authors concluded that intrinsic factors (salts, fats, moisture) were not enough to reduce high levels of *E. coli* O157:H7. In addition, Riordan et al. (1998) investigated the survival of *E. coli* O157:H7 during the manufacture of pepperoni, as well, and found that combinations of increased levels of salt and sodium nitrite with lower pH (≤ 5.0) significantly reduced levels of population counts of *E. coli* O157:H7 cells in the pepperoni; however, the declines (0.80-4.30 log CFU/g) were less than the target reduction of 5-logs (Billy, 1997; Reed, 1995).

Additional studies concerning pepperoni as a lower pH (≤ 5.0) food were conducted (Faith et al., 1997; Faith et al., 1998c). Investigators confirmed that the traditional processes of drying and fermenting pepperoni did not result in sufficient bacterial reduction. Faith et al. (1997, 1998c) reported that when slices of pepperoni were stored for at least 14 days at ambient temperature of 21°C (ca 70°F), packaged under air, a > 5.5 log CFU/g total reduction was achieved. Investigators also reported that the pathogen decreased at greater rates when the pepperoni slices were baked at temperatures > 275°F (ca. 135°C) (Faith et al., 1997, 1998c).

Besides pepperoni, Faith et al. (1998b) investigated the viability of inoculated *E. coli* O157:H7 cells on salami following conditioning, fermentation and drying of salami sticks. Various common salami-producing methods were investigated and compared. The study confirmed that fermentation and drying were only sufficient to effect a 1- to 2-

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log reduction of *E. coli* O157:H7 cells in fermented meats, in this case, salami sticks. Investigators recommended more aggressive methods be added to the food processing of fermented meat, such as post fermentation heat, extended fermentation and/or drying time at lower pHs and/or higher temperatures. In addition, the authors suggested that a "multiple barrier" procedure could enhance the safety of fermented meats (Faith et al., 1998b).

Keene et al. (1997) implicated homemade venison jerky as a source of pathogen transmission. The fate of *E. coli* O157:H7 was assessed during a laboratory experiment where jerky was dried at 51.7°C (125°F) and 62.8°C (145°F). When dried at 51.7°C, counts declined slowly in marinated jerky slices (< 2.0 log CFU/g), while at the higher dehydrator temperature at 62.8°C, counts declined over the first 6 to 8 h to levels undetectable by direct plating (< 10 CFU/g). Upon enrichment, however, *E. coli* O157:H7 could still be cultured in all dried samples (Keene et al., 1997). These experiments indicated low-temperature dehydration of ≤ 62.8°C (145°F) was not a reliable way to eliminate the pathogen from the dried jerky (Keene et al., 1997). The researchers recommended that, before dehydration, meat should be precooked to at least the same internal temperature recommended for commercially cooked wild game (74°C) (Keene et al., 1997). In this study, enumeration of surviving bacteria was conducted with Sorbitol MacConkey agar (SMAC) containing 0.1 4-methyl-umberlliferyl-D-glucoronide, (MUG) or modified SMAC (MSMAC) and although the strain of *E. coli* O157:H7 used in the study was not specified, it is presumed an isolate of the contaminated venison jerky was used. For the study conducted in this paper, an isolate of the contaminated venison

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jerky was obtained through Dr. M. P. Doyle, Center for Food Safety and Quality Enhancement, University of Georgia, Griffin, Georgia.

Harrison and Harrison (1996) reported that low-temperature dehydration (60°C/140°F) was adequate for destroying sufficient *E. coli* O157:H7 cells (at least 5 log units) when present in whole muscle beef jerky. They investigated the advantages of traditional jerky preparation (Reynolds and Williams, 1993) when compared to the current USDA-FSIS (1998) recommendation of heating meat to 71.1°C (160°F) before drying. The traditional jerky preparation consisted of raw meat pieces covered with a traditional marinade, refrigerated at 4°C (39°F) for 1 h, then dried for 10 h at 60°C. In the study, whole beef loin slices (15.0 x 1.5 x 1.5 cm) were divided into three groups; slices in two groups were inoculated with *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Typhimurium. On one-third of each strip, 0.1 ml of each inoculum (10^8) colony forming units--CFU/ml--was distributed on the top of the slices. The remaining group was treated with sterile Butterfield's phosphate buffer to mimic inoculation. The phosphate buffer-treated samples and half of the inoculated samples were marinated at 4°C (39°F) for 1 h. The remaining samples were placed in a shallow pan, covered with marinade and heated on a hot plate for an unspecified time to 71.1°C. The pan was removed and allowed to cool 10 min under a laminar flow hood to prevent contamination. All jerky pieces were dried at 60°C (140°F) for 10 h in home-type dehydrators. After 10 h of drying populations of *E. coli* O157:H7 on the inoculated, unheated meat decreased by 5.5 to 6.0 log units. Populations on the inoculated, pre-heated meat decreased by 5 log units during pre-heating, prior to drying and after 10 h of drying, populations decreased to undetectable levels (detection limit not stated) (Harrison

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and Harrison, 1996). The authors concluded that using a marinade in jerky preparation and drying at 60°C for 10 h were sufficient to reduce levels of *E. coli* O157:H7 by at least 5 log units on unheated and pre-heated product (Harrison and Harrison, 1996). In the study, Harrison and Harrison (1996) used a single strain of *E. coli* O157:H7 and enumeration of surviving cells was done with one selective medium, SMAC.

Harrison et al. (1998) investigated ground beef jerky inoculated with *E. coli* O157:H7, made with either a spice and cure mix or a spice mix only. Lean ground beef (227 g) was inoculated with a 5.0 ml (1.0 ml of each) five-strain *E. coli* O157:H7 composite (10^8 CFU/ml). After the inoculum was mixed with the ground meat, the meat was molded into strips (33.5 x 2.5 x 0.75 cm). The recovery rate of *E. coli* O157:H7 cells on two selective media, modified sorbitol MacConkey agar (MSMAC) or modified eosin methylene blue (MEMB) agar, was evaluated during the jerky-making process. The MSMAC was prepared by supplementing SMAC with MUG agent, while the MEMB was prepared using Levine's eosin methylene blue agar without lactose, but with added sorbitol, sodium chloride and sodium novobiocin (Clavero and Beuchat, 1995, 1996). Before drying in a home-type dehydrator, half of the inoculated strips were pre-heated in a convection oven to an internal temperature of 71.1°C (160°F) (time unspecified). Half of the pre-heated samples contained a spice mixture (ingredients not listed) and a cure mix (salt and sodium nitrite). The remaining pre-heated samples contained a spice mix only. The other half of inoculated jerky strips were not pre-heated, but half contained a spice and cure mix, while half contained a spice mix only. All jerky samples were dried in a 60°C (140°F) dehydrator for 8 h (unheated samples) or 6 h (pre-heated samples). It was found that a 5.2 log CFU/g reduction was obtained in unheated and pre-heated jerky

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containing a spice and cure mix (Harrison et al., 1998). With a spice mix only, log reductions were 4.3 and 4.8 log CFU/g, for the unheated and pre-heated jerky, respectively. When investigators compared enumeration methods, the jerky prepared with spice-cure mix achieved reductions of > 5.0 log CFU/g with MSMAC, while jerky prepared with a spice mix only achieved reductions of < 5.0 log CFU/g enumerated with MSMAC. For the unheated jerky prepared with the spice-cure mix, MEMB indicated significantly ($P < 0.05$) higher recovery rates than MSMAC after 6 and 8 h of drying. Investigators reported enumeration with MEMB agar may be a better alternative for research involving food preservation techniques and the elimination of *E. coli* O157:H7 from the food (Harrison et al., 1998). In addition, these findings suggested that if consumers are making jerky with ground meat and fail to add the jerky cure mix, the product could pose an increased risk of foodborne illness if the meat was contaminated with *E. coli* O157:H7 (Harrison et al., 1998).

Faith et al. (1998a) studied beef that was ground and formed into jerky at two levels of fat (5 and 20%), prepared with a spice mix and mixed with a five-strain *E. coli* O157:H7 inoculum (10^8 CFU/g). The mixture was molded into strips (15.0 x 5.0 x 0.4 cm) and dried in home-type dehydrators for various times intervals (2 to 20 h) at four dehydrator (air) temperatures (52°C/125°F, 57°C/135°F, 63°C/145°F or 68°C/155°F). The results indicated that drying times had to be increased well beyond the point considered visually acceptable at the lower drying temperatures and that pathogen reduction varied with the levels of fat (Faith et al., 1998a). While a ≥ 5-log reduction could eventually be obtained at any of the temperatures studied, only products with 20% fat and dried at 63°C and 68°C obtained ≥ 5-log reductions (6.72 and 7.07 log CFU/g) by

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the time the products were judged visually dry (8 and 6 h, respectively). In the lean ground beef (5%) products, a 4.8 log CFU/g reduction was achieved by the time the product was visually judged dry (4 h) at 68°C. At all other fat level/temperature combinations, log reductions ranged from 0.75 to 3.0 log CFU/g for products considered adequately dry by visual inspection (Faith et al., 1998a). Results indicated that the numbers of *E. coli* O157:H7 cells decreased at a greater rate in meat with lower fat (5%) and lower acid (pH 6.0) levels, than in jerky prepared from batter formulated at higher fat (20%) and higher (pH 6.4) acid levels. Since the higher fat jerky product took longer to become visually dry, the researchers concluded that jerky should be prepared from leaner cuts of meat (≤ 5%) and dried at temperatures ≥ 63°C for at least 8 h in order to achieve an appreciable reduction in *E. coli* O157:H7 (Faith et al., 1998a). It appears that there is a need for additional studies to evaluate procedures with the objective of achieving adequate (>5 log CFU/g) reduction in *E. coli* O157:H7 during jerky processing.

HURDLE TECHNOLOGY

The "multi-hurdle" concept employs several combined "hurdles" to help ensure that microorganisms can be controlled in a food (Leistner, 1987; Leistner, 1995). Using this concept, microorganisms should not be able to overcome hurdles that are present during food processing and storage. Thus, if the microorganism cannot "leap" the hurdles, the food will be microbiologically safe and stable (Leistner, 1995). With multi-hurdles, lower intensities of individual factors can be used than what would be needed, if the factors were used alone, since the combination of factors works synergistically to prevent spoilage (Leistner, 1987; Marth, 1998). Leistner (1987, 1995) recommended the use of a multihurdle strategy for food processing industries that involve fermented and

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dried meat, since a sequence of hurdles can lead to a stable product. The most important hurdles commonly used in food processing include temperature (high or low), water activity (a_w), acidity (pH), redox potential (E_h) and preservatives (organic acids, spices). Hurdles vary according to the goal of the processing step. Some hurdles can influence the safety of the food, while improving the flavor or taste of the product (Leistner, 1995).

As stated, organic acids are commonly used as antimicrobial agents, since the pH will contribute to help control growth of bacteria (Nychas, 1995; Leistner, 1995). However, when organic acids are used in food processing techniques, the concentration of the undissociated acid, in addition to the pH, must be considered, to ensure the inhibition of bacterial growth (Bell and Kyriakides, 1998). It is reported that when products are heat-treated and the product has a sub-optimal pH for *E. coli* O157:H7, while containing an organic acid, the treatment is more effective in inactivating the bacteria. The organic acid, acetic acid, has been reported to have qualities that work in decontaminating carcasses and sliced beef, and mixtures that contain acetic acid can also produce a lower pH on the surface of the meat (Smulders, 1995). Vinegar (5% acetic acid) was studied by Entani et al. (1998), who reported that it had a bactericidal effect on foodborne bacteria, including *E. coli* O157:H7. In the study, vinegar was inoculated with *E. coli* O157:H7 cells and it was found that *E. coli* O157:H7 cells in the logarithmic growth phase demonstrated a higher sensitivity to vinegar than cells in the stationary phase. In addition, Entani et al. (1998) investigated the bactericidal activity of vinegar with an increase of temperature. Investigators suggested that an effective method to prevent foodborne bacteria should include a vinegar treatment that is applied at 40 to 50°C (104 to 122°F). These temperatures would be more applicable and practical since

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heating vinegar to 75°C (167°F) may create an alteration of food taste, as well as a loss of nutritional value. However, researchers reported that heating vinegar to 75°C was a more effective method for sterilizing *E. coli* O157:H7 (Entani et al., 1998). When Entani et al. (1998) evaluated the application of acetic acid (2.5%) at room temperature of 20°C (68°F) to inoculated cells, a 3-log reduction of viable *E. coli* O157:H7 cells was seen after 739 min; however, when acetic acid (2.5%) was applied at 40°C (104°F), a 3-log reduction was seen after 14 min. In addition, investigators combined acetic acid (2.5%) with sodium chloride (NaCl) (5%) and a 3-log reduction was obtained after 5.27 min (Entani et al., 1998). However, when the acetic acid (2.5%) and NaCl (5%) were applied at 40°C, a 3 log reduction in viable *E. coli* O157:H7 cells was seen after 0.89 min (Entani et al., 1998). In addition to using various combinations of acetic acid (2.5%) and NaCl, at appropriate temperatures, Entani et al. (1998) recommended cells in the stationary phase should be used in experiments when investigating the applications of vinegar in food preparation. With this recommendation, *E. coli* O157:H7 cells in the stationary phase were used for this study. Since, organic acids can have greater antimicrobial effects at higher acid concentrations and at higher temperatures (Smulders, 1995), this study investigated the application of acetic acid (2.5%) to slices of beef jerky inoculated with *E. coli* O157:H7 and incubated to 57.5°C (135°F) for 20 sec.

In addition to acidity being used as a hurdle, spices and seasonings can be used a part of a multi-hurdle system. Spices and seasonings have long been used to add flavor to food products, but many spices also exhibit antimicrobial properties (Beuchat, 1994). One example of a spice family commonly used in the diet is from the *Allium* genus. This genus includes such plants and spices as garlic, onion and leek. Garlic has been used for

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centuries as a flavoring enhancement, but only in the past few decades has the antimicrobial and medicinal properties of garlic been established (Beuchat, 1994). In addition, garlic has been reported to kill *E. coli* O157:H7 when used at varying levels (Food Safety Consortium, 1998). However, unfortunately, to inhibit microorganisms, garlic and other spices must be added at concentrations that exceed levels normally recommended and concentrations of spices at less than 1% are not considered to be inhibitory to microorganisms (Beuchat, 1994). Riordan et al. (1998) recommended against simply increasing concentrations of existing antimicrobial ingredients (salts, spices, etc.) that are used in jerky preparation, due to the unpleasant and unacceptable taste qualities that result. However, when spices and seasonings are used in varying combinations, their antimicrobial activities are able to work synergistically in order to inhibit microorganisms. One way to combine different ingredients is through use of a marinade, which is typically common during jerky preparation. A jerky marinade often contains soy sauce, Worcestershire sauce, black pepper, garlic and onion powder, and Hickory smoked salt (Reynolds and Williams, 1993). Besides the spices and seasonings used in a jerky marinade, soy sauce has also been reported to have anti-*E. coli* O157:H7 effects (Masuda et al., 1998). Masuda et al. (1998) indicated that the inhibitory activity of soy sauce was due to the combination of ingredients found in the sauce (components of sodium chloride, ethanol, lactic acid and preservatives). When investigated, *E. coli* O157:H7 cells could not grow in soy sauce at 4, 18, and 30°C (39, 64 and 86°F), while cell survival was inhibited (partially or fully) in soy sauce at 30 and 18°C (Masuda et al., 1998). Authors concluded that the inhibition of *E. coli* O157:H7 in soy sauce was not determined by any one ingredient (sodium chloride, ethanol, pH, organic acid, or

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preservatives), temperature or time. On the contrary, the fate of the pathogen was due to the combined effects of all factors found in the soy sauce.

FOOD PRESERVATION

Not all foods are suitable for long periods of storage. Perishable foods generally have a shelf life of 5-7 days, under ideal conditions. However, shelf stable and not-perishable foods are products that have been preserved by heat, formulated with dry mixes or processed to have a lower a_w (Dethmers, 1979). Thus, food preservation is a term used to describe any measures used to keep food safe and acceptable over a reasonable period (Luck, 1993). The objective of food preservation is to minimize damage to the food, while ensuring the food is safe, enjoyable and full of nutritional value (Luck, 1993). A products shelf life is dependent on the ingredients, the processing techniques and storage packaging used, as well as the time, temperature and relative humidity throughout storage (Dethmers, 1979). Food preservation extends the shelf life of perishable foods, by making them shelf stable and preventing health hazards by limiting contamination with pathogenic microorganisms. The key to a safe food is the control of microorganisms present, or that could be present, in the food through the inactivation of pathogenic microorganisms (NRA, 1995). The term "food spoilage" refers to any change in a foodstuff that appreciably reduces its value, especially its nutritional value, sensory quality, palatability or usability (VanGarde and Woodburn, 1994; Luck, 1993). Four types of food spoilage can occur to a product during storage: chemical, enzymatic, physical, and microbiological (Dethmers, 1979; Luck, 1993). Due to the effects of foodborne pathogens, spoilage due to microbiological factors has impacted the food industries across the world. Bacteria, yeast, and molds can cause food

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spoilage due to microbiological reactions and are the main causes of deterioration of perishable foods (Dethmers, 1979). Spoilage microorganisms may be present from soil or other contaminant from harvest to consumption. In order to cause spoilage, the microorganism needs to be present in the food, survive, and have nutrients available in suitable and adequate quantities (Luck, 1993). While in the food, the microorganism should have favorable living conditions (temperature, water activity, presence or absence of oxygen, redox potential and pH values). Thus, there are four major strategies currently available for food preservation which: inhibit microbial growth, inactivate microorganisms in the food, promote growth of desired microorganisms or restrict access of microorganisms to germ-free products (Dillon and Board, 1994).

In order to inhibit or retard microbial growth in food, several methods can be used to achieve this purpose. One method available to retard growth of the bacteria is through a reduced water activity (a_w). Water activity (a_w) is the measure of the quantity of available water contained in a food and corresponds to the relative humidity of the ambient air (Leistner, 1987; Lenovich, 1987). Few bacteria grow at a_w below 0.9, while most yeast or molds do not grow below 0.83 or 0.80, respectively; only highly xerophilic yeast and molds tolerate 0.60. One way to lower water activity is through the addition of salts and sugars (Leistner, 1987; Lenovich, 1987). The free water in the food becomes bound, when the ingredients are added and the water activity is reduced to lower values, making conditions unfavorable for microorganisms.

Another method to achieve inhibition of microbial growth is through acidification. Acidic solutions, such as acetic or lactic, are added to help preserve the food from existing or potential microorganisms. The acidifying agents can be added in

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concentrations from 0.5 to 5.0%, but at these concentrations, their taste is perceptible. Acidulants can alter the pH of the foods, making microorganisms unable to live or reproduce; however, these acidic solutions have no inhibitory effect on many yeast and molds (Leistner, 1987; Lenovich, 1987). Other ways to achieve retardation of microbial growth are with modified atmospheres or adding preservatives to a food.

Food preservation incorporates various methods used to prevent microbiological food spoilage. Salting and/or curing are types of food preservation often used to preserve meats, vegetables and fruits (Luck, 1993). Salting uses high concentrations of salt, allowing moisture to be drawn out of microorganisms through osmotic pressure. Curing is similar to salting, except that this method involves the addition of other ingredients, such as sugars, spices, and sodium nitrite. In addition, pickling is a type of curing solution, but it uses a brine, vinegar or spice solution to help preserve the food (Luck, 1993). Refrigeration and/or freezing are another type of preservation method that inhibits the growth of microorganisms. The lower the ambient temperature that is used when refrigerating or freezing, there will be a greater inhibitory effect on the microorganisms (McWilliams, 1993; Luck, 1993). However, refrigeration and/or freezing may also influence the physical structure of food since slow freezing allows larger ice crystals to form between cells and upon thawing, the product can lose its juiciness (Luck, 1993).

In order to inactivate microorganisms, it is necessary to use food preservative strategies, such as pasteurization, canning, or irradiation. When using a heat treatment, all or certain microorganisms will be killed. Resistance of microorganisms to destruction is expressed as the decimal reduction time (D-value). The D-value is the time that is needed to kill 90% of a particular microorganisms at any given temperature; thus, the

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higher the D-value, the greater the heat tolerance (Dillon and Board, 1994; Luck, 1993). Bacterial reduction is dependent not on the temperature and the duration of the action, but also on the pH value, moisture content, and the presence or absence of certain food ingredients or additives (Marth, 1998; Luck, 1993). Finally, other strategies can use fermentation to promote the growth of desired microorganisms within a food product. In addition, aseptic packaging is often used for food products, in order to restrict the access of microorganisms to germ-free products (Dillon and Board, 1994).

Another method of food preservation is through the dehydration of a food product. Drying is the original method of food preservation and has been practiced for at least 3000 years (McWilliams, 1993; VanGarde and Woodburn, 1994). Drying food products outdoors in the sun was the original way to dehydrate foods. Currently, many home-type dehydrators are now widely available for personal use when drying foods. Home-type dehydrators were developed using the principles associated with dehydration by facilitating the transfer of water and restricting excess moisture during storage.

Dehydration is an effective method of food preservation, since food is dried to low levels of moisture (McWilliams, 1993). When foods are dried to moisture levels below 13%, the dried food can be stored at room temperatures for extended periods (McWilliams, 1993). However, food pieces must be dried thoroughly by using mild heat and evaporation. Dehydration is most effective when the food is dried in thin pieces and contains large surface areas. As the water in the food changes in state from liquid to vapor, heat is absorbed (heat of vaporization) and the surface is cooled (McWilliams, 1993). By using a mild heat, energy is added to the system, which helps maintain a sufficiently high temperature enabling evaporation to continue. Evaporation is enhanced

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when three drying conditions are met: warm air temperatures, air circulating above the food and a low relative humidity (McWilliams, 1993). Warm temperatures are necessary in order to provide the energy required for the heat of vaporization. The vapor pressure of water within the food is increased until it evaporates from the surface of the food. Circulating air is needed for the dehydration process since air currents passing over the fcod help to remove evaporating water. In addition, as the water evaporates from the food, a low humidity in maintained directly above the food (McWilliams, 1993).

When these three conditions are met, water within the food evaporates readily and efficiently because the air is now capable of holding considerably more water. The length of drying requirement depends on the nature of food being dried, the ingredients of food, as well as expected changes during storage and relies on the final a_w of the food product (VanGarde and Woodburn, 1994; McWilliams, 1993; Luck, 1993). In recent years, it was thought that dehydration inactivates microorganisms since they lack the free water necessary to grow. However, microorganism have been able to survive low a_w (CDC, 1995a, b, c).

MICROBIAL RESISTANCE

When microorganisms are exposed to food processing techniques that are sub-lethal or that are not adequate to inactivate the microorganism, cellular damage can occur. The cellular damage that is caused by such sub-lethal stresses will vary according to the type of stress and/or the microorganism. Common food processing techniques that cause cellular damage include heating (the most common stress), freezing, and drying. In addition, cellular damage can occur when chemicals are added, through acidification by either fermentation or direct addition of acid or through the addition of sodium chloride

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or sodium nitrite (Sofos, 1989; McFeters, 1989). Cellular damage to the microorganism may cause the microorganism to become stressed or injured. Stressed or injured cells are viable cells, capable of repairing the cellular damage, recovering from the stress that caused the damage.

Injured cells are unable to produce detectable growth under selective conditions that are suitable for the proliferation of uninjured ones, but injured cells maintain their ability to regain normal physiological activity when placed in an appropriate environment (McFeters, 1989). Dead cells cannot form a colony under any condition, but stressed bacteria, sustaining the cellular injury, can repair themselves. These cells will regain their ability to form a colony (or become infective) when they are incubated in an appropriate repair medium at appropriate temperatures (McFetters, 1989). Once these injured cells undergo repair and return to normal physiological function, these cells may cause unexpected health hazards (Semanchek and Golden, 1998).

Sub-lethally injured cells that arise from inadequate processing procedures or storage conditions will not readily grow when subjected to certain selective media conditions (McFetters, 1989; McCarthy et al., 1998), so the use of selective media for detection of recovered cells must be used with caution. Enumeration of stressed microorganisms requires the repair of injury in a nutrient-dense, non-inhibitory medium that is suitable for growth of both normal and environmentally stressed organisms (McFetters, 1989; Sofos, 1989; Semanchek and Golden, 1998). A nutrient-dense, non-inhibitory medium must be used to indicate the total viable populations present, as well as to eliminate any false changes in populations, due to the multiplication of cells in the sample.

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Sorbitol MacConkey (SMAC) agar is a selective medium often used to achieve the differential isolation of a microorganism, particularly for *E. coli* O157:H7 (McCarthy et al., 1998). SMAC has been reported to be inhibitory to stressed or injured *E. coli* O157:H7 cells, thus showing the least recovery of injured cells and the most reduction in log CFU/cm^2 (Clavero et al., 1998; McCarthy et al., 1998; Faith et al., 1998b; Abdul-Raouf et al., 1993a, b; Clavero and Beuchat, 1996). While investigating the survival of *E. coli* O157:H7, McCarthy et al. (1998) found greater than a 3-log difference between counts enumerated on SMAC compared with counts enumerated on tryptic soy agar (TSA). Results from the study indicated the ability to detect sub-lethally injured cells is crucial when considering *E. coli* O157:H7 (McCarthy et al., 1998). However, TSA is a general medium and not selective, accounting for some of the differences in log-reduction. Additional studies (Clavero et al., 1998; Clavero and Beuchat, 1996) made similar findings when SMAC was compared to other media. The recovery of viable cells on SMAC supplemented with 4-methyl-umberlliferyl-D-glucoronide--MUG (MSMAC) was significantly lower than viable cells recovered on TSA (Clavero and Beuchat, 1996). Recovery on MSMAC was significantly lower with increased heating times and decreased levels of pH and water activity/a_w, indicating injury was more extensive at lower levels of pH and a_w. In addition, non selective TSA generally supported better repair and colony formation by heated cells than another selective agar, modified eosin methlyene blue agar (MEMB) (Clavero and Beuchat, 1996). The poor recovery of viable cells on MSMAC was attributed to the presence of selective ingredients (such as bile salts) that inhibit the repair of injured cells (Clavero et al., 1998), underestimating thermal tolerance. Thus, the use of various culture media for detection of *E. coli*

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O157:H7 cells, both non-injured and injured, must continue to be compared, especially during processes involving varying levels of heat, acid and a_w.

Semanchek and Golden (1998) reported that it was important to employ techniques that optimize the detection and enumeration of both injured and uninjured organisms. Due to the fact that sub-lethally injured or stressed cells are able to recover and grow later (Levy, 1992), bacterial injury is important in developing, modifying and evaluating methodologies of food processing (thermal and irradiation treatment). If injured cells are ignored or improper recovery methods are implemented, food-processing methods may lead to an overestimation of lethal effects (Semanchek and Golden, 1998). With injured cells being potentially generated during home dehydrating, the use of appropriate culturing media must be considered (Semanchek and Golden, 1998). Since *E. coli* O157:H7 is infectious at a very low dose, any stressed or injured cells remaining in the food product can retain their virulence and potentially make the product a food safety hazard. If a jerky preparation method does not provide enough heat to adequately kill the cells, the pathogen may become injured and resistance to heat at sub-lethal heat treatments may develop (Levy, 1992). Any sub-lethal treatment or stress will cause injury to the microorganism or in this case, *E. coli* O157:H7 (Semanchek and Golden, 1998).

In order to survive, bacteria have acquired and maintained supplemental genetic information to cope with and adapt to the changing environments. Bacteria are continually faced with the Darwinian concept of the "survival of the fittest" (Birge, 1994), which implies that bacteria must be constantly evolving in order to ensure survival. For the bacteria to achieve survival, the organism must possess mechanisms

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that allow for continual cell replication and modification. In order to achieve this, bacteria have acquired extra genetic information through pieces of transferable genetic material called plasmids (Levy, 1992). Plasmids are defined as a DNA molecules that are less than chromosomal size and capable of self-replication (Birge, 1994). These plasmids exist as independent self-duplicating genetic elements that carry additional genes and enable bacteria to perform new functions (Levy, 1992). The plasmid cannot survive outside the bacterial cell and is dependent on the cell for life. Since the plasmid is dependent on the host, the plasmid provides the host cell with the survival traits necessary to ensure survival of both the host and plasmid (Levy, 1992). These plasmids will acquire traits allowing the bacteria to resist heat, chemicals, digestion, and even antibiotics.

Antibiotics have long been used by the medical field and are often prescribed as a quick treatment for various illnesses. However, the use of antibiotics may have resulted in the emergence of bacteria that, over time, become resistant to the very antibiotic that was designed to destroy the bacteria (Tollefson et al., 1998). During the 1950s, Japanese medical clinics found bacterial strains of *Shigella* resistant to several antibiotics being used (Tanaka et al., 1971; Birge, 1994). Upon further investigation several decades later, researchers found 70 to 80% of all *Shigella* strains were resistant to multiple drugs and other bacteria were also being found to show multi-drug resistance (Birge, 1994).

By introducing a large amount of antibiotic drugs into society, science has drastically altered the ecology of microbes. The prolonged use of antibiotics has introduced bacteria that are no longer killed by antibiotics (Levy, 1992). Most bacteria previously susceptible to antibiotics are now resistant to many different antibiotics

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(Birge, 1994). As bacteria became equipped with survival mechanisms, antibiotics were no longer able to destroy them. Consequently, these antibiotic-resistant strains caused infections not cured by drugs, creating a "microbial evolution" (Levy, 1992). Since antibiotics remain active after the target bacteria have been destroyed, resistant strains have an advantage and become more numerous since the antibiotic continues to kill off susceptible bacteria (Levy, 1992). It was once thought multiple drug resistance was the exception, now multiple drug resistance is the rule among bacteria.

CONSUMER EVALUATION

All people evaluate food quality, either consciously or unconsciously. Individuals make food choices in the marketplace based on previous experience with this food (Meilgaard et al., 1987). Food companies rely on market research to become informed on what the consumer desires in a food (Pearce, 1980). Each consumer evaluation is combined with other consumers and their responses dictate the quality and type of food produced throughout the nation. Food companies and processors must conduct very careful and thorough tests to ensure that current product formulations and processing techniques provide successful foods for the marketplace (Stone and Sidel, 1993). Consumer evaluation is used as routine checks of the food's quality throughout the production process (Merolli, 1980; Tassan, 1980). The food industry relies heavily on evaluation for the development of new products, while maintaining the quality control in current food items (Meilgaard et al., 1987; Moskowitz, 1988). Consumer evaluation can help a company evaluate a product upon distribution and help handle arising problems (Skinner, 1980). Researchers within the food companies use evaluation techniques to predict the acceptability of the food or to offer potential concerns with the product that

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were not addressed earlier. Food products can be evaluated on several characteristics; thus, depending on the intended purpose, characteristics may require different evaluation techniques (Meilgaard et al., 1987). An objective evaluation measures the physical properties of a food, such as texture or moisture content (Stone and Sidel, 1993; Moskowitz, 1988). However, physical properties can also be evaluated subjectively through a consumer panel.

Sensory or subjective evaluation is evaluation by individuals with a scoring system that is based on various characteristics using the senses (Lawless and Klein, 1991). Measurements can be determined by using the senses: sight, smell, taste, and touch (Lawless and Klein, 1991). Sensory evaluation is a vital source of information for research and quality control. Visual evaluation of a food gives the first indications concerning a food product. Often, the shape, texture, and color are observed through eyes first, even before the person has an opportunity to taste the food. Next, the smell of the food via the olfactory receptors preliminary cues to the acceptability of a food before it even enters the mouth. The olfactory sense of a human being is very keen. The individual variation in sensitivity exists as a consequence of nasal obstructions or sinus complications due to olfactory related problems. Taste is another measurement used in evaluation. There are four basic tastes: salty, bitter, sweet, sour and approximately 10,000 taste buds (most of which are found on the tongue). Finally, touch can provide a sense of acceptability and can be measured through texture (Lawless and Klein, 1991).

A food can have several characteristics requiring sensory evaluation, such as the flavor (taste and odor), texture, aroma, and appearance. First, flavor can be tested through a composite assessment of tastes that are blended with odor occurring in the

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mouth. Flavor is a very important attribute, yet often difficult to communicate (Lawless and Klein, 1991). Next, texture can be evaluated. Texture can be related to the food's appearance, as well as the mouthfeel, so one way to assess texture is to test the food's toughness or brittleness. This test can be evaluated by the number of chews required before the bite may be swallowed (Lawless and Klein, 1991). Another sensory characteristic often tested is aroma, which is the odor associated with the food. Aroma is important if the food needs to be served at warm or hot temperatures. According to Stone and Sidel (1993), aroma is often associated with the overall acceptability of the food. Finally, the appearance of the food can be evaluated. The appearance of the food is important since color triggers the connotation of particular flavors and provide acceptability of the exterior and interior appearance (cell size, uniformity, thickness) of the food (Lawless and Klein, 1991).

In order to conduct sensory evaluation, the Institute of Food Technologists (IFT) Sensory Evaluation Division (IFT, 1981) provided recommended guidelines for sensory evaluation. To create a panel for sensory evaluation, member selection should be based on factors identified as important for the specific study (IFT, 1981; Stone and Sidel, 1993). There are several types of taste panels and one type of taste panel is a consumer panel. Consumer taste panels can use either trained or untrained panelists. Trained sensory panelists are instructed extensively on procedures that will evaluate the samples to provide guidance for improving the product (IFT, 1981; Stone and Sidel, 1993; Meilgaard et al., 1987). A consumer panel with untrained panelists often selects people who happen to be available at a testing site. Untrained panelists are also willing to participate in the panel and have no preparation regarding evaluation of the product

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(Stone and Sidel, 1993). However, for both taste panels, an environment must be conductive to the testing.

When possible, the use of tasting booths is recommended, along with comfortable temperatures and air that is free of odors, fragrances, or aromas. When testing, smoking is prohibited and small sinks (or cups) should be available for spitting out samples (Meilgaard et al., 1987). In addition, if there are textual differences being evaluated, consideration to lighting is necessary, in order to mask any visual differences between products. Often, a colored light, such as red, is used for masking differences (Meilgaard et al., 1987). To avoid distractions, quiet surroundings are important and to allow concentration, testing booths should be set-up in different parts of the room, allowing no interaction among panelists.

Sensory evaluation may involve several types of tests. Normally, sensory evaluation tests are conducted for two main reasons: 1) to determine the consumer's ability to distinguish differences among products and 2) to determine the consumer preference/acceptability between new or old products (Stone and Sidel, 1993; Lawless and Klein, 1991; Meilgaard et al., 1987). Difference testing can be used to test the sensitivity of the judges, when ingredients are replaced or substituted. Within difference testing, numerous evaluation tests are available for consumer panels to evaluate (IFT, 1981). One test is the Single Sample Test, which presents a single sample early in an experimental project to determine initial acceptability, giving aid to decisions for future development of the product (IFT, 1981; Meilgaard et al., 1987). Another type of test is the Paired Comparison Test, in which the panelist must identify which of the two samples has the greater level of the designated characteristic. With this test, the panelist has a

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50% chance of being right, since they are presented with only two samples, the control and variable. The Duo-Trio test is another difference test, in which two samples are judged against one control, determining which of the two samples is different from the control (IFT, 1981; Meilgaard et al., 1987). Again with this test, the panelist only has two choices with a 50% chance of being right. A variation of the Duo-Trio Test is the Triangle Test. With the Triangle Test all three samples are presented simultaneously, and the panelist must identify which is the odd sample, giving them a 33% chance of being right (IFT, 1981; Meilgaard et al., 1987).

Preference or acceptance testing is another test used for sensory evaluation and is valuable in developing new food products or evaluating the quality of the new items (Moskowitz, 1988). This type of testing is also referred to as consumer testing, since a consumer will measure the amount of "like" or "dislike" for a product. The information obtained from the consumer testing is used to gain understanding of what food consumers prefer. Often a Rank test is used to rank products from the best liked or the most preferred to the least liked or the least preferred (IFT, 1981). A face or "smiley" scale is another example of a scale that has been used to measure the acceptance of products (Stone and Sidel, 1993; IFT, 1981). The most common preference or acceptance test used is the nine-point Hedonic Scale. This method is probably the most useful for consumer testing since it is easily understood by consumers and requires minimal instruction. In addition to the nine-point scale, other scoring methods have been used with the Hedonic scale and continues to be used to measure product liking and preference (Stone and Sidel, 1993). Considering the usefulness and effectiveness of the Hedonic scale in determining consumer acceptance, it was decided this test for sensory evaluation

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would be the most beneficial when conducting a taste panel on sensory characteristics typical of beef jerky products.

RED MEAT

When the meat is heated, there are changes in the appearance, flavor, texture and nutritive value, as well as changes in the existing microbial populations (Hamm, 1977; Foegeding, 1988). The most drastic changes in meat during heating are caused by muscle proteins (shrinkage, hardening of tissue, release of juice and discoloration) (Bouton and Harris, 1972).

When heated, the pigments of red meat are changed (Girard, 1992; McWilliams, 1993). There are two essential pigments associated with meat: hemoglobin and myoglobin (Girard, 1992). Hemoglobin is a very large, iron-containing compound consisting of four heme-polypeptide polymers linked together, and contributes to the color of meat. Heme contains four adjoining pyrrole rings linked to an atom of iron and is capable of complexing with other atoms to form new compounds resulting in alternative colors. Other color changes are the result in the valence of the iron itself (Girard, 1992; McWilliams, 1993).

Myoglobin is a purplish-red pigment consisting of heme, ferrous (Fe^{2+}) iron and a polypeptide polymer (globin) (Girard, 1992). Myoglobin is the predominant pigment in meat and contributes three times as much color as hemoglobin. The purple-red color of meat is the color of fresh meat when it is protected from contact with air. However, when meat is in the presence of oxygen, myoglobin adds two atoms of oxygen to form oxymyoglobin.

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Oxymyoglobin is an intense cherry red and is normally seen on the cut surfaces of meats (Girard, 1992; McWilliams, 1993). Availability of an abundance of oxygen favors the formation of oxymyoglobin and ensures that meat will have a pleasingly bright red color (Girard, 1992; McWilliams, 1993). In addition, plastic wrap that is permeable to oxygen helps ensure that red color appeals to consumers. Additives (citric acid and sodium erythorbate) can be added to maintain the oxymyoglobin when packaged in modified atmosphere packaging. However, if the oxygen supply available to myoglobin is limited or meat is exposed to fluorescent or incandescent light, a brownish-red pigment, metmyoglobin, forms (Girard, 1992; McWilliams, 1993). This less desirable color results from the oxidation of the iron atom to the ferric (3+) state by complexing with a molecule of water. However, metmyoglobin can be reduced back to myoglobin (Girard, 1992; McWilliams, 1993).

During heating, the pH of the meat generally increases (Hamm, 1977; Girard, 1992). A rise in pH is particularly noticeable when the temperatures are increased up to 70°C (158°F), but the duration of heating is of minor importance. In a living mammal, the animal tissue has a pH of 7.3, but upon slaughter, the normal pH of meat is 5.6 (McWilliams, 1993). However, when an animal experiences more fatigue at the time of slaughter, there is less glycogen available after slaughter, so there is less of a pH drop. In addition, the meat has high water binding and is flavorless. This meat is called dark, firm, and dry (DFD) and the pH of the meat remains high, 6.8-7.0. The resulting meat lacks flavor, and is tender, but mealy (McWilliams, 1993). Meat that is DFD has a decreased shelf life due to an increase in susceptibility to bacteria and it is not desirable meat, since DFD meat does not last, look, or taste good.

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Genetics, slaughter stress, and cool temperatures at slaughter can also affect meat. These effects result in meat that is pale, soft, exudative (PSE). When animals are stressed at the time of slaughter, the meat can have a higher temperature and lower pH. This problem is due to the denatured proteins in the meat, which change the ability to reflect light and bind water. Thus, there is a lot of free water available (exudative) and the meat is very pale because of the muscle fibers separating. In addition, lactic acid can remain in the muscle and decrease the pH, while at a higher body temperature. This meat also has a decreased shelf life and is susceptible to microbial growth.

It is reported that cooking losses are known to be much less in muscles of high pH meats (Bouton et al., 1976; Bouton and Harris, 1972) and cooking losses in cold shortened samples are significantly higher than in stretched muscle samples. Since stretched muscle samples have a greater water holding capacity, there is a decrease in the muscle fiber length, with less in the cold shortened samples (Bouton et al., 1976; Bouton and Harris, 1972). In addition, heating can affect the water-binding capacity of meat. When meats are heated, the fat in the meat is softened and melted and the proteins are denatured. The palatability of the meat also depends on the conditions used in heating. Heating meat results in water being lost or bound water in the meat being converted to free water (Bouton et al., 1976; Bouton and Harris, 1972). However, Davey and Gilbert (1974) stated that temperature dependent cooking can create toughness in beef and cooking in the strictest sense is heating meat to a sufficiently high temperature that will denature the proteins. Since the connective tissue of muscle is mainly collagen, upon homogenization the connective tissue is reduced to the form of shreds or clumps. In cooking beef, however, toughening phases can occur (Davey and Gilbert, 1974).

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Changes in texture and juiciness are due to the thermally driven denaturation (unfolding) of proteins. Denaturation is generally represented as a transition from a native (folded) to a denatured (unfolded disordered state). The thermally induced changes in muscle proteins include protein unfolding and protein-protein interactions that affect the textural properties of processed meats (Foegeding, 1988). Protein denaturation is when the spatial arrangement of the polypeptide chains within the molecule are changed from that typical of the native protein to a more disordered arrangement (Davey and Gilbert, 1974). In addition, structural stability of proteins may depend on pH and ionic conditions, so any variations in meat pH's and ion content could determine which unfolding path the protein may undergo (Davey and Gilbert, 1974). The irreversibility of thermal transitions can be due to the association of denatured molecules or the kinetic constraints on refolding and modification of amino acids and these transitions are major causes of protein irreversibility (Foegeding, 1988).

As meat prices continue to increase, consumers often take advantage of meat when it is less expensive and purchase large quantities. Due to the short shelf life before meat goes rancid, many individuals will preserve the meat by making beef jerky. However, consumers need to be aware of potential hazards associated with home dehydrating. This paper continues by focusing on the evaluation of processes that will destroy *E. coli* O157:H7 in whole muscle home dried beef jerky.

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CHAPTER III

EFFECT OF MARINADE AND DRYING TEMPERATURE ON SURVIVAL OF *ESCHERICHIA COLI* O157:H7 IN INOCULATED HOME DRIED BEEF SLICES

ABSTRACT

A 5-log reduction of *Escherichia coli* O157:H7 is recommended to be achieved during the drying process of beef jerky. There are discrepancies in the literature as to whether current practices for home dried jerky preparation can achieve such destruction. The objective of this study was to determine survival of inoculated *E. coli* O157:H7 populations during preparation of beef jerky with and without marinade and followed by drying at two temperatures (62.5 or 68.3°C). Lean beef slices (8.7 x 4.0 x 0.6 cm), from beef inside rounds with the subcutaneous fat removed, were inoculated with a four-strain composite of *E. coli* O157:H7 (5.7 to 7.5 log CFU/cm^2), stored (4°C, 24 h) and prepared according to one of three treatments: (1) no marinade, dried at 62.5°C; (2) marinade, dried at 62.5°C; and (3) marinade, dried at 68.3°C. The marinade consisted of 60 ml Kikkoman soy sauce, 15 ml Heinz Worcestershire sauce, 0.6 g Schilling black pepper, 1.25 g Kroger garlic powder, 1.5 g Kroger onion powder, and 4.35 g Spice Islands hickory smoke-flavored salt per kg of meat slices marinated (65 slices of 15 g each). Each inoculated meat slice received 1 ml of marinade, with 0.5 ml distributed evenly over each top and bottom surface. Marinade was spread with a sterile bent glass rod to ensure

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adequate coverage over the meat pieces. Marinated pieces were placed in aluminum pans, covered with heavy-duty aluminum foil, and stored (4°C, 24 h) before drying in two home-type food dehydrators for up to 10 h at 62.5 or 68.3°C.

Samples were analyzed (bacterial enumeration with selective and nonselective agar media, pH, and water activity-a_w) following inoculation, marinating (treatments 2 and 3), and at 4, 6, 8 and 10 h of drying. The study was replicated twice with three samples analyzed per replication. In addition, dried (10 h) jerky was stored (21°C) in 1-qt Ziploc freezer bags and analyzed at 30, 60 and 90 d.

Following marinating (treatments 2 and 3), bacterial population changes in the beef slices fluctuated slightly between agar media (by -0.3 to + 0.6 log CFU/cm^2). Significant ($P < 0.05$) bacterial destruction (2.1-4.1 log CFU/cm^2) was achieved during the first 4 h of drying, but little destruction occurred during the remaining 6 h of drying. After 10 h of drying, total bacterial reductions were similar between agar media and none of the three treatments achieved a 5-log reduction in bacteria (2.2-4.6 log CFU/cm^2). Following 10 h of drying, treatment 1 (no marinade, dried at 62.5°C) and 2 (marinade, dried at 62.5°C) resulted in log reductions of 3.2-3.4 and 2.2 logs CFU/cm^2, respectively. Treatment 3 (marinade, dried at 68.3°C) achieved log reductions of 3.0-4.6 log CFU/cm^2.

When stored jerky was analyzed, after 30 d of storage, there was a significant ($P < 0.05$) reduction in bacterial counts, which became very low or undetectable by direct plating (10 CFU/cm^2) compared to initial (after 10 h of drying) counts of 2.9-3.7 log CFU/cm^2. Following 60 and 90 d of storage, bacterial counts were the same as counts recovered after 30 d, which were very low (< 1.0 to 1.4 log CFU/cm^2). However, mold growth was evident on treatment 1 after 30 d of storage and continued to be evident up to

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90 d. Additional studies are needed to verify whether surviving bacterial cells became undetectable during storage after drying.

In order for a product such as beef jerky to be microbiologically stable during storage, the a_w should be ≤ 0.68. Water activity values following inoculation and marinating (treatments 2 and 3) ranged from 0.93 to 0.94, but decreased to 0.86 to 0.92 after 4 h of drying; with lower values being associated with treatment 3. After 10 h of drying, treatments 2 and 3 reached a_w levels recommended for microbiologically stable products, 0.65 and 0.64, respectively. In contrast, after 10 h of drying, treatment 1 reached an average a_w of 0.83. The higher amount of free water remaining in the unmarinated jerky was attributed to the lack of spices or seasonings available to act as water binding agents. The higher a_w explains the high amount of mold growth seen in treatment 1 (no marinade, dried at 62.5°C) during the storage of the jerky pieces. When jerky slices were analyzed following storage, the a_w for treatment 1 did not decrease with storage of 30-90 d. In contrast, the a_w for treatment 2 decreased slightly with storage (0.65 at 0 d to 0.59 at 90 d) and decreased even more for treatment 3 (from 0.64 at 0 d to 0.44 at 90 d).

Throughout the drying process, pH values ranged from 5.75 to 5.83 for treatment 1 (no marinade, dried at 62.5°C), 5.09 to 5.43 for treatment 2 (marinade, dried at 62.5°C) and 5.75 to 5.90 for treatment 3 (marinade, dried at 68.3°C). The pH differences seen among the treatments could be due to variations among the raw beef used in the study and to some extent to the use of jerky marinade.

Even though significant ($P < 0.05$) bacterial destruction occurred during the first 4 h of drying (2.1-4.1 log CFU/cm^2), additional drying time was necessary to achieve

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acceptable a_w values, enhancing the shelf-life stability of the product. The culture media used for recovery of bacteria resulted in some differences in counts determined, but bacterial enumeration often paralleled each other throughout the drying process. Based on the conditions and results of this study, it was concluded that the use of marinade with drying at 62.5 or 68.3°C for 10 h were not adequate for inactivation of 5-logs of inoculated *E. coli* O157:H7 in home dried beef jerky. However, when jerky was stored for at least 30 d at 21°C following 10 h of drying, a 5-log reduction in *E. coli* O157:H7 was achieved. This observation needs to be confirmed with additional studies. Since a 5-log reduction in *E. coli* O157:H7 was not achieved at either temperature, 62.5 or 68.3°C following 10 h of drying, methods must be developed to achieve adequate destruction during drying.

INTRODUCTION

There are a number of opportunities for cross-contamination throughout the processing, handling, and marketing of foods (Abdul-Raouf et al., 1993b). The concern increases when foods are eaten raw or after minimal or no heating. When consumers handle foods improperly, the potential for further contamination increases, and, if the potential for cross-contamination exists, precautions must be taken to ensure that the risk of foodborne illness is minimized. Public health problems associated with the foodborne pathogen enterohemorrhagic *Escherichia coli* O157:H7 are being recognized throughout the world (Wachsmuth, 1997). In the past decade, news releases of outbreaks of foodborne illnesses caused by *E. coli* O157:H7 have become common and seem to be increasing each year (CDC, 1999). The first documented occurrence of human illness

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associated with *E. coli* O157:H7 occurred in 1982, when two outbreaks of *E. coli* O157:H7, due to undercooked hamburger meat, were reported (Riley et al., 1983).

Escherichia coli O157:H7, in particular, has a low infectious dose rate and has been found to survive at 5°C (Clavero and Beuchat, 1996). Originally, outbreaks of *E. coli* O157:H7 seemed limited to undercooked ground meat products. However, as investigators continue to study *E. coli* O157:H7, other food sources have been found to be carriers of the pathogen or involved in foodborne illness. These foods included unpasteurized apple juice/cider (Besser et al., 1993), dry cured salami (CDC, 1995b), deer jerky (Keene et al., 1997), produce from manure-fertilized soil, yogurt (Morgan et al., 1993; Buchanan and Doyle, 1997), and radish and alfalfa sprouts (Taormina et al., 1999; Hara-Kudo et al., 1997).

In the past, the recommendations for preparing and heating jerky have been quite general. Jerky is a nutritious, convenient meat product consumers can make at home. In recent years, illnesses due to *Salmonella* and *E. coli* (CDC, 1995a, b, c; Keene et al., 1997) from homemade jerky have raised questions about the safety of products made using traditional drying methods (Reynolds and Williams, 1993). Because of these concerns, the United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) has mandated that manufacturers of dry and semi-dry fermented meats demonstrate a 5-log reduction of *E. coli* O157:H7 during processing (Billy, 1997; Reed, 1995). Existing food processing and preparation procedures are being evaluated based on these requirements. According to the USDA-FSIS, current requirements must include a step that will achieve a 5-log reduction in *E. coli* O157:H7 populations in order for dried and semi-dry fermented meats to be considered safe.

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Dried meats, such as jerky, may cause a greater threat to consumers as sources of contamination than fermented foods, because jerky can be easily prepared at home under limited precision and with less controlled equipment (Faith et al., 1998a). Studies on the survival of *E. coli* O157:H7 in dried jerky products (Harrison and Harrison, 1996; Harrison et al., 1998; Faith et al., 1998a; Keene et al., 1997) have produced variable results, depending in part on the type (beef vs. venison) and form (whole muscle vs. ground) of meat used in jerky formulation, as well as the dehydrator (air) temperature (51.7 to 68°C), the drying time (6 to 20 h) and the culture agar (tryptic soy agar, sorbitol MacConkey, sorbitol MacConkey supplemented with 4-methyl-umberlliferyl-D-glucoronide, or modified eosin methylene blue) used in the studies.

Harrison and Harrison (1996) determined the survival of *E. coli* O157:H7 in products made with a commonly used jerky preparation method (Reynolds and Williams, 1993) and the USDA suggested method of pre-heating meat to 71.1°C (160°F) before drying jerky (USDA -FSIS, 1998). Whole muscle beef was inoculated with *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Typhimurium and dried at 60°C (140°F) for 10 h in home-type dehydrators. On one-third of the top surface of each strip, 0.1 ml of inoculum (10^8 CFU/ml) was distributed before drying. No initial inoculum was given nor were the units of material (CFU/g, CFU/cm^2 or CFU/ml) specified. Inoculated samples were either marinated at 4°C (39°F) for 1 h or placed in a shallow pan, covered with jerky marinade and pre-heated on a hot plate to 71.1°C (time unspecified). After 10 h of drying, populations of *E. coli* O157:H7 on the inoculated, unheated meat decreased by 5.5 to 6.0 log units. Populations on the inoculated, pre-heated meat decreased by 5 log units during pre-heating prior to drying; after 10 h of drying, populations decreased to

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undetectable levels (detection limit not stated) (Harrison and Harrison, 1996). The authors concluded that using a marinade in jerky preparation and drying at 60°C for 10 h were sufficient to reduce levels of *E. coli* O157:H7 by at least 5 log units on unheated and pre-heated meat (Harrison and Harrison, 1996).

Keene et al. (1997) assessed the safety of meat dehydration methods using home-type dehydrators when marinated venison meat was inoculated with *E. coli* O157:H7. Results indicated that *E. coli* O157:H7 populations declined slowly in the marinated product dried at 51.7°C (125°F) for 10 h, while populations on the marinated product dried at 62.8°C (145°F) declined over the first 6-8 h to levels undetectable by direct plating (<10 CFU/g). However, initial inocula levels, 10^3 CFU/g and 10^5 CFU/g, differed for the two drying temperatures (51.7 and 62.8°C, respectively). Also, upon enrichment, *E. coli* O157:H7 could still be cultured from samples dried under both conditions. Based on these results, Keene et al. (1997) concluded that low-temperature ($\leq$ 62.8°C) dehydration was an unreliable means of eradicating *E. coli* O157:H7 from contaminated meat.

Harrison et al. (1998) studied ground beef jerky inoculated with *E. coli* O157:H7 and made with either a spice and cure mix or a spice mix only. Jerky was dried at 60°C (140°F) in a home-type dehydrator from a raw (unheated) stage or a pre-heated to 71.1°C (160°F) stage, for 8 or 6 h, respectively. The authors reported a 5.2 log CFU/g reduction in inoculated counts for the unheated and pre-heated jerky containing the spice and cure mix (Harrison et al., 1998). However, when the spice mix only was used, reductions were 4.3 and 4.8 log CFU/g, for the unheated and pre-heated jerky, respectively. These findings suggested that if consumers make jerky with ground beef and fail to add a cure

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mix, the jerky could pose an increased risk of foodborne illness if meat was contaminated with *E. coli* O157:H7 (Harrison et al., 1998).

Faith et al. (1998a) studied the survival of inoculated *E. coli* O157:H7 on beef with various levels of fat (5 and 20%) that was ground and formed into jerky and prepared with a spice mix. Jerky samples were dried for various times intervals (2 to 20 h) at four dehydrator (air) temperatures (52°C/125°F, 57°C/135°F, 63°C/145°F or 68°C/155°F). The study established that a 5-log reduction could eventually be obtained at any of the temperatures studied; however, only in the 20% fat products dried at 63 and 68°C was a 5-log reduction obtained by the time the product was judged visually dry (8 and 6 h, respectively). In the lean ground beef (5%) products, a 4.8 log CFU/g reduction was achieved by the time the product was visually judged dry (4 h) at 68°C. At all other fat level/temperature combinations, log reductions ranged from 0.75 to 3.0 log CFU/g for products considered adequately dry by visual inspection (Faith et al., 1998a). The researchers concluded that jerky should be prepared from leaner cuts of meat (≤ 5%) and dried at temperatures ≥ 63°C for at least 8 h in order to achieve an appreciable reduction in *E. coli* O157:H7 (Faith et al., 1998a).

With conflicting results reported concerning dried jerky, preparation methods (Reynolds and Williams, 1993) once considered adequate to prepare safe products are now being reconsidered and re-evaluated. The USDA-FSIS currently recommends the minimum of a 5-log bacterial reduction in dried and semi-dried fermented meats (Billy, 1997; Reed, 1995). The present study evaluated survival of inoculated *E. coli* O157:H7 cells during drying of whole muscle beef jerky prepared without marinade and dried at 62.5°C (145°F), or prepared with marinade and dried at 62.5°C (145°F) or at 68.3°C

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(155°F). Drying lasted for 10 h and survival of bacterial cells was determined by plating on two culture media.

MATERIALS AND METHODS

Study design

Treatments of home-dried jerky evaluated included: (1) no marinade, dried at 62.5°C; (2) marinade, dried at 62.5°C; and (3) marinade, dried at 68.3°C. The marinade consisted of 60 ml Kikkoman soy sauce, 15 ml Heinz Worcestershire sauce, 0.6 g Schilling black pepper, 1.25 g Kroger garlic powder, 1.5 g Kroger onion powder, and 4.35 g Spice Islands hickory smoke-flavored salt per kg of meat slices marinated (65 slices of 15 g each) (Reynolds and Williams, 1993) (pH = 4.3). The study was replicated twice with three samples analyzed per replication.

Preparation of bacterial inoculum

Four strains of *E. coli* O157:H7 (E0139, a venison jerky isolate provided by Dr. Michael Doyle, Center for Food Safety and Quality Enhancement, University of Georgia, Griffin, Georgia; ATCC 43895, 43890 and 43894 obtained from the American Type Culture Collection, Manassas, VA) were used to prepare a composite culture for inoculation of the meat slices before processing to make jerky. Cultures were maintained frozen at -18°C in tryptic soy broth (TSB) (Difco Laboratories, Detroit, MI) with 20% (v) added glycerol (Sigma Chemical Co., St. Louis, MO). Each strain was propagated individually on tryptic soy agar (TSA; pH 7.0) (Difco Laboratories, Detroit, MI) slants at 35°C before use in experiments. The experimental inocula were prepared by transferring a loopful of each culture into 9-ml tubes of tryptic soy broth (TSB) (pH 7.0) (Difco Laboratories) and incubating at 35°C for 18-24 h on the day prior to each experiment.

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The cultures used in the experiments averaged 5.0 x 10^8 CFU/ml. Immediately before use, the individual culture inocula were combined for inoculation of the meat to prepare jerky.

Preparation, inoculation and marinating of beef pieces

Vacuum packaged beef inside rounds (approximately 30 kg) were purchased frozen from local supermarket warehouses and stored at -18°C (-4°F) until use (i.e., within one week). The meat was removed from the freezer and cut, while still frozen, into slices (8.7 x 4.0 x 0.6 cm; 3.4 x 1.6 x 0.3 inches) with a band saw at the Colorado State University Meat Science Laboratory (Fort Collins, CO). Slices were randomly placed in 1-kg portions in plastic bags (20.3 x 25.4 cm; 0.75 mil nylon, 2.25 mil polyethylene laminate; oxygen transmission rate 3.5 cc/2.54 sq. m/24h; Kapak Heat Sealable, Fisher Scientific, Springfield, NJ). The bags were evacuated (29 mm/Hg), heat sealed (Multivac, Sepp Haggenmüller KG, Allgau, Germany), and then frozen (-18°C) to minimize potential changes until use (i.e., within two weeks). For each treatment, the top surface area (8.7 x 4.0 cm) of each meat slice was inoculated with 0.5 ml of the culture composite prepared as described above. The inoculum was spread with a sterile bent glass rod to ensure adequate coverage of the surface. Meat pieces were then turned using sterile forceps and the bottom surface area was inoculated with 0.5 ml of the composite culture, as described above. Inoculated slices were refrigerated (4°C, 24 h) in aluminum foil pans, covered with heavy-duty aluminum foil, to enhance adherence of pathogen cells to meat pieces. Inoculum levels reached were 5.7-7.5 log CFU/cm^2.

Following inoculation, meat slices were marinated (treatments 2 and 3). Each inoculated meat slice received 1 ml of the marinade, with each 0.5-ml portion distributed

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evenly over each top and bottom surface. Marinade was spread with a sterile bent glass rod to ensure adequate coverage over the meat pieces. Marinated pieces were placed in aluminum pans, covered with heavy-duty aluminum foil, and stored (4°C, 24 h) before drying.

Drying process and sample preparation

Two home food dehydrators (Gardenmaster® Dehydrator FD-1000, Nesco®/American Harvest®, Chaska, MN), with three dehydrator trays each, were used for the drying process. Dehydrators were preheated to the appropriate drying (air) temperature (62.5 or 68.3°C) for at least 15 min prior to placement of inoculated meat pieces on the drying trays. Meat slices were laid flat on dehydrator trays, without touching other slices. Inoculated meat slices (1 kg) were placed in each dehydrator randomly among trays; additional (0.5-kg) uninoculated, non-marinated meat slices were used to fill the dehydrator drying trays to capacity and placed in designated locations. Treatments were prepared on separate days and not combined in the dehydrators.

Circulating air within the dehydrators was monitored during the drying process with thermocouples (Type K Beaded Probes, MM Micromeasurements, Raleigh, NC) placed through the open circular top of each dehydrator with the sensor tip recording circulating air throughout the dehydrator. Temperatures were recorded on a Speedomax strip chart recorder (Leeds and Northup, Northwales, PA). In additional drying studies, the temperatures of meat surfaces were monitored using an infrared heat gun (Oakton Infrared Temperature Tester, Gainesville, FL). Every 30 min, throughout drying, dehydrators were cracked open, the heat gun was directed at the surface of the meat and

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temperatures were recorded. Three sets of two meat slices per treatment and per dehydrator were removed at each sampling time and placed in bags for analysis.

Microbiological analyses

Samples taken following inoculation for 24 h at 4°C and following marinating for 24 h at 4°C (treatments 2 and 3) were analyzed immediately. Samples taken at 4, 6, 8, and 10 h of drying were analyzed after storage at 21°C for 24 h. In addition, samples (25-30 pieces/bag) dried for 10 h were stored in 1-qt Ziploc freezer bags, closed and stored in the dark, at 21°C for 30, 60 and 90 d. Populations of bacteria, including counts of *E. coli* O157:H7, were determined by plating on tryptic soy agar (TSA) and sorbitol MacConkey (SMAC) agar (Difco Laboratories, Detroit, MI). Bags containing two slices of product per sample were weighed. Appropriate amounts of Butterfield's phosphate buffer were added to obtain a 1:10 sample dilution and bags were pummeled (IUL Instruments Masticator, Barcelona, Spain) for 120 s. Serial decimal dilutions were made with Butterfield's phosphate buffer and sample portions of 0.1 ml were spread plated in duplicate on two culture media. Following incubation at 35°C for 20 to 24 h, colonies were counted. The minimum detection limit was 100 CFU/cm^2. For stored samples (30-90 d), the minimum detection limit was 10 CFU/cm^2. All counts were converted to log CFU/cm^2. Typical colonies of *E. coli* (sorbitol negative) on SMAC were round and colorless (McCarthy et al., 1998). Six colonies per treatment per drying time were confirmed as *E. coli* serotype O157 using the *E. coli* O157 latex agglutination assay (OXOID Diagnostic Reagents, Hampshire, England).

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Physical analyses

Inoculated meat pieces (10-15 g) were analyzed for a_w and pH at each sampling time. Water activity was determined with a Rotronic Hygroskop DT water activity meter (Model D2100, Rotronic, Huntington, NY) calibrated with standard saturated salt solutions (1.000, distilled water; 0.973, K_2SO_4; 0.843, KCl; 0.753, NaCl; 0.743, $NaNO_3$ ± 0.01) (saturation points are 10.8% K_2SO_4; 26.5%, KCl; 26.5%, NaCl; 47.9%, $NaNO_3$). Inoculated meat slices were cut (0.5-1.0 g), placed in the a_w (PS14) cup and set in the a_w meter until the a_w reached a stable reading (via signal lights, 25°C/77°F). Each a_w reading was determined as a single value.

A Hanna Instruments, HI8424, (Woonsocket, RI) pH meter was used to determine the pH of inoculated meat pieces. Meat pieces were weighed in stomacher bags. Appropriate amounts of distilled water were added to obtain a dilution of 1:10 and bags were pummeled (IUL Instruments Masticator, Barcelona, Spain) for 120 s. Bags were allowed to sit for at least 15 min before each pH reading was determined in triplicate and averaged.

Statistical analyses

The experimental model of the study was a factorial split-plot design with 2 (number of dehydrators) x 3 (number of marinating/drying methods being tested) x 2 (number of culture media used for enumeration) x 6 (number of time intervals when meat slices were analyzed) factors and levels. Each mean value reported represented six determinations (3 values from 2 simultaneous trials/dehydrators). Bacterial counts of duplicate plates per dilution were averaged and CFU/cm^2 counts were converted into log values. Populations of surviving bacteria, including inoculated *E. coli* O157:H7 (log

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CFU/cm^2) were plotted versus processing time for each treatment. The decimal reduction times (D-values, 0 to 10 h) were calculated from survivor curves as outlined by Lund (1975) by linear regression to determine the best-fit line. Six mean values (3 values from 2 simultaneous trials/dehydrators) were used for calculating D-values and regression values.

Data were subjected to the Statistical Analysis System (SAS Institute version 6.1, Cary, NC) for analysis of variance of main (fixed) effects (dehydrator, treatment method, culture media and drying time), as well as significant interactions between the main (fixed) effects. When F values were significant ($P < 0.05$), least significant differences (LSD) in survival of bacterial population counts were determined using the mixed procedure (PROC MIXED) of SAS. All statistically significant effects were reported at the $P < 0.05$ level.

RESULTS AND DISCUSSION

Dehydrator and product temperatures

The average dehydrator (air) temperatures decreased by 15 to 20°C following placement of meat strips on dehydrator trays (Fig 3.1), then increased steadily until the desired temperature of 62.5 or 68.3°C (± 2.5°C) was reached, approximately 3 to 4 h after placement of meat strips in the dehydrator. Dehydrators maintained the desired (air) temperature of 62.5 or 68.3°C (± 2.5°C) throughout the length of the drying process. The surface temperatures of the meat strips (Appendix C, Table C. 3.1) equaled the desired air temperatures of 62.5 or 68.3°C (± 2.5°C) after 4.5 to 5 h in the dehydrator (Fig. 3.2) and stayed within these ranges for the reminder of the drying process.

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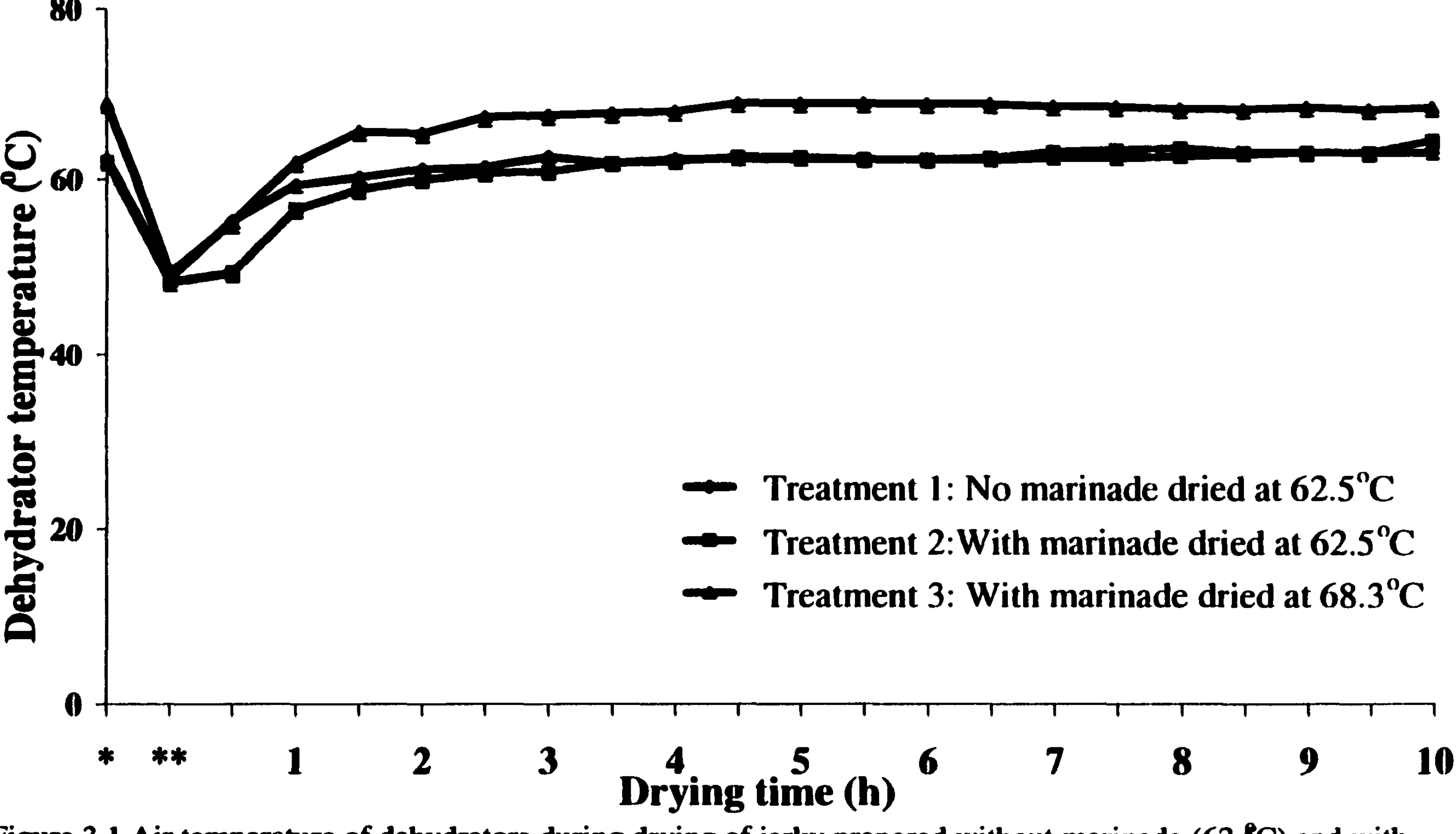

Figure 3.1 Air temperature of dehydrators during drying of jerky prepared without marinade (62.5°C) and with marinade (62.5 and 68.3°C); each point is the average of two determinations; error bars indicate standard deviation; * indicates 15 min dehydrator warm-up, ** indicates placement of jerky in dehydrator.

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Surface temperature of beef slices (°C)

80
60
40
20
0

Treatment 1: No marinade dried at 62.5°C
Treatment 2: With marinade dried at 62.5°C
Treatment 3: With marinade dried at 68.3°C

* ** 1 2 3 4 5 6 7 8 9 10

Drying time (h)

Figure 3.2 Surface temperature of beef slices during drying of jerky prepared without marinade (62.5°C) and with marinade (62.5 and 68.3°C); each point is the average of three determinations; error bars indicate standard deviation; * indicates 15 min dehydrator warm-up, ** indicates placement of jerky in dehydrator.

Temperature differences between dehydrators (Appendix C, Table C. 3.2) were greatest (5 to 15°C) during the first hour of drying, when the air temperature dropped with the addition of the beef jerky slices and then rose steadily until reaching the desired temperature (after 3-4 h of drying). Temperature differences were small (< 4°C) throughout the remainder of the drying process, regardless of treatment. Even though temperature differences between dehydrators were small (< 4°C), consumers and researchers should be aware that potential differences might exist between dehydrators, even between the same models of dehydrators (Faith et al., 1998a). In addition, dehydrators should be equipped with a thermometer to measure their internal temperature, ensuring accurate temperature measurements are maintained throughout the drying process (Faith et al., 1998a).

Effect of marinating and drying treatments

With the exception of dehydrator, the main factors (drying time, treatment method and culture media) had significant ($P < 0.05$) effects on bacterial populations recovered (Table 3.1). Significant interactions included treatment x drying time, treatment x culture media, drying time x culture media, treatment x drying time x culture media and treatment x dehydrator x drying time.

Bacterial reduction after marinating (treatments 2 and 3)

Following marinating at 4°C for 24 h, population counts did not change significantly ($P > 0.05$) with the exception of treatment 3, which had 0.6 log CFU/cm^2 higher counts on SMAC following marinating (Table 3.2). No other differences existed for treatment 3 or for treatment 2. However, it should be noted that treatment 3 had higher initial population counts following inoculation than did treatment 2.

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Table 3.1 Analysis of variance of microbial counts (determined with tryptic soy agar and sorbitol MacConkey agar) on beef jerky inoculated with *E. coli* O157:H7 (log CFU/cm^2) and processed with or without marinade at two different drying temperatures for different times in each of two dehydrators.

Variable	Tests of Fixed Effects			
	Numerator Degrees of Freedom	Denominator Degrees of Freedom	F-value	Pr>F
Treatment [a]	2	72	14.58	0.0001**
Drying Time [b]	5	72	335.43	0.0001**
Dehydrator [c]	1	72	2.05	0.1564
Culture Media [d]	1	72	21.88	0.0001**
Treatment * Drying Time	10	72	13.20	0.0001**
Drying Time* Dehydrator	5	72	1.54	0.1880
Treatment * Culture Media	2	72	3.31	0.0423**
Drying Time * Culture Media	5	72	3.56	0.0062**
Treatment *Dehydrator	2	72	0.10	0.9040
Dehydrator * Culture Media	1	72	4.25	0.0428**
Treatment * Drying Time * Culture Media	10	72	3.93	0.0003**
Treatment * Dehydrator * Drying Time	10	72	2.50	0.0124**
Treatment *Dehydrator * Culture Media	2	72	0.26	0.7680
Drying Time * Dehydrator * Culture Media	5	72	0.18	0.9687
Treatment * Drying Time * Dehydrator * Culture Media	10	72	0.61	0.8018

** $P < 0.05$

[a] Treatment 1: Without marinade, dried at 62.5°C; Treatment 2: With marinade, dried at 62.5°C; Treatment 3: With marinade, dried at 68.3°C

[b] Six time intervals during the drying process (after inoculation (4°C, 24 h), after marinating (4°C, 24 h) (treatments 2 and 3) and at 4, 6, 8, and 10 h of drying).

[c] Two home food dehydrators (Gardenmaster® Dehydrator FD-1000, Nesco®/American Harvest®, Chaska, MN)

[d] Tryptic soy agar (TSA) and sorbitol MacConkey agar (SMAC).

Table 3.2 Effect of beef jerky preparation procedure (with or without marinade; dried at 62.5 or 68.3°C), drying time (0-10 h) and culture media for bacterial enumeration (tryptic soy agar--TSA and sorbitol MacConkey agar--SMAC) on survival of inoculated *E. coli* O157:H7 (log CFU/cm^2) and natural bacterial flora.

	Beef Jerky Preparation Procedure and Agar Media[a]					
	1[b]		2[c]		3[d]	
Processing steps and drying times (h)	SMAC	TSA	SMAC	TSA	SMAC	TSA
Following inoculation	6.4^{AaX}	6.6^{AaY}	5.7^{AaY}	5.9^{AaZ}	6.3^{BbX}	7.5^{AaX}
(4°C, 24 h) *(a)*	(0.2)	(0.1)	(0.2)	(0.2)	(1.0)	(0.4)
Following marinating	*N/A*	*N/A*	5.4^{AaY}	5.5^{AaY}	6.9^{BaX}	7.5^{AaX}
(4°C, 24 h) *(b)*			(0.6)	(0.7)	(0.6)	(0.2)
Log Change (a-b)	***N/A***	***N/A***	***-0.3***	***-0.4***	***+0.6***	***0.0***
4	3.9^{BbX}	4.4^{AbX}	3.6^{AbX}	3.6^{AbcY}	2.8^{BdY}	3.4^{AbY}
	(0.3)	(0.4)	(0.5)	(0.4)	(0.4)	(0.3)
Log Change (b-4)	***N/A***	***N/A***	***-1.8***	***-1.7***	***-4.1***	***-4.1***
Log Change (a-4)	***-2.5***	***-2.3***	***-2.1***	***-2.3***	***-3.5***	***-4.1***
6	3.2^{BcX}	4.6^{AbX}	3.3^{AbX}	3.2^{AcY}	3.5^{AcX}	3.5^{AbY}
	(0.3)	(1.2)	(0.4)	(0.4)	(0.3)	(0.6)
8	3.4^{AcX}	3.3^{AcX}	3.7^{AbX}	3.6^{AbcX}	3.6^{AcX}	3.5^{AbX}
	(0.3)	(0.2)	(0.2)	(0.5)	(0.2)	(0.6)
10	3.2^{AcX}	3.3^{AcXY}	3.5^{AbX}	3.7^{AbX}	3.3^{AcX}	2.9^{AcY}
	(0.4)	(0.3)	(0.1)	(0.7)	(0.3)	(0.7)
Log Change(4-10)	***-0.7***	***-1.1***	***-0.1***	***+0.1***	***+0.5***	***-0.5***
Log Change (a-10)	***-3.2***	***-3.4***	***-2.2***	***-2.2***	***-3.0***	***-4.6***

[A-B] Means with different superscripts within a treatment in a row are significantly ($P < 0.05$) different.
[a-d] Means with different superscripts within each column are significantly ($P < 0.05$) different.
[X-Z] Means with different superscripts within each agar medium in a row are significantly ($P < 0.05$) different.
[a] Means represent three values from two dehydrators (n = 6) (standard deviation) of log colony forming units (CFU) cm^2; lowest detection limit by plating, 2 log CFU/cm^2
[b] No marinade, dried at 62.5°C
[c] With marinade, dried at 62.5°C
[d] With marinade, dried at 68.3°C

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Bacterial reduction after 4 h of drying

After 4 h of drying, treatment 1 (no marinade, dried at 62.5°C) achieved significant ($P < 0.05$) reductions of 2.5 and 2.3 log CFU/cm^2 with SMAC and TSA, respectively (Table 3.2). Bacterial counts determined with TSA were significantly ($P < 0.05$) higher than those of SMAC, indicating presence of potentially injured cells. However, as described in following paragraphs, when drying continued for 10 h, bacteria counts recovered between media were similar indicating absence of cell injury.

For treatment 2 (marinade, dried at 62.5°C), drying for 4 h after marinating achieved reductions of 1.8 and 1.7 log CFU/cm^2 with SMAC and TSA, respectively, and achieved corresponding total reductions (from initial counts) of 2.1 and 2.3 log CFU/cm^2. There were no differences between culture media for treatment 2.

After 4 h of drying, treatment 3 achieved 4.1 log CFU/cm^2 reductions with both media, with total reductions of 3.5 and 4.1 log CFU/cm^2 with SMAC and TSA, respectively. As with treatment 1, at 4 h bacterial counts determined with TSA were significantly ($P < 0.05$) higher than those determined with SMAC, indicating the presence of injured cells.

This study found that significant ($P < 0.05$) bacterial reductions (2.1-4.1 log CFU/cm^2) were achieved for all treatments after drying for 4 h. Bacterial reductions were similar when jerky was dried at 62.5°C, regardless if a jerky marinade was used (treatment 2) or not (treatment 1). This indicates that the use of a marinade in the jerky preparation did not provide additional destruction of inoculated bacteria. However, since it is unlikely that consumers would dry beef jerky without a jerky marinade, consumers should be cautioned to follow safe food handling when preparing beef jerky and using a

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jerky marinade. For the marinated products, bacterial reductions were greater at 4 h of drying for treatment 3 dried at 68.3°C (3.5-4.1 log CFU/cm^2) than treatment 2 dried at 62.5°C (2.1-2.3 log CFU/cm^2).

Bacterial reduction after 10 h of drying

After 10 h of drying, none of the treatments achieved the recommended 5-log reduction and all treatments had surviving bacteria (2.9-3.7 log CFU/cm^2) (Table 3.2). For all treatments, most bacterial reduction (68-95%) occurred during the first 4 h of drying in all situations. Between 4 and 10 h of drying, treatment 1 achieved reductions of 0.7 and 1.1 log CFU/cm^2 with SMAC and TSA, respectively, and corresponding total reductions of 3.2 and 3.4 log CFU/cm^2. Upon comparison between culture media, TSA counts were significantly ($P < 0.05$) higher than counts on SMAC after 6 h of drying, indicating the possibility of injured cells being present in this treatment. However, as drying progressed, bacterial counts on the two media became similar, indicating the potential destruction of injured cells.

Treatment 2 (marinade, dried at 62.5°C) did not achieve any additional significant destruction or growth between 4 and 10 h of drying (Table 3.2). After 10 h of drying, treatment 2 had achieved total log reductions of 2.2 log CFU/cm^2 with both media. The lack of difference between culture media suggests there was not a problem of injured cells occurring with treatment 2 as seen with treatment 1; however, greater log reductions were achieved with treatment 1. Possibly, the greater reduction in treatment 1 may have been due to the fact that treatment 1 had a significantly ($P < 0.05$) higher initial bacterial level than treatment 2, allowing for a greater reduction of bacteria to be observed.

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Between 4 and 10 h of drying, treatment 3 (marinade, dried at 68.3°C) achieved reduction/change of +0.5 and -0.5 log CFU/cm^2 with SMAC and TSA, respectively; achieving corresponding total reductions of 3.0 and 4.6 log CFU/cm^2. It is noteworthy that population counts increased ($P < 0.05$) between 4 and 6 h when enumerated on SMAC and remained at higher levels throughout the remainder of the drying process.

In this study, differences between plating media existed until competing bacteria and injured cells were inactivated; thus, making bacterial counts not different from each other (Table 3.2). Semanchek and Golden (1998) reported it is important to employ techniques that will optimize the detection and enumeration of both injured and uninjured organisms. Most microorganisms are destroyed, but some sub-lethally injured or stressed cells may survive and are able to recover and grow later (Levy, 1992). Poor recovery of injured cells may lead to an overestimation of lethal effects (Semanchek and Golden, 1998).

Results from this study support earlier findings that drying jerky at temperatures ≤ 62.5°C may not be adequate to minimize the potential risk of foodborne illness (Keene et al., 1997; Faith et al., 1998a). In this study, drying at 62.5°C for 10 h, with and without marinade, achieved 2.2 and 3.2-3.4 log reductions CFU/cm^2, respectively. However, in this study, it was found that drying at 68.3°C (treatment 3) for 10 h also was inadequate for achieving a 5-log reduction as only reductions of 3.0-4.6 log CFU/cm^2 were achieved. This disagrees with published results of other studies concerning jerky preparation (Harrison and Harrison, 1996; Keene et al., 1997; Faith et al., 1998a). Harrison and Harrison (1996) reported that the use of a marinade during jerky preparation and low-temperature dehydration (60°C) was adequate for destroying *E. coli* O157:H7 cells by at

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least 5 log units when present in whole muscle beef jerky. In this present study, while the use of a marinade and drying for 10 h at 62.5 and 68.3°C resulted in significant ($P < 0.05$) reductions of inoculated *E. coli* O157:H7 cells, a 5-log reduction of bacteria was not achieved. Faith et al. (1998a) investigated ground and formed beef jerky and concluded jerky should be prepared from lean cuts of meat (< 5%) and dried at temperatures ≥ 63.0°C for at least 8 h in order to achieve an appreciable reduction in inoculated *E. coli* O157:H7. In this study, marinated product dried at 68.3°C for 10 h achieved appreciable reductions (3.0-4.6 log CFU/cm^2), but these reductions did not meet the recommended 5-log reduction. Keene et al. (1997) studied dried venison jerky and found that drying at 62.8°C, reduced counts over the first 6 to 8 h to levels undetectable by direct plating (<10 CFU/g) when enumerated on SMAC. In this study, populations of *E. coli* O157:H7 decreased rapidly following the initial drying time (4 h), but as drying continued for up to 10 h, counts remained relatively constant. In addition, surviving levels of bacteria after 10 h of drying were 2.9-3.7 logs CFU/cm^2 for all treatments, by direct plating on SMAC and TSA.

D-Values

Although most of bacterial destruction occurred during the first 4 h of drying, regression values were calculated and D-values were determined for each treatment to cover the 10 h of drying period (Appendix D, Table D. 3.3). D-values for treatment 1 (no marinade, dried at 62.5°C) were 1.5 and 1.3 h for counts determined with SMAC and TSA, respectively, with corresponding correlation coefficients of 0.64 and 0.81. The D-values for treatment 2 (marinade, dried at 62.5°C) were 2.7 and 2.8, for SMAC and TSA, respectively, with corresponding correlation coefficients of 0.48 and 0.38. Finally, the D-

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values of treatment 3 (marinade, dried at 68.3°C) were 1.6 and 1.1, with correlation coefficients of 0.4 and 0.6. The low correlation coefficients are due to the lack of major changes in bacterial counts during 4-10 h of drying. Since most of the destruction occurred during the first 4 h of drying, it might be considered more acceptable to conduct additional trials with additional analyses between 0 and 4 h of drying in order to have more points for regression analysis only of the data collected during the first 4 h of drying. However, as it is indicated in following paragraphs, drying beyond 4 h was necessary to reach a_w levels needed for production of shelf-stable products. Nevertheless, there is a need to study potential reasons for lack of major bacterial destruction after 4 h of drying and to develop processes that will enhance drying during the latter stages of the process.

For all three treatments, population counts reached a plateau after 4 h of drying (2.8-4.4 log CFU/cm^2) and residual populations were not destroyed, even after 10 h of drying (2.9-3.7 log CFU/cm^2) (Fig. 3.3). The lack of further destruction perhaps was due to meat casehardening (McWilliams, 1993). Casehardening is a protective coating created on the surface of slices (McWilliams, 1993). This may have developed during the initial hours of drying at 62.5 and 68.3°C and provided protection to cells from further destruction. This effect may have been enhanced by the dry climate of Colorado. The relative humidity (Thermo-Hygro, VWR Scientific Products, West Chester, PA) within and outside the dehydrators during drying ranged from 4-10% and 19-24%, respectively.

The surviving bacteria "tail" phenomenon is evident in other studies investigating the survival of *E. coli* O157:H7 (Riordan et al., 1998; Faith et al., 1998a, 1997;

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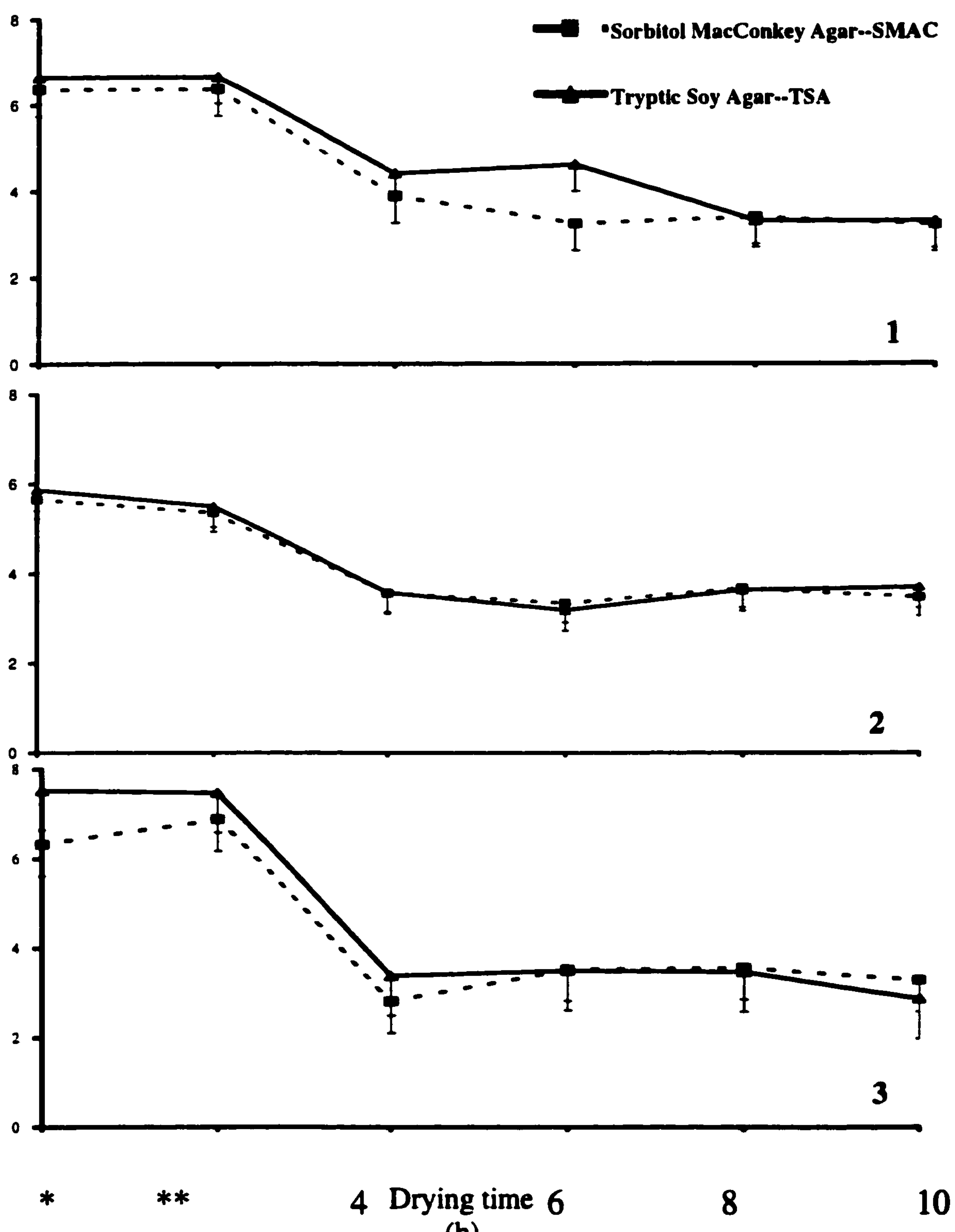

Figure 3.3 Changes in bacterial counts (Y-axis = log CFU/cm^2) on beef jerky before and during drying without marinade at 62.5°C (1) and with marinade at 62.5 (2) and 68.3°C (3). Counts were enumerated with tryptic soy agar (TSA) and sorbitol MacConkey agar (SMAC). Means are three values from two dehydrators (n=6). Data from Tables 3.2 and 3.3; error bars indicate standard deviation; * indicates inoculation (4°C, 24 h), ** indicates marinating (4°C, 24 h) for treatments (B) and (C). Lowest detection limit by plating, 2 log CFU/cm^2

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Ellajosyula et al., 1998; Harrison and Harrison, 1996). Further work must be done to remove the plateau and create continuous inactivation of the *E. coli* O157:H7 throughout drying. This indication of a plateau or tail effect, as described above, is a concern when home-drying meats. Previous research has not been done at higher elevations (>3000 ft) and the results of this research done in Colorado (4900) differs from research at lower elevations with higher humidity. Whether the plateau is a result of geographical differences (altitude, humidity) is a consideration; thus, more research should be done, providing additional information for consumers drying meat at higher elevations.

Effect of storage

Limited work has been done on the survival of inoculated *E. coli* O157:H7 during storage. Harrison and Harrison (1996) stored dried jerky for 8 weeks at 25°C and in three sterile desiccators containing sodium chloride (NaCl) solutions at 26.4, 19.0, and 9.3% used to yield a_w of 0.75, 0.84, and 0.94, respectively. Results indicated no detectable levels of *E. coli* O157:H7 by direct plating or enrichment following 8 weeks of storage, nor at any of the three a_w levels. In this study, jerky was analyzed following storage for 0 (following 10 h of drying), 30, 60 and 90 d at 21°C with room relative humidity of 19-24% (Table 3.3).

When stored jerky was analyzed, after 30 d of storage, there was a significant ($P < 0.05$) reduction in bacterial counts which became very low or undetectable by direct plating (10 CFU/cm^2), compared to initial (after 10 h of drying) counts of 2.9-3.7 log CFU/cm^2 (Table 3.3). Following 60 and 90 d of storage, counts were the same as counts recovered after 30 d, which were very low (< 1.0 to 1.4 log CFU/cm^2). After 30 d, treatment 1 had high amounts of visible mold growth, possibly a result of a_w values

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Table 3.3 Effect of storage (0, 30, 60 and 90 d) at 21°C, beef jerky preparation procedure (with or without marinade; dried at 62.5 or 68.3°C), drying time (0-10 h) and culture media for bacterial enumeration (tryptic soy agar--TSA and sorbitol MacConkey agar--SMAC) on survival of inoculated *E. coli* O157:H7 (log CFU/cm^2) and natural bacterial flora.

	Beef Jerky Preparation Procedure and Agar Media[a]					
	1[b]		2[c]		3[d]	
	SMAC	TSA	SMAC	TSA	SMAC	TSA
0[e]	3.2^{AaX} (0.4)	3.3^{AaXY} (0.3)	3.5^{AcX} (0.1)	3.7^{AaX} (0.7)	3.3^{AcX} (0.3)	2.9^{AaY} (0.7)
30	$< 1.0^{BbX}$ (0.1)	1.4^{AbX} (0.1)	$< 1.0^{AbX}$ (0.1)	$< 1.0^{AbY}$ (0.1)	$< 1.0^{AbX}$ (0.3)	$< 1.0^{AbY}$ (0.3)
60	$< 1.0^{BbX}$ (0.1)	1.4^{AbX} (0.1)	$< 1.0^{AbX}$ (0.1)	$< 1.0^{AbY}$ (0.1)	$< 1.0^{AbX}$ (0.1)	$< 1.0^{AbY}$ (0.1)
90	$< 1.0^{BbX}$ (0.1)	1.4^{AbX} (0.1)	$< 1.0^{AbX}$ (0.1)	$< 1.0^{AbY}$ (0.1)	$< 1.0^{AbX}$ (0.1)	$< 1.0^{AbY}$ (0.1)

[A-B] Means with different superscripts within each medium in a row are significantly ($P < 0.05$) different.

[a-b] *Means with different superscripts within each column are significantly ($P < 0.05$) different.*

[X-Y] Means with different superscripts within each agar medium in a row are significantly ($P < 0.05$) different.

[a] Means represent three values from two dehydrators (n = 6) (standard deviation) of log colony forming units (CFU) cm^2; lowest detection limit by plating, 2 log CFU/cm^2 for 0 d and three values (n=3) (standard deviation) of log colony forming units (CFU) cm^2; lowest detection limit by plating, 1 CFU/cm^2 for 30-90 d SMAC and TSA.

[b] No marinade, dried at 62.5°C

[c] With marinade, dried at 62.5°C

[d] With marinade, dried at 68.3°C

[e] Following 10 h of drying (21°C, 24 h)

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> 0.80 and after 60 and 90 d, mold growth was covering all pieces. Since most molds do not grow below a_w values of 0.80, the a_w must be below this level. The high amount of free water available in the jerky product, made conditions favorable for mold growth (Leistner, 1995, 1987; Lenovich, 1987). The other treatments (2 and 3) did not have visible mold growth during storage. Additional studies are needed to verify whether surviving bacterial cells become undetectable during storage after drying.

Product water activity and pH

Water activity is an index of the quantity of available or free water in a food that can be used in chemical reactions and microbial growth (Leistner, 1987; Lenovich, 1987). Drying beef jerky is a food preservation technique based on removal of moisture and decrease of the a_w. Microbial growth can be inhibited by the combination of reduced a_w and low pH levels. It is essential that beef jerky be dried to an a_w acceptable for storage and proper shelf life. In order for a product such as beef jerky to be microbiologically stable, the a_w should be ≤ 0.68 (Leistner, 1987, 1995). The Code of Federal Regulations (USDHHS-FDA, 1999) states that acidified foods can have $a_w > 0.85$ when the finished equilibrium pH is 4.6 or below. However, in this study, all finished jerky products had pH values ranging from 5.39 to 6.00 after the pre-drying and drying processes; thus, the pH was not low enough to classify these products as acid or acidified foods. Therefore, the a_w of these products must reach low enough (≤ 0.68) levels in the jerky to be considered shelf-stable (Leistner, 1987, 1995).

Water activity values before and after marinating (treatments 2 and 3) ranged from 0.93 to 0.94 (Table 3.4); after 4 h of drying, values decreased to 0.86 to 0.92; with lower values being associated with treatment 3. At 10 h of drying, treatments 2

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Table 3.4 Effect of beef jerky preparation procedure (with or without marinade; dried at 62.5 or 68.3°C) and pre-drying, drying (0-10 h) and storage (30, 60, and 90 d) on mean water activity (a_w) values of *E. coli* O157:H7 (log CFU/cm^2) inoculated beef slices.

	Pre-Drying		Drying time (h)[a]				Storage (d)		
Jerky Treatment	Following inoculation (4°C, 24 h)	Following marinating (4°C, 24 h)	4	6	8	10	30	60	90
1[b]	0.93 (0.01)	N/A	0.92 (0.01)	0.91 (0.01)	0.90 (0.01)	0.83 (0.03)	0.88	0.89	0.85
2[c]	0.94 (0.01)	0.93 (0.01)	0.90 (0.01)	0.80 (0.04)	0.69 (0.07)	0.65 (0.05)	0.65	0.61	0.59
3[d]	0.93 (0.01)	0.94 (0.01)	0.86 (0.02)	0.74 (0.02)	0.66 (0.06)	0.64 (0.04)	0.44	0.50	0.44

[a] Means represent one value from two dehydrators (n = 2) (standard deviation) for pre-drying and drying time and one dehydrator (n=1) for 30-90 d.
[b] No marinade, dried at 62.5°C
[c] With marinade, dried at 62.5°C
[d] With marinade, dried at 68.3°C

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(marinade, dried at 62.5°C) and 3 (marinade, dried at 68.3°C) reached average a_w values recommended for storage of microbiologically safe products (0.65 and 0.64, respectively). Jerky slices for treatments 2 and 3 remained at or below these levels when analyzed following storage for 30-90 d (0.44-0.65). As would be expected, no evidence of mold growth occurred during storage up to 90 d for treatments 2 and 3 (marinated, then dried at 62.5 or 68.3°C).

In contrast, after 10 h of drying, treatment 1 (no marinade, dried at 62.5°C) reached an average a_w value of only 0.83 and remained at or near this level following storage for 30-90 d (0.85-0.88). The higher amount of free water remaining in the unmarinated jerky may be attributed to the lack of spices or seasonings, available to act as water binding agents. This treatment would not be recommended for use, due to the limited shelf-life stability. In addition, the high amount of mold growth seen after storage for 30-90 d supports further evidence that this treatment would not be an appropriate way to dry jerky.

Throughout the drying process, pH values ranged from 5.75 to 5.83 for treatment 1 (no marinade, dried at 62.5°C), 5.09 to 5.43 for treatment 2 (marinade, dried at 62.5°C) and 5.75 to 5.90 for treatment 3 (marinade, dried at 68.3°C) (Table 3.5). The pH differences seen among the treatments could be due to variations among the raw beef used in the study. The three treatments were performed on separate days and different vacuum-packaged beef inside rounds were used for the separate treatments. However, initial pHs taken on the raw beef ranged from 5.6 to 5.9, falling in the range of acceptable pHs for raw, whole muscle beef (McWilliams, 1993). Following jerky being stored for 30-90 d, the pH values for the stored products from treatments 1 and 2 appeared higher

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Table 3.5 Effect of beef jerky preparation procedure (with or without marinade, dried at 62.5 or 68.3°C), and pre-drying, drying (0-10 h) and storage (30, 60, and 90 d) on mean pH values of *E. coli* O157:H7 (log CFU/cm^2) inoculated beef slices.

Jerky Treatment	Pre-drying		Drying time (h)[a]				Storage (d)		
	Following inoculation (4°C, 24 h)	Following marinating (4°C, 24 h)	4	6	8	10	30	60	90
1[b]	5.75 (0.06)	N/A	5.83 (0.01)	5.83 (0.03)	5.86 (0.01)	5.83 (0.01)	6.76	6.37	6.89
2[c]	5.36 (0.00)	5.20 (0.01)	5.09 (0.12)	5.19 (0.17)	5.25 (0.06)	5.43 (0.08)	6.12	5.77	5.67
3[d]	5.90 (0.00)	5.76 (0.00)	5.86 (0.11)	5.86 (0.13)	5.75 (0.01)	5.81 (0.21)	5.75	5.78	5.81

[a] Means represent triplicate readings from one sample each from two dehydrators (n = 2) (standard deviation) for pre-drying and drying time and one dehydrator (n=1) for 30-90 d

[b] No marinade, dried at 62.5°C

[c] With marinade, dried at 62.5°C

[d] With marinade, dried at 68.3°C

All raw pre-inoculated meat ranged in pH from 5.60 to 5.90

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than the pHs obtained for the products from the same treatments, throughout the drying process. However, the pH values for treatment 3 after 30-90 d were similar to the pH values obtained during the drying process.

CONCLUSIONS

Bacterial reductions during the first 4 h of drying were 2.3-2.5, 2.1-2.3 and 3.5-4.1 log CFU/cm^2 for treatments 1-3, respectively, but after 10 h of drying, corresponding total bacterial reductions were 3.2-3.4, 2.2, and 3.0-4.6 log CFU/cm^2. Significant ($P < 0.05$) bacterial destruction was achieved during the first 4 h of drying, but little and often non-significant destruction occurred during the remaining 6 h of drying. These results may indicate casehardening occurred on the surface of the jerky, preventing further bacterial destruction. In addition, counts enumerated with SMAC were significantly ($P < 0.05$) lower than counts enumerated on TSA for treatments 1 and 3 at several times during the first 6 h of drying. These lower counts with SMAC possibly indicated the presence of injured cells or cells that were being inhibited by the agar medium; however, as drying time progressed, differences in counts between culture media became smaller. Although significant ($P < 0.05$) bacterial destruction occurred during the first 4 h of drying, additional drying time was necessary to achieve acceptable a_w values, enhancing the shelf-life stability of the product.

Based on the conditions and results of this study, it was concluded that drying beef jerky for 10 h at 62.5 or 68.3°C resulted in significant ($P < 0.05$) reductions of inoculated *E. coli* O157:H7 cells, but a 5-log reduction of bacteria was not obtained. However, when jerky was stored for at least 30 d at 21°C following 10 h of drying, a 5-log reduction in *E. coli* O157:H7 was achieved. Therefore, since a 5-log reduction in

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inoculated *E. coli* O157:H7 was not achieved after drying for 10 h at 62.5 (with and without marinade) or at 68.3°C, methods must be developed to obtain a 5-log reduction in the pathogen. The following chapter focuses on four pre-drying treatments designed to achieve a 5-log reduction in inoculated beef jerky slices, each using two pre-drying steps.

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CHAPTER IV

SURVIVAL OF INOCULATED *ESCHERICHIA COLI* O157:H7 ON BEEF JERKY DRIED AT 62.5°C FOLLOWING APPLICATION OF FOUR PRE-DRYING TREATMENTS

ABSTRACT

The United States Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) recommends a 5-log reduction of *E. coli* O157:H7 for processed dried and semidry fermented foods to be considered safe. Discrepancies exist, however, as to whether current preparation practices for home dried jerky lead to such destruction. Four pre-drying treatments, designed to enhance destruction of *E. coli* O157:H7 before and during drying, were developed and evaluated. The objective of this study was to determine survival of inoculated *E. coli* O157:H7 populations in beef jerky using the four pre-drying jerky treatments, followed by drying in home-type dehydrators for up to 10 h at 62.5°C.

Inoculated meat slices were subjected to each of the four pre-drying jerky treatments, each consisting of two pre-drying steps: 1--dipping in boiling water (95°C, 15 sec), followed by marinating (4°C, 24 h); 2--seasoning with pickling spices (4°C, 24 h), followed by dipping in hot pickling brine (78°C, 90 sec); 3--dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec), followed by marinating (4°C, 24 h); 4--marinating (4°C,

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24 h), followed by dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec). The marinade used in treatments 1, 3 and 4 consisted of 60 ml Kikkoman soy sauce, 15 ml Heinz Worcestershire sauce, 0.6 g Schilling black pepper, 1.25 g Kroger garlic powder, 1.5 g Kroger onion powder, and 4.35 g Spice Islands hickory smoke-flavored salt per kg of meat slices marinated (65 slices of 15 g each). Each inoculated meat slice received 1.0 ml of marinade, with 0.5 ml distributed evenly over each top and bottom surface. The pickling spices used in treatment 2 consisted of 27 g Morton iodized salt, 12 g Food Club granulated sugar and 1.9 g Schilling black pepper per kg of meat slices and the pickling brine consisted of 108 g Morton iodized salt, 48 g Food Club granulated sugar and 5.8 g Schilling black pepper per gallon of water. For treatment 2, pickling spices were evenly distributed (0.6 g per slice) over the top and bottom surface of inoculated meat slices.

Pre-treated beef slices were dried in two home-type dehydrators for up to 10 h at 62.5°C. Samples were analyzed (bacterial enumeration with selective and nonselective agar media, pH, and water activity-a_w) after inoculation, after each pre-drying step, and at 4, 6, 8 and 10 h of drying. The study was replicated twice with three samples analyzed per replication. In addition, after 10 h of drying, jerky from all four treatments was stored (21°C) in 1-qt Ziploc freezer bags and analyzed after 30, 60 and 90 d.

Depending on initial inoculum level (5.3 to 7.6 CFU/cm^2) and agar medium used (tryptic soy agar—TSA and sorbitol MacConkey agar—SMAC), mean reductions in counts after 10 h of drying were 4.3-4.5, 5.7-5.8, 4.9-5.2 and 4.7-4.8 log CFU/cm^2 for treatments 1-4, respectively. Treatment 2 resulted in the greatest mean log reduction before drying (3.1-4.1 log CFU/cm^2) and after 10 h of drying (5.7-5.8 log CFU/cm^2).

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Whereas, treatment 2 achieved ≥ 5-log bacterial reductions at 4 h of drying, an acceptable a_w value (< 0.68) was not achieved until after 8 h of drying. After 10 h of drying, treatments 3 and 4 reached average a_w values < 0.68 (0.50 and 0.59, respectively); however, these treatments achieved reductions of only 4.9-5.2 and 4.7-4.8 log CFU/cm^2, respectively. After 10 h of drying, treatment 1 had a_w of 0.75 and did not achieve a 5-log reduction (4.3 and 4.5 log CFU/cm^2). After 30 d of storage, counts in all jerky treatments were significantly ($P < 0.05$) lower (< 1.0 log CFU/cm^2) than those at 0 d (0.8 to 2.7 log CFU/cm^2). These counts remained similar after 60 and 90 d of storage. This observation needs further confirmation.

For each treatment, significant ($P < 0.05$) bacterial reductions occurred within the first 4 h of drying, but they maintained a plateau between 4 and 10 h of drying, regardless of the level of bacterial reduction seen after 4 h of drying. This finding indicates the importance of achieving a 5-log reduction by 4 h of drying at 62.5°C. However, drying must continue since a_w values after 4 h of drying ranged from 0.83 to 0.92 for jerky treatments allowing for growth of molds, yeast and some bacteria. Drying needed to continue to achieve an acceptable a_w levels for microbiologically stable jerky products (≤ 0.68).

These results are useful in developing guidelines for jerky preparation by consumers. After 8 h of drying, treatment 2 achieved bacterial reductions ≥ 5 log CFU/cm^2, as well as acceptable a_w values (≤ 0.68). Thus, treatment 2 would be an acceptable treatment for home-drying jerky, provided that the product is of acceptable quality. Treatment 1 should be re-investigated, using higher inoculum levels to determine whether a 5-log reduction could be achieved. In addition, the use of

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preservatives could be investigated to enhance the treatment's shelf life, since the product had high a_w values. Treatments 3 and 4 achieved significant ($P < 0.05$) reductions, but < 5-logs on selective media (SMAC) after 10 h of drying. These treatments showed promise as acceptable treatments, but further work must be done. Future work should investigate these treatments to improve destruction by modifying exposure and/or levels of acetic acid used. Acidulants, such as ascorbic or citric, could be investigated as supplements or substitutions.

INTRODUCTION

Buchanan and Doyle (1997) stated that the foodborne pathogen *Escherichia coli* O157:H7 presents a problem to current preventive strategies for food safety protocols, since it has a low infectious dose and can result in severe disease characteristics (hemorrhagic colitis--HC and hemolytic uremic syndrome--HUS). In addition, the pathogen is unevenly distributed in raw ingredients, making spot testing of the pathogen unreliable (Tilden et al., 1996). Thus, the most desirable step for processors and consumers is a lethal processing step that successfully minimizes the risk of *E. coli* O157:H7 contamination (Buchanan and Doyle, 1997).

Outbreaks in the mid 1990s were associated with *E. coli* O157:H7 in dried and fermented meats (CDC, 1995b). These outbreaks prompted federal action mandating that manufacturers of such products demonstrate a 5-log reduction of *E. coli* O157:H7 during processing (Billy, 1997; Reed, 1995). According to the USDA-FSIS, a validated step in processing is required to obtain a 5-log reduction in *E. coli* O157:H7 populations for processed fermented meat food systems or a 7-log reduction for roasted beef to be considered safe.

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Research was done to investigate the survival of *E. coli* O157:H7 cells in dried and fermented meat (Hinkens et al., 1996; Riordan et al., 1998; Faith et al., 1997, 1998b, c; Buege and Luchansky, 1999; Luchansky and Buege, 1998). Their results indicated that traditional fermentation processes, which do not include a thermal step in processing, did not achieve a 5-log reduction of inoculated *E. coli* O157:H7 cells. Based on these results, dried meats may potentially cause a greater threat to consumers due to contamination with pathogens surviving the process (Faith et al., 1998a). Dried meats, such as dried jerky, are easily prepared at home by consumers since they do not require extensive equipment or precision in methods (Faith et al., 1998a). Thus, it is important to investigate the preparation of dried jerky to determine if the potential threat is valid and to develop processing methods that lead to a safer product.

The "multi-hurdle" concept is an approach that tries to control the level of microorganisms that may be present in food (Leistner, 1987, 1995). This concept allows several "hurdles" to be combined to work together to help ensure that microorganisms can be controlled. Using this concept, existing microorganisms should not be able to overcome hurdles that are present during food processing. Thus, if the microorganism cannot "leap" the hurdles, the food will be microbiologically safe and stable (Leistner, 1995). With multi-hurdles, lower intensities of individual factors can be used than what would be needed if the factors were used alone, since the combination of ingredients works synergistically to prevent spoilage (Leistner, 1987; Marth, 1998). Leistner (1987, 1995) recommended the use of a multi-hurdle strategy for food processing industries that involve fermented and dried meat, since a sequence of hurdles can lead to a stable product. The most important hurdles commonly used in food processing include

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temperature (high or low), water activity (a_w), acidity (pH), redox potential (E_h) and preservatives (organic acids, spices) (Leistner, 1995). Hurdles vary according to the goal of the processing step. Some hurdles can influence the safety of the food, while improving the flavor or taste of the product.

As stated above, organic acids are commonly used as antimicrobial agents since they contribute to the control of growth of bacteria (Leistner, 1995; Nychas, 1995). It is reported heat treatments are more effective in inactivating the bacteria when the product has a sub-optimal pH (Bell and Kyriakides, 1998). Acetic acid has been reported to reduce bacteria on sliced beef and can also produce a lower pH on the surface of the meat (Smulders, 1995). Vinegar (5% acetic acid) was studied by Entani et al. (1998), who reported that it had a bactericidal effect on foodborne bacteria, including *E. coli* O157:H7. In the study, vinegar was inoculated with *E. coli* O157:H7 cells and it was found that *E. coli* O157:H7 cells in the logarithmic growth phase demonstrated a higher sensitivity to vinegar than cells in the stationary phase. In addition, Entani et al. (1998) investigated the bactericidal activity of vinegar with an increase of temperature. Investigators suggested that an effective method to prevent food poisoning should include a vinegar treatment that is applied at 40 to 50°C (104 to 122°F). These temperatures would be more applicable since heating vinegar to 75°C (167°F) may create an alteration of food taste, as well as loss of nutritional value. However, researchers reported that heating vinegar to 75°C was a more effective method for inactivating *E. coli* O157:H7 (Entani et al., 1998). When Entani et al. (1998) evaluated the application of acetic acid (2.5%) at 20°C (68°F) to inoculated cells, a 3-log reduction in viable *E. coli* O157:H7 cells was seen after 739 min; however, when acetic acid (2.5%) was applied at 40°C

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(104°F), a 3-log reduction was seen after 14 min. In addition, investigators combined acetic acid (2.5%) with sodium chloride (NaCl) (5%) and a 3-log reduction was obtained after 5.27 min (Entani et al., 1998). However, when the acetic acid (2.5%) and NaCl (5%) were applied at 40°C, a 3 log reduction in viable *E. coli* O157:H7 cells was seen after 0.89 min (Entani et al., 1998). In addition to using various combinations of vinegar and NaCl at appropriate temperatures, Entani et al. (1998) recommended cells in the stationary phase should be used in experiments when investigating the applications of vinegar in food preparation. With this recommendation, *E. coli* O157:H7 cells in the stationary phase were used for this study. Since organic acids can have greater antimicrobial effects at higher acid concentrations and at higher temperatures (Smulders, 1995), this study investigated the application of acetic acid (2.5%) to slices of beef jerky inoculated with *E. coli* O157:H7 at 57.5°C (135°F) for 20 sec.

In addition to acidity being used as a hurdle effect, spices are also used as a hurdle effect. Spices and seasonings have long been used to add flavor to food products, but many spices also exhibit antimicrobial properties (Beuchat, 1994). One example of a spice family commonly used in the diet is from the *Allium* genus. This genus includes such plants and spices as garlic, onion and leek. Garlic has been used for centuries for flavor enhancement, but only in the past few decades has the antimicrobial and medicinal properties of garlic been established (Beuchat, 1994). In addition, garlic has been reported to kill *E. coli* O157:H7 when used at varying levels (Food Safety Consortium, 1998). However, to inhibit microorganisms, garlic and other spices must be added at concentrations that exceed levels normally recommended and concentrations of spices at less than 1% are not considered to be inhibitory to microorganisms (Beuchat, 1994).

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Riordan et al. (1998) recommended against simply increasing concentrations of existing antimicrobial ingredients (salts, spices, etc.) that are used in jerky preparation, due to the unpleasant and unacceptable taste qualities that result. However, when spices and seasonings are used in varying combinations, their antimicrobial activities are able to work synergistically in order to inhibit microorganisms. One way to combine different ingredients is through use of a marinade, which is typically seen during jerky preparation. A jerky marinade often contains soy sauce, Worcestershire sauce, black pepper, garlic and onion powder, and Hickory smoked salt (Reynolds and Williams, 1993). Besides the spices and seasonings used in a jerky marinade, soy sauce has also been reported to have anti-*E. coli* O157:H7 effects (Masuda et al., 1998). Masuda et al. (1998) indicated that the inhibitory activity of soy sauce was due to the combination of ingredients found in the sauce (components of sodium chloride, ethanol, lactic acid and preservatives). When investigated, *E. coli* O157:H7 cells could not grow in soy sauce at 4, 18, and 30°C (39, 64 and 86°F). Soy sauce reduced the cell numbers of bacteria at 18°C to a lesser extent than at 30°C, but to a greater extent than at 4°C (Masuda et al., 1998). The authors concluded that the inhibition of *E. coli* O157:H7 in soy sauce was not determined by any one ingredient (sodium chloride, ethanol, pH, organic acid, or preservatives), temperature or time. On the contrary, the fate of the pathogen was due to the combined effects of all factors found in the soy sauce and at various storage temperatures.

Studies on the survival of *E. coli* O157:H7 in dried jerky products (Harrison and Harrison, 1996; Harrison et al., 1998; Faith et al., 1998a; Keene et al., 1997) have produced variable results, depending in part on the type (beef vs. venison) and form (whole muscle vs. ground) of meat used in jerky formulation, as well as the dehydrator

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(air) temperature (51.7 to 68°C), the drying time (6 to 20 h) and the culture agar (tryptic soy agar, sorbitol MacConkey, sorbitol MacConkey supplemented with 4-methyl-umberlliferyl-D-glucoronide, or modified eosin methylene blue) used in the studies. The current recommendation from the USDA-FSIS Meat Poultry Hotline (1998) is that when drying meat, the meat should be heated to 71.1°C (160°F) before drying. However, this recommendation of pre-heating the meat raises concern, since consumers may object to the cooked flavor of jerky prepared in this manner. Only two studies (Harrison and Harrison, 1996; Harrison et al., 1998) have reported results of jerky pre-heated prior to drying.

In the first study, Harrison and Harrison (1996) investigated whole muscle beef jerky prepared using a traditional method (Reynolds and Williams, 1993) or a pre-heating (71.1°C) method (USDA-FSIS, 1998). Harrison and Harrison (1996) divided beef slices into three groups and inoculated two groups with *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Typhimurium. On one-third of the top surface of each strip, 0.1 ml of each inoculum (10^8 CFU/ml) was distributed on the top of the slices. No initial inoculum was given nor were the units of material (CFU/g, CFU/cm^2, or CFU/ml) specified. The remaining group had sterile Butterfield's phosphate buffer distributed on the top of the slices, to mimic inoculation. The phosphate buffer-treated samples and half of the inoculated samples were marinated at 4°C (39°F) for 1 h. The other half of inoculated samples were placed in a shallow pan, covered with marinade and pre-heated to 71.1°C on a hot plate (time unspecified). The pan was removed and allowed to cool for 10 min under a laminar flow hood to prevent contamination. After 10 h of drying, populations of *E. coli* O157:H7 on the inoculated, unheated meat decreased by 5.5 to 6.0

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log units. Populations on the inoculated, pre-heated meat decreased by 5 log units during pre-heating prior to drying; after 10 h of drying, populations decreased to undetectable levels (no detection limit stated) (Harrison and Harrison, 1996). Authors concluded that using a marinade in jerky preparation and drying at 60°C (140°F) for 10 h were sufficient to reduce levels of *E. coli* O157:H7 by at least 5 log units on unheated meat and pre-heated meat (Harrison and Harrison, 1996).

The second study investigated a pre-heating method applied to ground beef jerky inoculated with *E. coli* O157:H7 and made with either a spice and cure mix or a spice mix only (Harrison et al., 1998). Lean ground beef was inoculated with *E. coli* O157:H7 and the meat was molded into strips. Before drying in home-type dehydrators, half of the inoculated jerky was pre-heated to 71.1°C (160°F) in a convection oven (time unspecified). Half of these pre-heated strips contained a spice (ingredients not listed) and cure mix (salt and sodium nitrite), while the other half contained a spice mix only. The remaining inoculated jerky strips were not pre-heated and half contained a spice and cure mix, while half contained a spice mix only. All jerky strips were dried in a 60°C (140°F) dehydrator for 6 h (pre-heated strips) or 8 h (unheated strips). The authors found that populations of inoculated *E. coli* O157:H7 were reduced by 5.2 log CFU/g for the pre-heated and unheated strips that contained the spice and cure mix (Harrison et al., 1998). In contrast, reductions of *E. coli* O157:H7 populations in pre-heated and unheated jerky strips containing the spice mix only were 4.8 and 4.3 log CFU/g, respectively. Thus, inactivation of *E. coli* O157:H7 cells was enhanced through the addition of a cure mix to a spice mix. Inactivation was slightly enhanced when jerky was pre-heated (71.1°C) prior to drying and contained only a spice mix (Harrison et al., 1998). These findings

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suggested that if consumers make jerky with ground meat and fail to add the jerky cure mix, the product could pose an increased risk of foodborne illness if the meat was contaminated with *E. coli* O157:H7 (Harrison et al., 1998). With conflicting results reported concerning dried jerky, it appears that there is a need for additional studies to evaluate procedures achieving adequate (≥ 5 log CFU/g) reduction in inoculated *E. coli* O157:H7 during jerky processing.

The results of the study reported in Chapter III indicated bacterial reductions during the first 4 h of drying were 2.3-2.5, 2.1-2.3 and 3.5-4.1 log CFU/cm^2 for treatments 1-3, respectively; after 10 h of drying, corresponding reductions were 3.2-3.4, 2.2, and 3.0-4.6 log CFU/cm^2. Significant ($P < 0.05$) bacterial destruction was achieved during the first 4 h of drying, but little and often non-significant destruction occurred between 4 and 10 h of drying. These results may indicate casehardening occurred on the surface of the jerky (McWilliams, 1993), preventing further bacterial destruction. Even though significant ($P < 0.05$) bacterial destruction occurred during the first 4 h of drying, additional drying time was necessary to achieve acceptable a_w values, enhancing the shelf-life stability of the product. Based on the conditions and results of this study, it was concluded that drying beef jerky for 10 h at 62.5 or 68.3°C resulted in significant ($P < 0.05$) reductions of inoculated *E. coli* O157:H7 cells, but a 5-log reduction of bacteria was not obtained.

In the study reported here, four different pre-drying treatments, incorporating multi-hurdle strategies, were explored to improve pathogen destruction, while maintaining sensory properties typical of beef jerky. Specifically, the objective of the present study was to determine survival of inoculated *E. coli* O157:H7 populations in

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beef jerky, using four pre-drying treatments and drying in home-type dehydrators for up to 10 h at 62.5°C (145°F). Since the drying process may result in injured cells, recovery of surviving bacterial cells was evaluated with two agar media.

MATERIALS AND METHODS

Study design

Inoculated beef slices were stored at 4°C for 24 h and then subjected to four pre-drying treatments, with each involving two steps: 1--dipping in boiling water (95°C, 15 sec), followed by marinating (4°C, 24 h); 2--seasoning with pickling spices (4°C, 24 h), followed by dipping in hot pickling brine (78°C, 90 sec); 3--dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec), followed by marinating (4°C, 24 h); 4--marinating (4°C, 24 h), followed by dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec). The study was replicated twice with three samples analyzed per replication.

The marinade consisted of 60 ml Kikkoman soy sauce, 15 ml Heinz Worcestershire sauce, 0.6 g Schilling black pepper, 1.25 g Kroger garlic powder, 1.5 g Kroger onion powder, and 4.35 g Spice Islands hickory smoke-flavored salt per kg of meat slices marinated (65 slices of 15 g each) (Reynolds and Williams, 1993) (pH = 4.3). Each inoculated meat slice received 1.0 ml of marinade, with 0.5 ml distributed evenly over each top and bottom surface. The pickling spices used in treatment 2 consisted of 27 g Morton iodized salt, 12 g Food Club granulated sugar and 1.9 g Schilling black pepper per kg of meat slices and the pickling brine consisted of 108 g Morton iodized salt, 48 g Food Club granulated sugar and 5.8 g Schilling black pepper per gallon of water. For treatment 2, pickling spices were evenly distributed (0.6 g per slice) over the

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top and bottom surface of inoculated meat slices (Cooperative Extension Service, University of Wyoming, 1984).

Preparation of bacterial inoculum

Four strains of *E. coli* O157:H7 (E0139, a venison jerky isolate, provided by Dr. Michael Doyle, Center for Food Safety and Quality Enhancement, University of Georgia, Griffin, Georgia; ATCC 43895, 43890 and 43894 obtained from the American Type Culture Collection, Manassas, VA) were used to prepare a composite inoculum for inoculation of the meat slices before processing to make jerky. Cultures were maintained frozen at -18°C in tryptic soy broth (TSB) (Difco Laboratories, Detroit, MI) with 20% (v) added glycerol (Sigma Chemical Co., St. Louis, MO). Each strain was propagated individually on tryptic soy agar (TSA; pH 7.0) (Difco Laboratories, Detroit, MI) slants at 35°C before use in experiments. The experimental inocula were prepared by transferring a loopful of each culture into 9-ml tubes of tryptic soy broth (TSB) (pH 7.0) (Difco Laboratories) and incubating at 35°C for 18-24 h on the day prior to each experiment. The cultures used in the experiments averaged 1.7 x 10^9 CFU/ml. Immediately before use, the individual culture inocula were combined for inoculation of the meat to prepare jerky.

Preparation and inoculation of beef pieces

Vacuum packaged beef inside rounds (approximately 30 kg) were purchased frozen from local supermarket warehouses and stored at -18.0°C (-4°F) until use (i.e., within one week). The meat was removed from the freezer and cut, while still frozen, into slices (8.7 x 4.0 x 0.6 cm; 3.4 x 1.6 x 0.3 inches) with a band saw at the Colorado State University Meat Science Laboratory (Fort Collins, CO). Slices were randomly

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placed in 1-kg portions in plastic bags (20.3 x 25.4 cm; 0.75 mil nylon, 2.25 mil polyethylene laminate; oxygen transmission rate 3.5 cc/2.54 sq. m/24h; Kapak Heat Sealable, Fisher Scientific, Springfield, NJ). The bags were evacuated (29 mm/Hg), heat sealed (Multivac, Sepp Haggenmüller KG, Allgau, Germany), and frozen (-18°C) to minimize potential changes until use (i.e., within two weeks). For each treatment, the top surface area (8.7 x 4.0 cm) of each meat slice was inoculated with 0.5 ml of the culture composite prepared as described above. The inoculum was spread with a sterile bent glass rod to ensure adequate coverage of the surface. Meat pieces were then turned using sterile forceps and the bottom surface area was inoculated with 0.5 ml of the composite culture, as described above. Inoculated slices were refrigerated (4°C, 24 h) in aluminum foil pans covered with heavy-duty aluminum foil, to enhance adherence of pathogen cells to meat pieces before drying. Inoculum levels reached were 5.3-7.6 log CFU/cm^2.

Pre-drying treatment preparation

Treatment 1

Pre-drying step 1: Inoculated beef slices were placed in a single layer on the bottom of a 2-qt steamer basket, submerged in a 2-qt stainless steel pot of boiling water (95°C, 15 sec), removed rapidly and drained. Remaining meat pieces were submerged using the same method. After two uses, water was emptied and replaced with fresh water.

Pre-drying step 2: Within 30 min following the dipping process, each inoculated meat slice received 1 ml of marinade, with each 0.5 ml portion distributed evenly over each top and bottom surface. Marinade was spread with a sterile bent glass rod to ensure

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adequate coverage of the meat pieces. Marinated pieces were placed in aluminum pans, covered with heavy-duty aluminum foil, and stored (4°C, 24 h) before drying.

Treatment 2

Pre-drying step 1: Pickling spices (0.6 g per inoculated meat slice) were sprinkled evenly over inoculated meat slices (top and bottom). The slices were pounded with a rubber mallet, covered with heavy-duty aluminum foil and stored (4°C, 24 h).

Pre-drying step 2: Pickling brine was prepared in a 2-qt stainless steel pot and heated to 78°C (172°F). Inoculated beef slices were placed in a single layer on the bottom of a 2-qt steamer basket submerged (90-sec), removed rapidly and drained. Remaining meat pieces were submerged using the same method. After two uses, brine was emptied and replaced with fresh brine. Immediately following the dipping process, meat slices were dried.

Treatment 3

Pre-drying step 1: In a 2-qt stainless steel pot, equal parts (3 c) of distilled white vinegar (5% acetic acid) and distilled water were combined and heated on a hot plate to 57.5°C (135°F). Inoculated beef slices were placed in a single layer on the bottom of a 2-qt steamer basket, submerged (20-sec), removed rapidly and drained. Remaining meat pieces were submerged using the same method. After two uses, the solution was emptied and replaced with fresh solution.

Pre-drying step 2: Within 30 min of the dipping process, each inoculated meat slice received 1 ml of marinade, with each 0.5 ml portion distributed evenly over each top and bottom surface. Marinade was spread with a sterile bent glass rod to ensure adequate

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coverage of the meat pieces. Marinated pieces were placed in aluminum pans, covered with heavy-duty aluminum foil, and stored (4°C, 24 h) before drying.

Treatment 4

Pre-drying step 1: Each inoculated meat slice received 1 ml of marinade, with each 0.5 ml portion distributed evenly over each top and bottom surface. Marinade was spread with a sterile bent glass rod to ensure adequate coverage of the meat pieces. Marinated pieces were placed in aluminum pans, covered with heavy-duty aluminum foil, and stored (4°C, 24 h).

Pre-drying step 2: In a 2-qt stainless steel pot, equal parts (3 c) of distilled white vinegar (5% acetic acid) and distilled water were combined and heated on a hot plate to 57.5°C (135°F). Inoculated beef slices were placed in a single layer on the bottom of a 2-qt steamer basket submerged (20-sec), removed rapidly and drained. Remaining meat pieces were submerged using the same method. After two uses, the solution was emptied and replaced with fresh solution. Immediately following the dipping process, meat slices were dried.

Drying process and sample preparation

Two home food dehydrators (Gardenmaster® Dehydrator FD-1000, Nesco®/American Harvest®, Chaska, MN), with three dehydrator trays each, were used for the drying process. Dehydrators were preheated to 62.5°C/145°F (air) for at least 15 min prior to placement of the inoculated, pre-treated meat pieces on the drying trays. Meat slices were laid flat on dehydrator trays, without touching other slices. Inoculated meat slices (1 kg) were placed in each dehydrator randomly among trays; additional (0.5-kg) uninoculated, non-treated meat slices were used to fill the dehydrator drying trays to

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capacity and placed in designated locations. Treatments were prepared on separate days and not combined in the dehydrators.

Circulating air temperature within the dehydrators was monitored during the drying process with thermocouples (Type K Beaded Probes, MM Micromeasurements, Raleigh, NC) placed through the open circular top of each dehydrator, with the sensor tip recording circulating air throughout the dehydrator. Temperatures were recorded on a Speedomax strip chart recorder (Leeds and Northup, Northwales, PA). In additional drying studies, the temperatures of meat surfaces were monitored using an infrared heat gun (Oakton Infrared Temperature Tester, Gainesville, FL). Throughout drying, every 30 min, dehydrators were cracked open, the heat gun was directed at the surface of the meat and temperatures were recorded. Three sets of two meat slices each per treatment and per dehydrator were removed at each sampling time and placed in bags for analysis.

Microbiological analyses

Samples taken after inoculation (4°C, 24 h) and after each pre-drying step were analyzed immediately. Samples taken at 4, 6, 8, and 10 h of drying were analyzed following storage at 21°C for 24 h. In addition, samples (25-30 pieces/bag) dried for 10 h were stored in 1-qt Ziploc freezer bags, closed and stored in the dark, at 21°C for 30, 60 and 90 d. Populations of bacteria, including inoculated *E. coli* O157:H7 were determined by plating on tryptic soy agar (TSA) and sorbitol MacConkey (SMAC) agar (Difco Laboratories). Bags containing two slices of product per sample were weighed. Appropriate amounts of Butterfield's phosphate buffer were added to obtain a 1:10 sample dilution and bags were pummeled (IUL Instruments Masticator, Barcelona, Spain) for 120 s. Serial decimal dilutions were made and spread plated in duplicate on

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two culture media. Following incubation at 35°C for 20 to 24 h, colonies were counted. The minimum detection limit for all samples was 10 CFU/cm^2 for treatments 1-4. All counts were converted to log CFU/cm^2. Typical colonies for *E. coli* (sorbitol negative) on SMAC were round and colorless (McCarthy et al., 1998). Six colonies per treatment per drying time were confirmed as *E. coli* serotype O157 using the *E. coli* O157 latex agglutination assay (OXOID Diagnostic Reagents, Hampshire, England).

Physical analyses

Inoculated meat pieces (10-15 g) were analyzed for a_w and pH at each sampling time. Water activity was determined with a Rotronic Hygroskop DT water activity meter (Model D2100, Rotronic, Huntington, NY) calibrated with standard saturated salt solutions (1.000, distilled water; 0.973, K_2SO_4; 0.843, KCl; 0.753, NaCl; 0.743, $NaNO_3$ ± 0.01) (saturation points are 10.8% K_2SO_4; 26.5%, KCl; 26.5%, NaCl; 47.9%, $NaNO_3$). Inoculated meat slices were cut (0.5-1.0 g), placed in the a_w (PS14) cup and set in the a_w meter until the a_w reached a stable reading (via signal lights, 25°C). Each a_w reading was determined as a single value.

A Hanna Instruments, HI8424, (Woonsocket, RI) pH meter was used to determine the pH of the inoculated meat pieces. Meat pieces were weighed in stomacher bags. Appropriate amounts of distilled water were added to obtain a dilution of 1:10 and bags were pummeled (IUL Instruments Masticator, Barcelona, Spain) for 120 s. Bags were allowed to sit for at least 15 min before each pH reading was determined in triplicate and averaged.

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Statistical analyses

The experimental model of the study was a factorial split-plot design with 2 (number of dehydrators) x 4 (number of treatments using two pre-steps) x 2 (number of culture media used for enumeration) x 7 (number of time intervals when meat slices were analyzed) factors and levels. Each mean value reported represented six values (3 determinations from 2 simultaneous trials/dehydrators). Bacterial counts of duplicate plates per dilution were averaged and CFU/cm^2 counts were converted into log values. Populations of surviving bacteria including inoculated *E. coli* O157:H7 (log CFU/cm^2) were plotted versus processing time for each treatment. The decimal reduction times (D-values, 0 to 10 h) were calculated from survivor curves as outlined by Lund (1975) by linear regression to determine the best-fit line. Six mean values (3 values from 2 simultaneous trials/dehydrators) were used for calculating D-values and regression values were calculated.

Data were subjected to the Statistical Analysis System (SAS Institute version 6.1, Cary, NC) for analysis of variance of main (fixed) effects (dehydrator, treatment method, media and drying time), as well as significant interactions between the main (fixed) effects. When F values were significant ($P < 0.05$), least significant differences (LSD) in survival of bacterial population counts were determined using the mixed procedure (PROC MIXED) of SAS. All statistically significant effects were reported at the $P < 0.05$ level.

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RESULTS AND DISCUSSION

Dehydrator and product temperatures

The average dehydrator (air) temperature decreased by 10 to 17°C following placement of the meat strips on dehydrator trays (Fig 4.1). Then, the dehydrator (air) temperature increased steadily until the desired temperature of 62.5°C (± 2.5°C) was reached, approximately 3 to 4 h after placement of meat strips in the dehydrator. The dehydrators maintained the temperature 62.5°C (± 2.5°C) throughout the length of the drying process. Using data reported in Chapter III (Fig. 3.2), the surface temperatures of the meat strips equaled the desired temperatures of 62.5°C (± 2.5°C) after 4.5 to 5 h in the dehydrator and remained within this target range for the reminder of the drying process. Considering the same dehydrators were used in these experiments, it is assumed meat slices equaled the desired temperature of 62.5°C (± 2.5°C) within 4.5 to 5 h of drying for these treatments, as well. Temperature differences between dehydrators (Appendix D, Table D. 4.1) were slight (1.8 to 2.4°C) among the four pre-drying treatments throughout the drying process. However, consumers and researchers should be aware that potential differences may exist between dehydrators, even between the same models of dehydrators (Faith et al., 1998a). In addition, proper recording procedures should be employed to check the temperature of the dehydrator, ensuring accurate temperature measurements are maintained throughout the drying process (Faith et al., 1998a).

Effect of pre-drying steps and drying

With the exception of dehydrator, the main factors (drying time, treatment method and culture media) had significant effects ($P < 0.05$) on bacterial populations recovered

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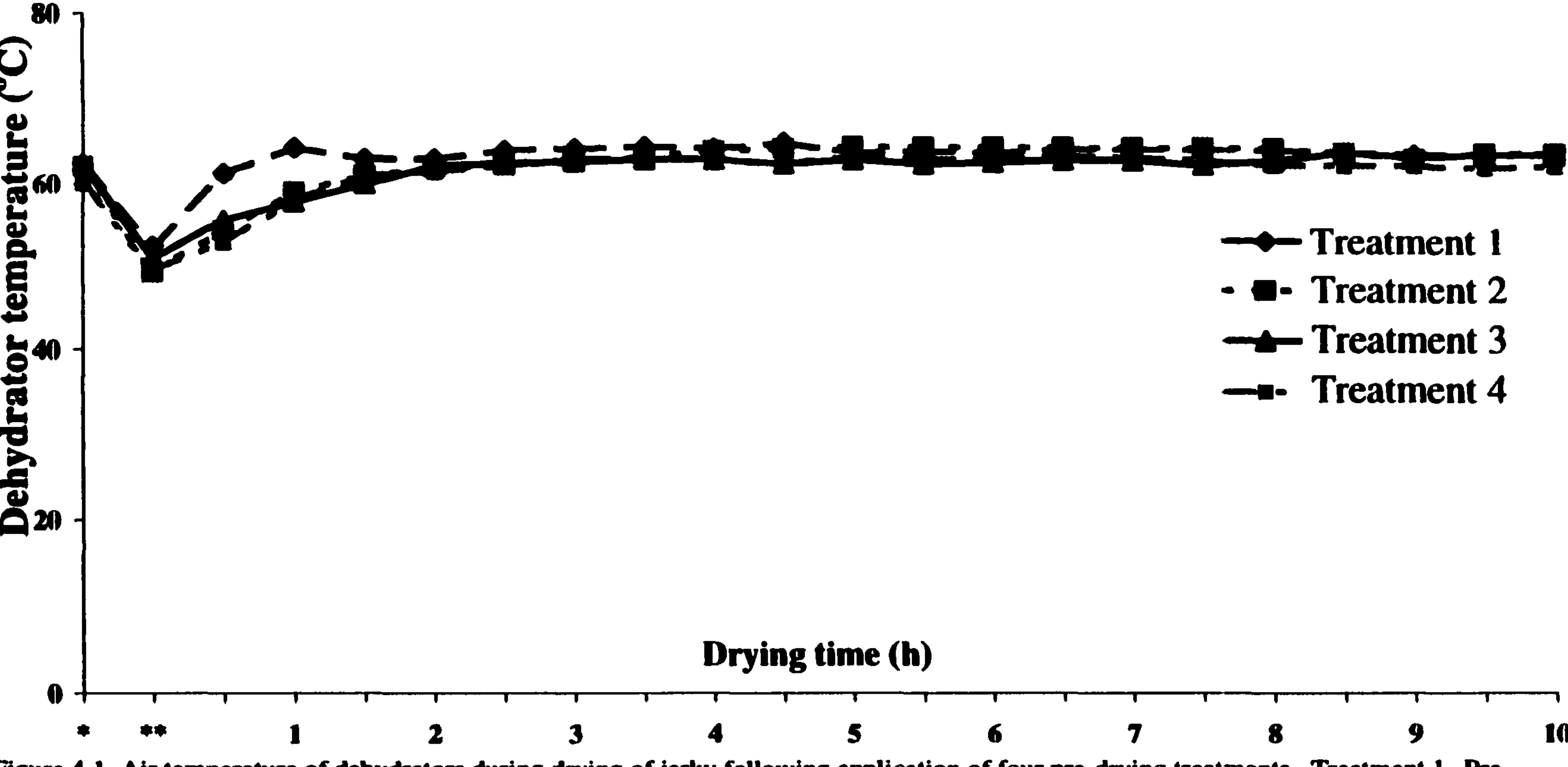

Figure 4.1 Air temperature of dehydrators during drying of jerky following application of four pre-drying treatments. Treatment 1--Pre-drying step 1: dipping in hot water (96°C, 15 sec), pre-drying step 2: marinating (4°C, 24 h); Treatment 2--Pre-drying step 1: seasoning with pickling spices (4°C, 24 h), pre-drying step 2: dipping in hot pickling brine (78°C, 90 sec); Treatment 3--Pre-drying step 1: dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec), pre-drying step 2: marinating (4°C, 24 h); Treatment 4--Pre-drying step 1: marinating (4°C, 24 h), pre-drying step 2: dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec). Each point is the average of two determinations; error bars indicate standard deviation; * indicates 15 min dehydrator warm-up; ** indicates placement of jerky in the dehydrator.

(Table 4.1). Significant interactions were treatment x drying time, treatment x culture media, drying time x culture media and treatment x drying time x culture media.

Bacterial reduction after pre-drying step 1

Following pre-drying step 1, treatment 1 (slices dipped in boiling water at 95°C) achieved significant ($P < 0.05$) reductions of 2.4 and 3.1 log CFU/cm^2 for SMAC and TSA, respectively (Table 4.2). Treatment 2 (slices seasoned with pickling spices at 4°C for 24 h) showed significant ($P < 0.05$) and non-significant reductions (0.8 and 0.1 log CFU/cm^2) with SMAC and TSA, respectively. Treatment 3 (slices dipped in 2.5 % acetic acid at 57.5°C) achieved significant ($P < 0.05$) reductions of 0.8 and 0.6 log CFU/cm^2 with SMAC and TSA, respectively. In comparison, treatment 4 (slices marinated at 4°C for 24 h) showed slight increases in counts (0.1 and 0.2 log CFU/cm^2 for SMAC, and TSA, respectively).

Bacterial reduction following pre-drying step 2

Following pre-drying step 2, treatment 1 (slices marinated at 4°C for 24 h) showed significant ($P < 0.05$) increases in counts (1.1 log CFU/cm^2 with SMAC and TSA) compared to counts after pre-drying step 1 (Table 4.2). Thus, net reductions following both pre-drying steps were 1.3 and 2.0 log CFU/cm^2. In contrast, treatment 2 (slices dipped in hot (78°C) pickling brine for 90 sec) achieved reductions of 2.3 and 3.0 log CFU/cm^2 with SMAC, and TSA, respectively, compared to pre-drying step 1; net log reductions for both pre-drying steps were 3.1 and 4.1 log CFU/cm^2. Treatment 3 (slices marinated at 4°C for 24 h) resulted in no significant bacterial reduction/change (+ 0.1 and -0.2 log CFU/cm^2 with SMAC and TSA, respectively) and achieved total reductions of 0.7 and 0.8 log CFU/cm^2 with SMAC and TSA, respectively. In addition, following pre-

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Table 4.1 Analysis of variance of microbial counts (determined with tryptic soy agar, and sorbitol MacConkey agar) on beef jerky inoculated with *E. coli* O157:H7 (log CFU/cm^2) and processed with four pre-drying treatments at 62.5°C (drying temperature) for different times in each of two dehydrators.

Variable	Tests of Fixed Effects			
	Numerator Degrees of Freedom	Denominator Degrees of Freedom	F-value	Pr>F
Treatment [a]	3	112	331.77	0.0001**
Drying Time [b]	6	112	1034.50	0.0001**
Dehydrator [c]	1	112	0.43	0.5118
Culture Media [d]	1	113	94.84	0.0001**
Treatment * Drying Time	18	112	32.20	0.0001**
Drying Time* Dehydrator	6	112	0.71	0.6454
Treatment * Culture Media	3	113	3.51	0.0177**
Drying Time * Culture Media	6	112	2.37	0.0339**
Treatment * Dehydrator	3	112	0.62	0.6060
Dehydrator * Culture Media	1	113	0.43	0.5115
Treatment * Drying Time * Culture Media	18	112	5.12	0.0001**
Treatment * Drying Time* Dehydrator	18	112	1.31	0.1924
Treatment * Dehydrator * Culture Media	3	113	0.99	0.4016
Drying Time * Dehydrator * Culture Media	6	112	2.44	0.0293**
Treatment * Drying Time * Dehydrator * Culture Media	18	112	1.47	0.1134

** $P < 0.05$

[a] Two-pre-drying steps: Treatment 1--Pre-drying step 1: dipping in boiling water (95°C, 15 sec), pre-drying step 2: marinating (4°C, 24 h); Treatment 2--Pre-drying step 1: seasoning with pickling spices (4°C, 24 h), pre-drying step 2: dipping in hot pickling brine (78°C, 90 sec); Treatment 3--Pre-drying step 1: dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec), pre-drying step 2: marinating (4°C, 24 h); Treatment 4--Pre-drying step 1: marinating (4°C, 24 h), pre-drying step 2: dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec).

[b] Seven time intervals during the drying process: following inoculation (4°C, 24 h), following each pre-drying step and at 4, 6, 8, and 10 h of drying)

[c] Two home food dehydrators (Gardenmaster® Dehydrator FD-1000, Nesco®/American Harvest®, Chaska, MN)

[d] Tryptic soy agar (TSA) and sorbitol MacConkey agar (SMAC).

Table 4.2 Effect of beef jerky preparation procedure (dried at 62.5°C), drying time (0-10 h) and culture media for bacterial enumeration (tryptic soy agar--TSA and sorbitol MacConkey agar--SMAC) on survival of inoculated *E. coli* O157:H7 (log CFU/cm^2) and natural bacterial flora.

Processing steps & drying times (h)	Beef Jerky Preparation Procedure and Agar Media[a]							
	1[b]		2[c]		3[d]		4[e]	
	SMAC	TSA	SMAC	TSA	SMAC	TSA	SMAC	TSA
Following inoculation (4°C, 24 h) ***(a)***	5.3^{BaY} (0.6)	6.0^{AaY} (0.3)	7.1^{BaX} (0.2)	7.6^{AaX} (0.1)	7.3^{AaX} (0.1)	7.5^{AaX} (0.3)	7.1^{AaX} (0.5)	7.5^{AabX} (0.6)
Following Pre-drying step 1 ***(b)***	2.9^{AcZ} (0.4)	2.9^{AcZ} (0.5)	6.3^{BbY} (0.7)	7.5^{AaX} (0.0)	6.5^{BbY} (0.2)	6.9^{AbY} (0.1)	7.2^{BaX} (0.3)	7.7^{AaX} (0.3)
Log Change (a-b)	***-2.4***	***-3.1***	***-0.8***	***-0.1***	***-0.8***	***-0.6***	***+0.1***	***+0.2***
Following Pre-drying step 2 ***(c)***	4.0^{AbY} (0.2)	4.0^{AbY} (0.2)	4.0^{AcY} (0.5)	3.5^{BbZ} (0.9)	6.6^{AbX} (0.1)	6.7^{AbX} (0.1)	6.4^{BbX} (0.6)	7.0^{AbX} (0.2)
Log Change (b-c)	***+1.1***	***+1.1***	***-2.3***	***-3.0***	***+0.1***	***-0.2***	***-0.8***	***-0.7***
Log Change (a-c)	***-1.3***	***-2.0***	***-3.1***	***-4.1***	***-0.7***	***-0.8***	***-0.7***	***-0.5***
4	1.5^{AdY} (0.2)	1.8^{AdeY} (0.5)	1.5^{AdY} (0.4)	1.8^{AcY} (0.4)	2.4^{BcX} (0.1)	3.1^{AcX} (0.5)	2.7^{AcdX} (0.2)	3.0^{AcX} (0.3)
Log Change (c-4)	***-2.5***	***-2.2***	***-2.5***	***-1.7***	***-4.2***	***-3.6***	***-3.7***	***-4.0***
Log Change (a-4)	***-3.8***	***-4.2***	***-5.6***	***-5.8***	***-4.9***	***-4.4***	***-4.4***	***-4.5***
6	1.5^{BdZ} (0.1)	2.2^{AdY} (0.6)	1.7^{AdZ} (0.3)	2.0^{AcY} (0.3)	2.5^{BcY} (0.4)	3.0^{AcX} (0.4)	3.0^{AcX} (0.1)	2.9^{AcX} (0.3)
8	0.6^{BeZ} (0.5)	2.1^{AdeY} (0.4)	1.8^{AdY} (0.8)	2.0^{AcY} (0.6)	2.4^{AcX} (0.2)	2.3^{AdY} (0.3)	2.9^{AcdX} (0.3)	3.0^{AcX} (0.2)
10	0.8^{BeZ} (0.6)	1.7^{AeY} (0.7)	1.4^{BdY} (0.9)	1.8^{AcY} (0.7)	2.4^{AcX} (0.2)	2.3^{AdX} (0.2)	2.4^{AdX} (0.2)	2.7^{AcX} (0.4)
Log Change (4-10)	***-0.7***	***-0.1***	***-0.1***	***0.0***	***0.0***	***-0.8***	***-0.3***	***-0.3***
Log Change (a-10)	***-4.5***	***-4.3***	***-5.7***	***-5.8***	***-4.9***	***-5.2***	***-4.7***	***-4.8***

[A-B] Means with different superscripts within a treatment row are significantly ($P < 0.05$) different.
[a-e] Means with different superscripts within each column are significantly ($P < 0.05$) different.
[X-Z] Means with different superscripts within each agar medium in a row are significantly ($P < 0.05$) different.
[a] Means represent three values from two dehydrators (n = 6) (standard deviation) of log colony forming units (CFU) cm^2; lowest detection limit by plating, 1 log CFU/cm^2 for treatments 1-4.
[b] Pre-drying step 1: dipping in boiling water (95°C, 15 sec); pre-drying step 2: marinating (4°C, 24 h)
[c] Pre-drying step 1: seasoning with pickling spices (4°C, 24 h); pre-drying step 2: dipping in hot pickling brine (78°C, 90 sec)
[d] Pre-drying step 1: dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec); pre-drying step 2: marinating (4°C, 24 h)
[e] Pre-drying step 1: marinating (4°C, 24 h); pre-drying step 2: dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec)

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drying step 2, treatment 4 (slices dipped in 2.5 % acetic acid at 57.5°C) achieved significant ($P < 0.05$) bacterial log reductions of 0.8 and 0.7 log CFU/cm^2 with SMAC and TSA, respectively; corresponding total pre-drying reductions were 0.7 and 0.5 log CFU/cm^2. These results do not support results indicating soy sauce had anti-*E. coli* O157:H7 effects (Masuda et al., 1998). While treatment 1 achieved bacterial reductions of 2.4-3.1 log CFU/cm^2 following pre-drying step 1, significant ($P < 0.05$) growth was seen following pre-drying step 2 (+1.1 log CFU/cm^2). This significant growth indicated that cells were able to recover when marinated (4°C, 24 h) and possibly, the jerky marinade (Reynolds and Williams, 1993) supplied the environment necessary for the inoculated cells to recover and multiply.

In this study, treatment 2 (slices dipped in pickling brine at 78°C for 90 sec) had significant ($P < 0.05$) bacteria reduction, but a 5-log reduction was not achieved. Treatment 1 (slices dipped in boiling water (95°C) for 15 sec) had significant ($P < 0.05$) bacteria reduction, but a 5-log reduction was also not achieved.

Bacterial reduction after 4 h of drying

After 4 h of drying, treatment 1 achieved reductions of 2.5 and 2.2 log CFU/cm^2 with SMAC and TSA, respectively, compared to counts after both pre-drying steps; corresponding total reductions were 3.8 and 4.2 log CFU/cm^2 (Table 4.2). In contrast, treatment 2 achieved log reductions of 2.5 and 1.7 log CFU/cm^2 with SMAC, and TSA, respectively, with total reductions of 5.6 and 5.8 log CFU/cm^2 on SMAC and TSA, respectively. After 4 h of drying, treatment 3 achieved reductions of 4.2 and 3.6 log CFU/cm^2 with SMAC and TSA; corresponding total reductions were 4.9 and 4.4 log CFU/cm^2. In addition, treatment 4 achieved reductions of 3.7 and 4.0 log CFU/cm^2 with

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SMAC and TSA, with total reductions of 4.4 and 4.5 log CFU/cm^2 with SMAC and TSA, respectively.

Results after exposure to vinegar do not support those of Entani et al. (1998), who reported that vinegar (5% acetic acid) had a bactericidal effect on foodborne bacteria, including *E. coli* O157:H7. Entani et al. (1998) also reported that when acetic acid (2.5%) and NaCl (5%) were heated, there was a marked effect on the destruction of foodborne bacteria. In this study, although exposure to vinegar (57.5°C) had a slight effect on the inoculum, subsequent drying for 4 h (62.5°C) reduced counts by a total of 4.4 to 4.9 log CFU/cm^2.

Bacterial reduction after 10 h of drying

Between 4 and 10 h of drying, treatment 1 resulted in reductions of 0.7 and 0.1 log CFU/cm^2 on SMAC and TSA, respectively; total reductions after 10 h of drying were 4.5 and 4.3 log CFU/cm^2 with SMAC, and TSA, respectively (Table 4.2). Similarly, no significant bacterial destruction occurred between 4 to 10 h of drying for treatments 2, 3 and 4 (0.0 and 0.8 log CFU/cm^2). Of all treatments, treatment 2 had the highest total (inoculum minus counts after 10 h of drying) bacterial reduction after 10 h of drying (5.7 and 5.8 log CFU/cm^2 for SMAC, and TSA, respectively). Treatments 3 and 4 reached total bacterial reductions of 4.7 to 5.2 log CFU/cm^2.

Results of this study are in partial agreement with results from Harrison and Harrison (1996) and Harrison et al. (1998). Harrison and Harrison (1996) investigated whole muscle beef jerky prepared using a traditional method (Reynolds and Williams, 1993), as well as pre-heating the meat to 71.1°C (160°F) (time unspecified) before drying (USDA-FSIS, 1998). Harrison and Harrison (1996) found that pre-heated and unheated

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meat had a 5-log reduction when dried at 60°C (140°F) for 10 h in a home-type dehydrator. In this study, meat heated at 78°C (172°F) for 90 sec, then dried for 10 h at 62.5°C (145°F) achieved ≥ 5-log reduction (5.7-5.8 log CFU/cm^2, for SMAC and TSA, respectively). However, meat pre-heated at 95°C (203°F) for 15 sec did not reach a 5-log reduction (4.5-4.3 log CFU/cm^2, for SMAC and TSA, respectively). Differences of time and temperature during the pre-heating processes in this study could have accounted for differences in log reductions. In addition, the marinating step (4°C, 24 h) following the boiling water dip could have allowed sufficient time for the heat shocked bacteria to regenerate and become stronger.

Harrison et al. (1998) evaluated ground beef jerky prepared with a spice (ingredients not listed)/cure (salt and sodium nitrite) mix and dried at 60°C (140°F) for either 6 or 8 h. The authors indicated that reductions of 5.2 log CFU/g were achieved in pre-heated and unheated jerky, containing the spice and cure mix. In this study, a cure mix was not evaluated and only treatment 2 included jerky preparation using spices (salt, sugar and black pepper). However, it must be noted that treatment 2 was the only treatment that achieved ≥ 5-log reduction. Thus, it is presumed that the combination of pre-drying steps 1 and 2--seasoning with pickling spices (4°C, 24 h) and dipping in hot pickling brine (78°C, 90 sec)--were responsible for the significant ($P < 0.05$) bacterial reduction seen. However, it must be noted that previous research has not been done at higher elevations (4900) or lower relative humidity. Thus, these geographical factors (elevation and humidity) may account for some of the differences noted, as described in paragraphs below.

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D-values

Treatments were designed to combine several processing techniques used in preservation or the use of a "multiple hurdle" concept (Leistner, 1987, 1995). The multiple hurdle concepts incorporated common preservative techniques (such as temperature, acidity, and water activity) in combination that result in a safer and more stable food product (Riordan et al., 1998). By combining several preservation techniques, chances an existing microorganism will survive or reproduce in the food product are decreased (Leistner, 1987, 1995).

In this study, regression values were calculated and D-values (Appendix D, Table 4.2) were determined for each jerky treatment for the total preparation time (pre-drying steps 1 and 2 with drying time) and drying time (0 to 10 h) period (Fig 4.2). For total preparation time, the D-values for treatment 1 were 1.4 and 1.7 h with SMAC and TSA, respectively, with corresponding correlation coefficients of 0.81 and 0.65. For drying time, the D-values for treatment 1 were 1.4 and 2.3 h for counts determined with SMAC and TSA, respectively, with corresponding correlation coefficients of 0.72 and 0.52. For treatment 2, D-values for total preparation time were 1.0 and 0.9 h with SMAC and TSA, respectively, and corresponding correlation coefficients of 0.82 and 0.75; for drying time, corresponding D-values were 2.0 and 3.2, with correlation coefficients of 0.52 and 0.47. Total preparation time D-values for treatment 3 were 1.0 h for both media and correlation coefficients were 0.78 and 0.86 with SMAC and TSA, respectively. For drying time, the D-values for treatment 3 were 1.2 and 1.0 h for SMAC and TSA, respectively, with correlation coefficients of 0.5 and 0.68. Finally, for total preparation time, treatment 4 had D-values of 1.1 and 1.0 h with SMAC and TSA, respectively, and corresponding

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*Sorbitol MacConkey Agar--SMAC
Tryptic Soy Agar--TSA
1
2
3
4
* ** *** 4 6 8 10
Drying time (h)

Figure 4.2 Changes in bacterial counts (Y- axis = log CFU/cm^2) on beef jerky during pre-drying preparation. Treatments of inoculated meat slices included (1) Pre-drying step 1: dipping in boiling water (95°C, 15 sec), pre-drying step 2: marinating (4°C, 24 h); (2) Pre-drying step 1: seasoning with pickling spices (4°C, 24 h), pre-drying step 2: dipping in hot pickling brine (78°C, 90 sec); (3) Pre-drying step 1: dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec), pre-drying step 2: marinating (4°C, 24 h); (4) Pre-drying step 1: marinating (4°C, 24 h), pre-drying step 2: dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec). Counts were enumerated with tryptic soy agar (TSA) and sorbitol MacConkey agar (SMAC). Means are three values from two dehydrators (n=6). Data from Tables 4.2 and 4.3; error bars indicate standard deviation; * indicates inoculation (4°C, 24 h), ** indicates following pre-drying step 1; *** indicates following pre-drying step 2. Lowest detection limit by plating, 1 log CFU/cm^2

correlation coefficients of 0.81 and 0.79; for drying time, corresponding D-values were 1.3, and 1.2 with correlation coefficients of 0.57, and 0.55. Due to the low correlation coefficients, these D-values may underestimate destruction that occurred during the first 4 h of drying.

It is interesting to note the "tail" present in the survivor curves. For all treatments, population counts reached a plateau after 4 h. Even after 10 h of drying, a residual population of cells was not destroyed (0.8-2.7 log CFU/cm^2). Perhaps, there was no further destruction of bacteria due to casehardening (McWilliams, 1993) occurring on the surface of the jerky slices when dried at low heat (62.5°C) for several hours (0-4 h). As the temperature of circulating air within the dehydrator continued to climb toward the desired temperature (62.5°C), initial drying may have created a protective coating (McWilliams, 1993) making continuous destruction unachievable. This effect may have been enhanced by the dry climate of Colorado. The relative humidity (Thermo-Hygro, VWR Scientific Products, West Chester, PA) within and outside the dehydrators ranged from 4-10% and 19-24%, respectively.

Effect of culture media

As indicated, two culture media were used for bacterial enumeration: TSA and SMAC. TSA is a general-purpose medium used for enumeration of living bacterial cells, as well as injured cells, or total bacterial populations (McCarthy et al., 1998). Because counts obtained with nonselective TSA may include bacterial species other than inoculated *E. coli* O157:H7 present on the meat, SMAC was used as more selective for sorbitol negative bacteria indicating *E. coli* O157:H7 cells and related organisms (McCarthy et al., 1998). However, SMAC has been reported to be inhibitory to stressed

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E. coli O157:H7 cells and showed the least recovery of injured cells, with the most reduction in log CFU/cm^2 (Clavero et al., 1998; McCarthy et al., 1998; Faith et al., 1998b; Abdul-Raouf et al., 1993a, b). In this study, TSA tended to yield higher counts than SMAC, but overall there was no evidence of major bacterial injury, except for treatment 1 at 6, 8 and 10 h of drying (Table 4.2).

Effect of storage

Limited work has been done on the survival of inoculated *E. coli* O157:H7 during storage of jerky. Harrison and Harrison (1996) found no detectable levels of *E. coli* O157:H7 by direct plating or enrichment following 8 weeks of storage at 25°C. However, their results indicated a 5-log reduction of bacteria had been achieved in their drying procedures. In this study, jerky was analyzed following storage for 30, 60 and 90 d (Table 4.3). For all jerky treatments, after 30 d of storage, significant ($P < 0.05$) bacterial reduction was achieved since counts became undetectable (< 1.0 log CFU/cm^2) by direct plating, compared to counts at 0 d (0.8-2.7 log CFU/cm^2). After 60 and 90 d, populations were not different from populations obtained after 30 d. There was indication of mold growth on some pieces from treatment 1 after 30, 60 and 90 d of storage. There was no evidence of mold growth on the other treatments.

Product water activity and pH

Water activity is a measure of the quantity of available water in food that is available to take part in chemical reactions and microbial growth (Leistner, 1987; Lenovich, 1987). Drying beef jerky is a food preservation technique, which removes moisture and decreases a_w. The growth of some microorganisms is inhibited by reduced pH levels and the combination of lower pH with the reduction of a_w provides addition

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Table 4.3 Effect of storage (0, 30, 60 and 90 d) at 21°C, beef jerky preparation procedure (dried at 62.5°C), drying time (0-10 h) and culture media for bacterial enumeration (tryptic soy agar--TSA and sorbitol MacConkey agar--SMAC) on survival of inoculated *E. coli* O157:H7 (log CFU/cm^2) and natural bacterial flora.

Storage (d)	Beef Jerky Preparation Procedure and Agar Media[a]							
	1[b]		2[c]		3[d]		4[e]	
	SMAC	TSA	SMAC	TSA	SMAC	TSA	SMAC	TSA
0	0.8^{BaZ} (0.6)	1.7^{AaY} (0.7)	1.4^{AaY} (0.9)	1.8^{AaY} (0.7)	2.4^{AaX} (0.2)	2.3^{AaX} (0.2)	2.4^{AaX} (0.2)	2.7^{AaX} (0.4)
30	$< 1.0^{AbX}$ (0.1)	$< 1.0^{AbX}$ (0.0)	$< 1.0^{AbX}$ (0.0)	$< 1.0^{AbX}$ (0.0)	$< 1.0^{AbX}$ (0.0)	$< 1.0^{AbX}$ (0.0)	$< 1.0^{AbX}$ (0.0)	$< 1.0^{AbX}$ (0.0)
60	$< 1.0^{AbX}$ (0.1)	$< 1.0^{AbX}$ (0.1)	$< 1.0^{AbX}$ (0.0)	$< 1.0^{AbX}$ (0.0)	$< 1.0^{AbX}$ (0.0)	$< 1.0^{AbX}$ (0.0)	$< 1.0^{AbX}$ (0.1)	$< 1.0^{AbX}$ (0.1)
90	$< 1.0^{AbX}$ (0.1)	$< 1.0^{AbX}$ (0.1)	$< 1.0^{AbX}$ (0.0)	$< 1.0^{AbX}$ (0.0)	$< 1.0^{Ab}$ (0.1)	$< 1.0^{AbX}$ (0.1)	$< 1.0^{AbX}$ (0.1)	$< 1.0^{AbX}$ (0.1)

[A-B] Means with different superscripts within each treatment in a row are significantly ($P < 0.05$) different.
[a-b] Means with different superscripts within each column are significantly ($P < 0.05$) different.
[X-Z] Means with different superscripts within each agar medium in a row are significantly ($P < 0.05$) different.
[a] Means represent three values from two dehydrators (n = 6) (standard deviation) of log colony forming units (CFU) cm^2 for 0 d and three values (n=3) (standard deviation) for 30-90 d; lowest detection limit by plating, 1 log CFU/cm^2, for all treatments.
[b] Pre-drying step 1: dipping in boiling water (95°C, 15 sec); pre-drying step 2: marinating (4°C, 24 h)
[c] Pre-drying step 1: seasoning with pickling spices (4°C, 24 h); pre-drying step 2: dipping in hot pickling brine (78°C, 90 sec)
[d] Pre-drying step 1: dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec); pre-drying step 2: marinating (4°C, 24 h)
[e] Pre-drying step 1: marinating (4°C, 24 h); pre-drying step 2: dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec)

protection against growth of bacteria (Leistner, 1987; Lenovich, 1987). It is essential that beef jerky be dried to an a_w acceptable for proper shelf-life (Leistner, 1987). In order for a product such as beef jerky to be microbiologically stable, the a_w should be ≤ 0.68 (Leistner, 1987).

In this study, average a_w values ranged from 0.93 to 0.94 following inoculation and remained relatively constant through both pre-drying steps (Table 4.4). After 4 h of drying, average a_w values decreased to 0.89, 0.84, 0.84 and 0.87 for treatments 1-4, respectively, and declined steadily throughout the drying period. After 8 h of drying, the average a_w for treatment 2 (0.68) indicated a microbiologically stable jerky product (Leistner, 1987), while treatments 3 and 4 achieved acceptable average a_w values after 10 h of drying (0.50 and 0.59, respectively). After 10 h of drying, treatment 1 achieved an average a_w value of only 0.75. However, following storage for 90 d the average a_w decreased to 0.64. In addition, the a_w from jerky slices for the other treatments (2-4) fluctuated throughout storage when compared to the final a_w.

In this study, pH values varied throughout the drying process from 5.56 to 6.04, 5.76 to 6.23, 5.20 to 5.75, and 5.26 to 5.68 for treatments 1 to 4, respectively (Table 4.5). Differences existed between treatments 1 and 2, but, differences were slight (0.42 to 0.58). In addition, the pH values varied among the treatments during storage (30-90 d). Differences could be due to variations among the beef raw meat used for the samples, although initial pH values taken on the raw beef ranged from 5.6 to 5.9, within the range of acceptable pH for raw whole muscle beef (McWilliams, 1993).

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Table 4.4 Effect of beef jerky preparation procedure (dried at 62.5°C), pre-drying and drying time (0-10 h) and storage (30, 60 and 90 d) on mean water activity (a_w) values of *E. coli* O157:H7 (log CFU/cm^2) inoculated beef slices.

	Pre-Drying			Drying time (h) [a]				Storage (d)		
Jerky Treatment	Following inoculation (4°C, 24 h)	Following Pre-drying step 1	Following Pre-drying Step 2	4	6	8	10	30	60	90
1 [b]	0.94 (0.00)	0.94 (0.00)	0.93 (0.00)	0.89 (0.04)	0.87 (0.03)	0.89 (0.02)	0.75 (0.11)	0.73	0.67	0.64
2 [c]	0.93 (0.01)	0.93 (0.01)	0.93 (0.00)	0.84 (0.02)	0.70 (0.05)	0.68 (0.00)	0.59 (0.21)	0.65	0.63	0.61
3 [d]	0.93 (0.00)	0.92 (0.00)	0.93 (0.00)	0.84 (0.02)	0.81 (0.02)	0.76 (0.04)	0.50 (0.04)	0.61	0.55	0.54
4 [e]	0.94 (0.01)	0.93 (0.00)	0.93 (0.01)	0.87 (0.00)	0.81 (0.02)	0.71 (0.10)	0.59 (0.02)	0.62	0.53	0.52

[a] Means represent one value from two dehydrators (n = 2) (standard deviation) for pre-drying and drying time and one dehydrator (n=1) for 30-90 d.

[b] Pre-drying step 1: dipping in boiling water (95°C, 15 sec); pre-drying step 2: marinating (4°C, 24 h)

[c] Pre-drying step 1: seasoning with pickling spices (4°C, 24 h); pre-drying step 2: dipping in hot pickling brine (78°C, 90 sec)

[d] Pre-drying step 1: dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec); pre-drying step 2: marinating (4°C, 24 h)

[e] Pre-drying step 1: marinating (4°C, 24 h); pre-drying step 2: dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec)

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Table 4.5 Effect of beef jerky preparation procedure (dried at 62.5°C), pre-drying and drying times (0-10 h) and storage (30, 60 and 90 d) on mean pH values of *E. coli* O157:H7 (log CFU/cm^2) inoculated beef slices.

	Pre-Drying			Drying time (h) [a]				Storage (d)		
Jerky Treatment	Following inoculation (4°C, 24 h)	Following Pre-drying step 1	Following Pre-drying step 2	4	6	8	10	30	60	90
1 [c]	5.56 (0.00)	5.78 (0.00)	5.46 (0.18)	5.73 (0.04)	5.74 (0.00)	6.04 (0.10)	5.92 (0.14)	6.22	5.86	5.66
2 [d]	5.83 (0.10)	5.76 (0.07)	5.91 (0.00)	6.09 (0.06)	6.04 (0.01)	6.23 (0.06)	6.00 (0.13)	6.05	6.02	5.97
3 [e]	5.75 (0.04)	5.38 (0.11)	5.20 (0.00)	5.52 (0.06)	5.54 (0.01)	5.60 (0.05)	5.48 (0.05)	5.46	5.22	5.48
4 [f]	5.68 (0.19)	5.46 (0.12)	5.26 (0.07)	5.43 (0.05)	5.48 (0.20)	5.45 (0.20)	5.39 (0.01)	5.45	5.14	5.56

[a] Means represent triplicate readings from one sample each from two dehydrators (n = 2) (standard deviation) for pre-drying and drying time and one dehydrator (n=1) for 30-90 d.

[b] Pre-drying step 1: dipping in boiling water (95°C, 15 sec); pre-drying step 2: marinating (4°C, 24 h)

[c] Pre-drying step 1: seasoning with pickling spices (4°C, 24 h); pre-drying step 2: dipping in hot pickling brine (78°C, 90 sec)

[d] Pre-drying step 1: dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec); pre-drying step 2: marinating (4°C, 24 h)

[e] Pre-drying step 1: marinating (4°C, 24 h); pre-drying step 2: dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec)

All raw pre-inoculated meat ranged in pH from 5.60 to 5.90

CONCLUSIONS

Because of mandates from the USDA-FSIS, manufacturers of dried and semi-dried fermented meats are required to reach a 5-log reduction in bacteria to ensure the safety of the meat products. Using that mandate as a guideline, food-processing techniques have been evaluated for their potential to achieve this goal. The "multi-hurdle" concept is a useful strategy in food processing, since it often involves combinations of antimicrobial factors that lead to safe products. When factors are combined, they can act as barriers or hurdles to prevent microbial growth. In this study, beef jerky inoculated with *E. coli* O157:H7 was subjected to four treatments, each using two pre-drying steps. After 4 h of drying, significant ($P < 0.05$) destruction was achieved (3.8-4.2, 5.6-5.8, 4.4-4.9, and 4.4-4.5, for treatments 1-4, respectively). Although, bacterial counts continued to decline slowly during the 10 h of drying, a $\geq$ 5-log reduction was only achieved for treatment 2 (5.7-5.8 log CFU/cm^2). After 10 h of drying, treatments 1, 3 and 4 achieved log reductions of 4.3-4.5, 4.9-5.2, and 4.7-4.8, respectively. In addition, after 8 h of drying, treatment 2 achieved reductions $\geq$ 5 log CFU/cm^2, as well as obtaining a_w values acceptable for microbiologically safe products ($\leq$ 0.68). Thus, treatment 2 is recommended for preparation of home-dried jerky, providing the consumer finds the product acceptable.

Treatment 1 is not recommended and should be re-investigated, using higher inoculum levels to determine whether a 5-log reduction could be achieved. In addition, the use of preservatives could be investigated to enhance the treatment's shelf life, since the product had a high a_w value.

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After 10 h of drying, treatments 3 and 4 achieved log reductions of 4.7-5.2 log CFU/cm^2. These treatments showed promise as acceptable treatments, but further work must be done. Future work should further investigate these treatments possibly to improve destruction by modifying exposure and/or levels of acetic acid used. Acidulants, such as ascorbic or citric acid, could be investigated as supplements or substitutions. After 30 d of storage, counts in all jerky treatments were significantly ($P < 0.05$) lower ($<$ 1.0 log CFU/cm^2) than those at 0 d (0.8 to 2.7 log CFU/cm^2). This observation needs further confirmation.

The following chapter focuses on the four pre-drying treatments subjected to consumer evaluation for Appearance, Dryness, Texture, Flavor and Overall Acceptability.

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CHAPTER V

SENSORY PROPERTIES OF BEEF JERKY PROCESSED UNDER VARIOUS CONDITIONS

ABSTRACT

The United States Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) recommends a 5-log reduction in *Escherichia coli* O157:H7 in processed dried and semidry fermented foods to be considered safe. Discrepancies exist as to whether current practices for home dried jerky preparation can achieve such reduction. Therefore, USDA-FSIS recommends pre-heating meat to 71.1°C (160°F) before drying at 54.5-60°C (130-140°F). However, consumers may object to the pre-heated flavor of jerky prepared in this manner.

The objective of this study was to evaluate consumer responses to uninoculated beef jerky prepared following four treatments, each involving two pre-drying steps and found to result in a 4.3 to 5.8 log CFU/cm^2 reduction of inoculated *E. coli* O157:H7 populations.

Meat slices were subjected to four pre-drying treatments, involving two pre-drying steps each: 1--dipping in boiling water (95°C, 15 sec), followed by marinating (4°C, 24 h); 2--seasoning with pickling spices (4°C, 24 h), followed by dipping in hot pickling brine (78°C, 90 sec); 3--dipping in warm acetic acid solution (57.5°C, 2.5%, 20

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sec), followed by marinating (4°C, 24 h); 4--marinating (4°C, 24 h), followed by dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec).

The marinade used in treatments 1, 3, and 4 consisted of 60 ml Kikkoman soy sauce, 15 ml Heinz Worcestershire sauce, 0.6 g Schilling black pepper, 1.25 g Kroger garlic powder, 1.5 g Kroger onion powder, and 4.35 g Spice Islands hickory smoke-flavored salt per kg of meat slices marinated (65 slices of 15 g each). Each meat slice received 1 ml of marinade, with 0.5 ml distributed evenly over each top and bottom surface. The pickling spices used in treatment 2 consisted of 27 g Morton iodized salt, 12 g Food Club granulated sugar and 1.9 g Schilling black pepper per kg of meat slices and the pickling brine consisted of 108 g Morton iodized salt, 48 g Food Club granulated sugar and 5.8 g Schilling black pepper per gallon of water. For treatment 2, the pickling spices were evenly distributed (0.6 g per slice) over the top and bottom surface of meat slices.

Product was dried in home-type dehydrators at 62.5°C (air). All treatments were dried for 10 h, except for treatment 2, which was dried for 8 and 10 h. A taste panel was conducted (n=120) at three central locations. Sensory characteristics (Appearance, Dryness, Texture, Flavor, and Overall Acceptability) were evaluated on a 7-point scale and a commercial jerky was used as a reference control.

Commercial jerky received significantly ($P < 0.05$) higher scores (4.9 to 5.6) than experimental products (3.4 to 5.5) for most sensory characteristics. Although differences among experimental products were slight, treatment 4 was preferred ($P < 0.05$) over treatment 2, dried for 10 h. This was unfortunate, since treatment 2 was the only treatment found to achieve ≥ 5-log reduction of inoculated *E. coli* O157:H7 (Chapter IV).

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Consumer results in this study indicated moderate acceptance of the five experimental jerky products. This information is useful in forming recommendations to produce flavorful home-dried jerky, while minimizing the risk of *E. coli* O157:H7.

INTRODUCTION

Consumer evaluation is used as routine checks of the food's quality throughout the production process (Merolli, 1980; Tassan, 1980). The food industry relies heavily on sensory evaluation for the development of new products, in order to maintain the quality of food items (Meilgaard et al., 1987; Moskowitz, 1988). Consumer evaluation can help a company evaluate a product before distribution and help handle arising problems (Skinner, 1980). Researchers within food companies use evaluation techniques to predict the acceptability of a food or potential concerns with a product that were not addressed earlier. Food products can be evaluated on several characteristics; depending on the intended purpose, these characteristics may require different evaluation techniques (Meilgaard et al., 1987). An objective evaluation measures the physical properties of a food, such as texture or moisture content (Stone and Sidel, 1993; Moskowitz, 1988). However, properties can also be evaluated subjectively through a consumer panel or trained panel.

Sensory or subjective evaluation is done by individuals using a scoring system that is based on various characteristics using the senses (Lawless and Klein, 1991). Measurements can be determined by using the senses: sight, smell, taste, and touch (Lawless and Klein, 1991). Sensory evaluation is a vital source of information for research and quality control. A food can have several characteristics requiring sensory evaluation, including flavor (taste and odor), texture, aroma, and appearance. First,

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flavor can be tested through a composite assessment of tastes that are blended with odor occurring in the mouth. Flavor is a very important attribute, yet one that is often difficult to communicate (Lawless and Klein, 1991). Next, texture can be evaluated. Texture can be related to the food's appearance, as well as the mouthfeel, so one way to assess texture is to test the food's toughness or brittleness. This test can be evaluated by the number of chews required before the bite may be swallowed (Lawless and Klein, 1991). Another sensory characteristic often tested is aroma, which is the odor associated with the food. Aroma is important if the food needs to be served at warm or hot temperatures. According to Stone and Sidel (1993), aroma is often associated with the overall acceptability of the food. Finally, the appearance of the food can be evaluated. The appearance of the food is important since color triggers the connotation of particular flavors and provides acceptability of the exterior and interior appearance (cell size, uniformity, thickness) of the food (Lawless and Klein, 1991).

The Institute of Food Technologists (IFT) Sensory Evaluation Division (IFT, 1981) has provided recommended guidelines for sensory evaluation. Member selection should be based on factors identified as important for the specific study (IFT, 1981; Stone and Sidel, 1993). There are several types of taste panels and one type of taste panel is a consumer panel. Consumer taste panels may consist of trained or untrained panelists. Trained sensory panelists are instructed extensively on procedures that will evaluate the samples and provide guidance for improving the product (IFT, 1981; Stone and Sidel, 1993; Meilgaard et al., 1987). A consumer panel with untrained panelists often selects people who happen to be available at a testing site, are willing to participate in the panel

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and have no preparation regarding evaluation of the product (Stone and Sidel, 1993). However, for both taste panels, the environment must be conductive to the testing.

When conducting sensory evaluation, several types of tests may be used. One type is preference testing. This test is used to determine the consumer preference or acceptability between new or old products. Preference testing is valuable in developing new food products or evaluating the quality of new items. Another type of sensory test is difference testing. Difference testing is used to determine the consumer's ability to distinguish differences among products and can be used to test the sensitivity of the judges when ingredients are replaced or substituted (Stone and Sidel, 1993; Lawless and Klein, 1991; Meilgaard et al., 1987).

The United States Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) recommends a 5-log reduction in *E. coli* O157:H7 for the processing of fermented and semidry foods to be considered safe (Billy, 1997; Reed, 1995). Discrepancies exist as to whether current practices for home dried jerky preparation can achieve a 5 log reduction of inoculated bacteria (Harrison and Harrison, 1996; Harrison et al., 1998; Faith et al., 1998a; Keene et al., 1997). Current recommendation state meat slices should be pre-heated to 71.1°C (160°F), before drying at 54.5-60°C (130-140°F) (USDA-FSIS, 1998). While safe, many consumers may object to the heated flavor of jerky prepared in this manner. To date, limited research has been conducted on sensory evaluation of beef jerky (Farouk and Swan, 1999; Carr et al., 1997; Sutton et al., 1993) and no research has been conducted assessing the consumer acceptance to pre-heated jerky preparation methods.

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The objective of this study was to evaluate consumer responses to uninoculated beef jerky prepared following four jerky pre-drying treatments, each involving two pre-drying steps. These treatments were found (Chapter IV) to result in a 4.3 to 5.8 log CFU/cm^2 reduction of inoculated *E. coli* O157:H7 populations. Commercial beef jerky served as a control in the sensory evaluation, since the traditional method of home drying beef jerky did not result in a 5 log reduction of inoculated *E. coli* O157:H7 cells (Chapter III). A taste panel (n=120) was conducted at three central locations to determine the acceptability of the products. Treatment methods were evaluated and five sensory characteristics typical of beef jerky were compared.

MATERIALS AND METHODS

Study design

Meat slices were subjected to four pre-drying treatments, each involving two pre-drying steps: 1--dipping in boiling water (95°C, 15 sec), followed by marinating (4°C, 24 h); 2--seasoning with pickling spices (4°C, 24 h), followed by dipping in hot pickling brine (78°C, 90 sec); 3--dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec), followed by marinating (4°C, 24 h); 4--marinating (4°C, 24 h), followed by dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec). The products were dried in home-type dehydrators at 62.5°C (air). All treatments were dried for 10 h, except for treatment 2, which was dried for 8 and 10 h.

The marinade used in treatments 1, 3, and 4 consisted of 60 ml Kikkoman soy sauce, 15 ml Heinz Worcestershire sauce, 0.6 g Schilling black pepper, 1.25 g Kroger garlic powder, 1.5 g Kroger onion powder, and 4.35 g Spice Islands hickory smoke-flavored salt per kg of meat slices marinated (65 slices of 15 g each) (Reynolds and

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Williams, 1993) (pH = 4.3). Each meat slice received 1 ml of marinade, with 0.5 ml distributed evenly over each top and bottom surface. The pickling spices used in treatment 2 consisted of 27 g Morton iodized salt, 12 g Food Club granulated sugar and 1.9 g Schilling black pepper, per kg of meat slices and the pickling brine consisted of 108 g Morton iodized salt, 48 g Food Club granulated sugar and 5.8 g Schilling black pepper per gallon of water (Cooperative Extension Service, University of Wyoming, 1984). For treatment 2, the pickling spices were evenly distributed (0.6 g per slice) over the top and bottom surface of meat slices.

Preparation of beef pieces

Vacuum packaged beef inside rounds (approximately 30 kg) were purchased frozen from local supermarket warehouses and stored at -18.0°C until use (i.e., within one week). The meat was removed from the freezer and cut, while still frozen, into slices (8.7 x 4.0 x 0.6 cm; 3.4 x 1.6 x 0.3 inches) with a band saw at the Colorado State University Meat Science Laboratory (Fort Collins, CO). Slices were randomly placed in 1-kg portions in plastic bags (20.3 x 25.4 cm; 0.75 mil nylon, 2.25 mil polyethylene laminate; oxygen transmission rate 3.5 cc/2.54 sq. m/24h; Kapak Heat Sealable, Fisher Scientific, Springfield, NJ). The bags were evacuated (29 mm/Hg), heat sealed (Multivac, Sepp Haggenmüller KG, Allgau, Germany), then frozen (-18°C) to minimize potential changes until use (i.e., within two weeks).

Jerky preparation

Treatment 1

Pre-drying step 1: Beef slices were placed in a single layer on the bottom of a 2-qt steamer basket, submerged in a 2-qt stainless steel pot of boiling water (95°C, 15 sec),

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removed rapidly and drained. Remaining meat pieces were submerged using the same method. After two uses, water was emptied and replaced with fresh water.

Pre-drying step 2: Within 30 min following the dipping process, each meat slice received 1 ml of marinade, with each 0.5 ml portion distributed evenly over each top and bottom surface. Marinade was spread with a sterile bent glass rod to ensure adequate coverage of the meat pieces. Marinated pieces were placed in aluminum pans, covered with heavy-duty aluminum foil, and stored (4°C, 24 h) before drying.

Treatment 2

Pre-drying step 1: Pickling spices (1.6 g per meat slice) were sprinkled evenly over meat slices (top and bottom). The slices were pounded with a rubber mallet, covered with heavy-duty aluminum foil and stored (4°C, 24 h).

Pre-drying step 2: Pickling brine was prepared in a 2-qt stainless steel pot and heated to 78°C (172°F). Beef slices were placed in a single layer on the bottom of a 2-qt steamer basket submerged (90-sec), removed rapidly and drained. Remaining meat pieces were submerged using the same method. After two uses, brine was emptied and replaced with fresh brine. Immediately following the dipping process, meat slices were dried.

Treatment 3

Pre-drying step 1: In a 2-qt stainless steel pot, equal parts (3 c) of distilled white vinegar (5% acetic acid) and distilled water were combined and heated on a convection oven burner to 57.5°C (135°F). Beef slices were placed in a single layer on the bottom of a 2-qt steamer basket submerged (20-sec), removed rapidly and drained. Remaining meat

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pieces were submerged using the same method. After two uses, the solution was emptied and replaced with fresh solution.

Pre-drying step 2: Within 30 min of the dipping process, each meat slice received 1 ml of marinade, with each 0.5 ml portion distributed evenly over each top and bottom surface. Marinade was spread with a sterile bent glass rod to ensure adequate coverage of the meat pieces. Marinated pieces were placed in aluminum pans, covered with heavy-duty aluminum foil, and stored (4°C, 24 h) before drying.

Treatment 4

Pre-drying step 1: Each meat slice received 1 ml of marinade, with each 0.5 ml portion distributed evenly over each top and bottom surface. Marinade was spread with a sterile bent glass rod to ensure adequate coverage of the meat pieces. Marinated pieces were placed in aluminum pans, covered with heavy-duty aluminum foil, and stored (4°C, 24 h).

Pre-drying step 2: In a 2-qt stainless steel pot, equal parts (3 c) of distilled white vinegar (5% acetic acid) and distilled water were combined and heated on a convection oven burner to 57.5°C (135°F). Beef slices were placed in a single layer on the bottom of a 2-qt steamer basket submerged (20-sec), removed rapidly and drained. Remaining meat pieces were submerged using the same method. After two uses, the solution was emptied and replaced with fresh solution. Immediately following the dipping process, meat slices were dried.

Drying process and sample preparation

Two home food dehydrators (Gardenmaster® Dehydrator FD-1000, Nesco®/American Harvest®, Chaska, MN), with three dehydrator trays each, were used

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for the drying process. Dehydrators were preheated to 62.5°C/145°F (air) for at least 15 min prior to placement of the pre-treated meat pieces on drying trays. Treated meat slices were laid flat on the dehydrator trays, without touching other slices. Dehydrator drying trays were filled to capacity. Treatments were prepared on separate days and not combined in the dehydrators.

Circulating air within the dehydrators was monitored during the drying process with calibrated thermometers (Taylor Bi-Therm Thermometers, Oakbrook, IL) placed through the open circular top of each dehydrator, such that the sensor tip recorded the temperature of circulating air throughout the dehydrator.

Preparation of product for evaluation

After 8 (treatment 2 only) and 10 h of drying dehydrators were turned off. All meat pieces were removed aseptically and placed in 1-qt Ziploc freezer bags. Bags were left open, allowing meat pieces to cool in the bag at 21°C for 24 h. Commercial beef jerky (Oberto Homestyle Jerky, Oberto Sausage Company, Seattle, WA) was purchased (Sam's Warehouse, Fort Collins, CO) and used as a control. Ingredients listed on the label (Appendix E, Figure E. 5.1) of the Oberto Homestyle jerky included beef, corn syrup, dextrose, water, hydrolyzed soy protein, salt, soy flour, flavorings, sodium erythorbate and sodium nitrite.

Using a table of random numbers (O' Mahony, 1986), three-digit numbers were selected for the five jerky treatments and the one commercial beef jerky. Using these assigned numbers, individual Ziploc Snack-size bags were labeled with a three-digit number and given a color-code (Table 5.1). After all bags were labeled, one piece of jerky, corresponding to the designated number and color, was selected randomly and

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placed in a bag. Once all jerky pieces were placed in bags, one bag from each of the five jerky treatments, as well as one from the commercial jerky, was placed in a 1-qt Ziploc freezer bag. In addition to the six bags of jerky pieces, three unsalted crackers and two napkins were included in the bag. All work was done in a kitchen designed for food preparation (Colorado State University, Fort Collins, CO).

The Human Research Committee, Office of Regulatory Compliance, approved the scorecard used for sensory evaluation (Appendix A, Human Research Committee Approval). The scorecard included six characteristics for sensory evaluation, along with the corresponding description terms, as well as seven demographic/behavioral questions (Appendix B, Beef Jerky Taste Panel Scorecard). Directions were also provided explaining the evaluation process.

Evaluation collection

Subjects (n=120) were faculty, staff, students or visitors of Colorado State University (Fort Collins, CO). Subjects were asked to participate in a taste panel, lasting no longer than 30 minutes. Subjects signed the Informed Consent Form (Appendix A, Informed Consent Form) and received a scorecard, a tray containing the bag of six jerky samples and a plastic cup of tepid water.

A randomized complete block design (RCB) was used to randomly distribute the order of the presentation of jerky samples for sensory evaluation (Meilgaard et al., 1987). Sample order was equally distributed so that order of tasting was not a confounding factor. Each jerky mean score was evaluated (n=120) according to six sensory characteristics.

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Table 5.1. Jerky coding for the Consumer Taste Panel (n=120) of commercial jerky and jerky prepared following four pre-drying treatments and dried for 8 (treatment 2 only) and 10 h at 62.5°C (air) in home-type dehydrators.

Jerky treatment	Drying time (h)	Color-code	Three-digit number
• 1	10	Blue	957
• 2	8	Green	885
• 2	10	Pink	169
• 3	10	Yellow	478
• 4	10	White	646
• Commercial	N/A	Red	533

N/A = not applicable

Physical analyses

Meat pieces (10-15 g) from each jerky treatment were analyzed for water activity-a_w and pH. Water activity was determined with a Rotronic Hygroskop DT water activity meter (Model D2100, Rotronic, Huntington, NY) calibrated with standard saturated salt solutions (1.000, distilled water; 0.973, K_2SO_4; 0.843, KCl; 0.753, NaCl; 0.743, $NaNO_3$ ± 0.01) (saturation points are 10.8% K_2SO_4; 26.5%, KCl; 26.5%, NaCl; 47.9%, $NaNO_3$). Meat slices were cut (0.5-1.0 g), placed in the a_w (PS14) cup and set in the a_w meter until the a_w reached a stable reading (via signal lights, 25°C). Each a_w reading was determined as a single value.

A Hanna Instruments, HI8424, (Woonsocket, RI) pH meter was used to determine the pH of the inoculated meat pieces. Meat pieces were weighed in stomacher bags. Appropriate amounts of distilled water were added to obtain a dilution of 1:10 and bags were pummeled (IUL Instruments Masticator, Barcelona, Spain) for 120 s. Bags were allowed to sit for at least 15 min before each pH reading was determined in triplicate and averaged.

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Statistical analyses

Data were subjected to the Statistical Analysis System (SAS Institute version 6.1, Cary, NC) for analysis of variance of main (fixed) effects (jerky treatments), as well as significant interactions between the treatments by demographic/behavior variables. When F values were significant ($P < 0.05$), least significant differences (LSD) for each main effect were determined using the mixed procedure (PROC MIXED) of SAS. The sensory characteristic "Moisture Content" was omitted from statistical analysis due to the combination of two different sensory properties (Moisture and Oiliness). All statistically significant effects were reported at the $P < 0.05$ level.

RESULTS AND DISCUSSION

The taste panel was conducted for three consecutive days (n=120) and results of panel responses to the demographic/behavior questions are shown on Table 5.2. Of the total respondents, 43% were male, with 57% being female. The majority of the subjects were students of Colorado State University (54%) and 67% of the respondents indicated that they were under 35 years of age. Most (70%) of the respondents had never made jerky, but 83% of the respondents indicated they liked to eat jerky. However, the majority of the subjects indicated that they only eat jerky once a year (58%), while 29% of the subjects responded that they eat jerky at least once a month. Finally, 54% of the subjects reported that they purchased jerky at least yearly, while 22 and 23% of the subjects reported that they purchase jerky monthly or that they never purchase jerky, respectively.

Mean scores for commercial jerky were significantly ($P < 0.05$) higher than mean scores for all the experimental products for four sensory characteristics: "Appearance",

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Table 5.2 Comparison of Consumer Taste Panel (n=120) for the results of the Beef Jerky Taste Panel Demographic/Behavior Questions for commercial jerky and jerky prepared following four pre-drying treatments and dried for 8 (treatment 2 only) and 10 h at 62.5°C (air) in home-type dehydrators

	Results of Beef Jerky Taste Panel Demographic/Behavior Questions	
Demographic /Behavior Question	n	%
Gender		
Male	51	43
Female	69	57
Status		
Faculty	21	18
Staff	22	18
Student	65	54
Other	12	10
Age		
Over 35 Years Old	39	33
Under 35 Years Old	81	67
Have You Ever Made Jerky		
Yes	36	30
No	84	70
Do You Like to Eat Jerky		
Yes	100	83
No	20	27
How Often Do You Eat Jerky		
At Least Once/Week	1	1
At Least Once/Month	35	29
At Least Once/Year	70	58
Never	14	12
How Often Do You Purchase Jerky		
At Least Once/Week	1	1
At Least Once/Month	26	22
At Least Once/Year	65	54
Never	28	23

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"Texture", "Flavor" and "Overall Acceptability" (Table 5.3). For "Appearance", the commercial jerky was rated as "moderately acceptable" (5.4 ± 1.4), while the other products received mean scores indicating "slightly acceptable" (4.4 ± 1.4 to 4.6 ± 1.3). For "Texture", the commercial jerky received a mean score of "chewy and tough" (5.6 ± 1.4) and the experimental products had lower mean scores ($P < 0.05$) of "tough-brittle and dry" to "chewy and very tough" (3.6 ± 1.5 to 4.2 ± 1.4). The commercial jerky had a mean score indicating "moderately acceptable" (4.9 ± 1.5) for "Flavor", while the mean scores of the experimental products were more in the "slightly acceptable" range (3.7 ± 1.4 to 4.3 ± 1.5). Finally, for "Overall Acceptability", the commercial jerky had a mean score indicating "moderate acceptability" (4.9 ± 1.5), whereas the mean scores for the experimental products were in the "slightly unacceptable" to "slightly acceptable" range (3.4 ± 1.4 to 3.9 ± 1.5).

Mean scores of the commercial jerky were not significantly higher than all products for the sensory characteristic, "Dryness." For "Dryness", the mean score for commercial jerky was similar to mean scores for treatments 3 and 4 (5.4 ± 1.7 to 5.6 ± 1.4), but significantly ($P < 0.05$) higher than mean scores for treatments 1, 2: 8 and 10 h (4.9 ± 1.4 to 5.0 ± 1.6).

Among the five experimental products, there were no differences in mean scores for "Appearance." For "Flavor" and "Overall Acceptability", mean scores for treatment 4 (4.3 ± 1.5 and 3.9 ± 1.5) were significantly ($P < 0.05$) higher than scores for treatment 2: 10 h (3.7 ± 1.4 and 3.4 ± 1.4, respectively). In addition, for "Texture", treatment 4 (4.2 ± 1.4) was significantly ($P < 0.05$) higher than treatment 2:10 h and treatment 1 (3.6 ± 1.5 and 3.7 ± 1.6, respectively). Finally, mean scores for "Dryness" for treatment 4 (5.5 ±

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Table 5.3 Comparison of Consumer Taste Panel (n=120) palatability scores for commercial jerky and jerky prepared following four pre-drying treatments and dried for 8 (treatment 2 only) and 10 h at 62.5°C (air) in home-type dehydrators.

	Beef Jerky Preparation Procedure[a]					
	1[b]	2[c]		3[d]	4[e]	Commercial[f]
Sensory Property	10 h	8 h	10 h	10 h	10 h	N/A
Appearance[g]	4.5 ± 1.5^{B}	4.4 ± 1.5^{B}	4.4 ± 1.4^{B}	4.6 ± 1.3^{B}	4.5 ± 1.5^{B}	5.4 ± 1.4^{A}
Dryness[h]	5.0 ± 1.6^{BC}	4.9 ± 1.5^{C}	4.9 ± 1.4^{C}	5.4 ± 1.7^{ABC}	5.5 ± 1.5^{AB}	5.6 ± 1.4^{A}
Texture[i]	3.7 ± 1.6^{C}	3.9 ± 1.5^{BC}	3.6 ± 1.5^{C}	3.9 ± 1.4^{BC}	4.2 ± 1.4^{B}	5.6 ± 1.1^{A}
Flavor[g]	4.1 ± 1.7^{BC}	3.9 ± 1.5^{BC}	3.7 ± 1.4^{C}	4.2 ± 1.6^{BC}	4.3 ± 1.5^{B}	4.9 ± 1.5^{A}
Overall Acceptability[g]	3.7 ± 1.6^{BC}	3.7 ± 1.4^{BC}	3.4 ± 1.4^{C}	3.8 ± 1.5^{BC}	3.9 ± 1.5^{B}	4.9 ± 1.5^{A}

[A-C] Means with different superscripts within rows are significantly ($P < 0.05$) different.
[a] Means represent values from n = 120 ± standard deviation
[b] Dipping in boiling water (95°C, 15 sec), followed by marinating (4°C, 24 h)
[c] Seasoning with pickling spices (4°C, 24 h), followed by dipping in hot pickling brine (78°C, 90 sec)
[d] Dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec), followed by marinating (4°C, 24 h)
[e] Marinating (4°C, 24 h), followed by dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec)
[f] *Oberto "Homestyle" Jerky*
[g] Evaluation description terms were 7-1, with 7: Extremely acceptable and 1: Very unacceptable
[h] Evaluation description terms were 7-1, with 7: Dry, fibrous when torn and 1: Dry, too tough to break
[i] Evaluation description terms were 7-1, with 7: Chewy, tender and 1: Crumbly, very dry

1.5) were significantly ($P < 0.05$) higher than mean scores for treatments 2: 8 and 10 h (4.9 ± 1.5 and 4.9 ± 1.4). There were no further significant differences among experimental products.

Although differences among experimental products were slight, treatment 4 was preferred ($P < 0.05$) over treatment 2: 10 h for four of the five sensory characteristics. This was unfortunate since treatment 2 was the only pre-drying treatment found to achieve ≥ 5-log reduction of inoculated *E. coli* O157:H7 (Chapter IV).

Consumer results indicated moderate acceptance of all experimental jerky products. The less than favorable mean scores for the experimental products could be attributed primarily to physical differences. Although all samples were considered beef jerky, the jerky prepared in the home-type dehydrators looked and tasted different than the commercial jerky used in the sensory test. Subjects who purchased commercial jerky regularly may not be accustomed to the properties of homemade jerky, and therefore may not be optimistic toward these products.

In order to assess differences in response to jerky products by demographic/behavior variables, sensory characteristic scores were analyzed by response to the seven-demographic/behavior questions. No significant interactions existed between commercial jerky and the five experimental products for the four demographic/behavior questions related to the subject's gender, work status, age and if the subject had ever made jerky. Thus, it was concluded that these four variables did not influence the subject's evaluation of the jerky products. In contrast, the subject's response to three questions: "Do you like to eat jerky," "How often do you eat jerky," and "How

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often do you buy jerky" did significantly ($P < 0.05$) affect the subject's evaluation of jerky products.

Do you like to eat jerky: Yes or No

Responses to this question influenced scores for "Appearance" and "Flavor" (Table 5.4). Subjects responding "Yes" gave significantly ($P < 0.05$) higher mean scores for "Appearance" and "Flavor" to the commercial jerky than to the experimental products. In contrast, subjects responding "No" or that they did not like to eat jerky gave similar scores to all products for "Flavor" and similar scores to all products except treatments 2: 8 and 10 h for "Appearance." When comparisons were made within treatments, subjects responding "Yes" gave significantly ($P < 0.05$) higher mean scores than subjects responding "No" to the commercial jerky, treatments 2: 8 and 10 h and treatment 4 for "Appearance" and significantly ($P < 0.05$) higher mean scores to the commercial jerky and treatment 4 for "Flavor." These results indicated that subjects responding "No" had preconceived ideas about the "Appearance" and "Flavor" of jerky that would be acceptable to them. It was concluded that these subjects already had a dislike for jerky or had preconceived ideas concerning the products and indicates consumers respond with lower acceptability to the flavor associated with pre-heated jerky. Responses to other sensory characteristics did not vary by subject's response to the question of whether they liked to eat jerky or not.

How often do you eat jerky: Never, Yearly, or Monthly/Weekly

The subject's response to flavor varied by how often the subject ate jerky. For this comparison, subjects responding "Monthly" or "Weekly" were combined in one category to increase cell size (Table 5.5). Subjects responding "Yearly" gave

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Table 5.4 Comparison of Consumer Taste Panel (n=120) "Appearance" and "Flavor" scores for commercial jerky and jerky prepared following four pre-drying treatments and dried for 8 (treatment 2 only) and 10 h at 62.5°C (air) in home-type dehydrators by whether or not they like jerky.

		Do you like to eat jerky: Yes or No			
		Appearance[a]		Flavor[a]	
Jerky Treatments	Drying Time (h)	Yes n = 100	No n = 20	Yes n =100	No n =20
		0.1[g]	0.3	0.2	0.3
1[b]	10	4.6^{Ab}	4.0^{Aa}	4.2^{Abc}	3.9^{Aa}
2[c]	8	4.6^{Ab}	3.3^{Bb}	3.9^{Ac}	3.7^{Aa}
2[c]	10	4.5^{Ab}	3.2^{Bb}	3.7^{Ac}	3.7^{Aa}
3[d]	10	4.7^{Ab}	4.4^{Aa}	4.3^{Ab}	3.6^{Aa}
4[e]	10	4.7^{Ab}	3.9^{Ba}	4.5^{Ab}	3.6^{Ba}
Commercial[f]	N/A	5.6^{Aa}	4.3^{Ba}	5.2^{Aa}	3.7^{Ba}

[A-B] Means with different superscripts within rows of yes/no response are significantly ($P < 0.05$) different.
[a-c] Means with different superscripts within columns are significantly ($P < 0.05$) different
[a] Evaluation description terms were 7-1, with 7: Extremely acceptable and 1: Very unacceptable
[b] Dipping in boiling water (95°C, 15 sec), followed by marinating (4°C, 24 h)
[c] Seasoning with pickling spices (4°C, 24 h), followed by dipping in hot pickling brine (78°C, 90 sec)
[d] Dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec), followed by marinating (4°C, 24 h)
[e] Marinating (4°C, 24 h), followed by dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec)
[f] *Oberto "Homestyle" Jerky*
[g] Standard error of mean

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Table 5.5 Comparison of Consumer Taste Panel (n=120) "Flavor" scores for commercial jerky and jerky prepared following four pre-drying treatments and dried for 8 (treatment 2 only) and 10 h at 62.5°C (air) in home-type dehydrators by frequency of jerky consumption.

		How often do you eat jerky: Never, Yearly, or Monthly/Weekly		
		Flavor[a]		
Jerky treatment	Drying time (h)	Never n = 14	Yearly n = 70	Monthly/ Weekly n = 36
		0.2[g]	0.5	0.6
1[b]	10	3.8^{Aa}	4.2^{Abc}	4.1^{Ab}
2[c]	8	3.4^{Ba}	4.2^{Abc}	3.4^{Bc}
2[c]	10	3.4^{Ba}	4.0^{Ac}	3.3^{Bc}
3[d]	10	3.4^{Ba}	4.3^{Abc}	4.2^{Ab}
4[e]	10	3.4^{Ba}	4.5^{Ab}	4.3^{Ab}
Commercial[f]	N/A	3.1^{Ba}	5.2^{Aa}	5.3^{Aa}

[A-B] Means with different superscripts within rows between columns are significantly ($P < 0.05$) different
[a-c] Means with different superscripts within columns are significantly ($P < 0.05$) different
[a] Evaluation description terms were 7-1, with 7: Extremely acceptable and 1: Very unacceptable
[b] Dipping in boiling water (95°C, 15 sec), followed by marinating (4°C, 24 h)
[c] Seasoning with pickling spices (4°C, 24 h), followed by dipping in hot pickling brine (78°C, 90 sec)
[d] Dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec), followed by marinating (4°C, 24 h)
[e] Marinating (4°C, 24 h), followed by dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec)
[f] *Oberto "Homestyle" Jerky*
[g] Standard error of mean

significantly ($P < 0.05$) higher mean scores to treatments 2: 8 and 10 h for "Flavor" than did subjects who ate jerky more often or not at all. In addition, subjects responding "Yearly", as well as subjects responding "Monthly/Weekly", gave significantly ($P < 0.05$) higher mean scores to the commercial jerky and treatments 3 and 4 than did subjects that responded "Never." It was concluded that subjects responding "Never" were more critical of all the jerky products. This was evident with the significantly ($P < 0.05$) lower mean scores given to the commercial product for "Flavor" than given by those who ate jerky more often. Other sensory characteristics did not vary by frequency of jerky consumption.

How often do you buy jerky: Never, Yearly or Monthly/Weekly

Response to the dryness characteristics varied by how often the subject purchases jerky. Again, due to number of subjects responding "Weekly" (n=1), subjects who responded "Monthly" or "Weekly" were combined in one category (Table 5.6). For "Dryness", subjects responding "Yearly" gave significantly ($P < 0.05$) higher mean scores for treatment 2: 8 h than those subjects who responded "Never." In addition, subjects responding "Monthly/Weekly" gave significantly ($P < 0.05$) higher mean scores for the commercial jerky than those subjects responding "Never." Although significant ($P < 0.05$) differences were small (0.6-0.9 pt.), subjects responding "Never" could be responding to a lack of exposure to qualities typical of commercial beef jerky or could be responding out of a displeasure for the product. However, for treatment 3, subjects responding "Never" gave significantly ($P < 0.05$) higher mean scores than the subjects responding "Yearly." It should be noted that "Dryness" used descriptive terms in the sensory evaluation and the descriptive terms could have been inferred differently by the

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Table 5.6 Comparison of Consumer Taste Panel (n=120) "Dryness" scores for commercial jerky and jerky prepared following four pre-drying treatments and dried for 8 (treatment 2 only) and 10 h at 62.5°C (air) in home-type dehydrators by frequency of jerky purchase.

		How do you buy jerky: Never, Yearly, or Monthly/Weekly		
		Dryness[a]		
Jerky treatment	Drying time (h)	Never n = 28	Yearly n = 65	Monthly/Weekly n = 27
		0.5[g]	0.8	0.9
1[b]	10	5.1^{Aab}	4.9^{Aa}	4.9^{Ab}
2[c]	8	4.3^{Bc}	5.2^{Aa}	4.6^{ABb}
2[c]	10	4.8^{Ab}	4.9^{Aa}	5.2^{Ab}
3[d]	10	5.9^{Aa}	5.1^{Ba}	5.4^{ABab}
4[e]	10	5.2^{Aab}	5.5^{Aa}	5.7^{Aa}
Commercial[f]	N/A	5.3^{Bab}	5.6^{ABa}	6.1^{Aa}

[A-B] Means with different superscripts within rows between columns are significantly ($P < 0.05$) different

[a-c] Means with different superscripts within columns are significantly ($P < 0.05$) different

[a] Evaluation description terms were 7-1, with 7: Dry, fibrous when torn and 1: Dry, too tough too break

[b] Dipping in boiling water (95°C, 15 sec), followed by marinating (4°C, 24 h)

[c] Seasoning with pickling spices (4°C, 24 h), followed by dipping in hot pickling brine (78°C, 90 sec)

[d] Dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec), followed by marinating (4°C, 24 h)

[e] Marinating (4°C, 24 h), followed by dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec)

[f] *Oberto "Homestyle" Jerky*

[g] Standard error of mean

subjects. There were no other differences that existed among the other sensory characteristics.

What gender are you: Male or Female & How old are you: Under 35 or Over 35

Subjects responding "Female & Over 35" gave significantly ($P < 0.05$) lower mean scores to commercial jerky for "Flavor" (Appendix E, Table E. 5.1) than did those subjects responding "Male & Over 35", "Male & Under 35" or "Female & Under 35." No other differences existed among responses to the jerky samples.

Do you like to eat jerky: Yes or No & How often do you buy jerky: Never, Yearly or Monthly/Weekly

Subjects responding "Yes and Yearly" gave significantly higher ($P < 0.05$) mean scores to treatment 2: 8 h than subjects responding "Yes and Never" for "Overall Acceptability" (Appendix E, Table E. 5.2). Subjects responding "No and Yearly" gave significantly ($P < 0.05$) higher mean scores to the commercial jerky and treatment 2: 10 h than those subjects responding "No and Never." In addition, subjects responding "No and Yearly" gave significantly ($P < 0.05$) higher scores for treatment 1 than those subjects responding "No and Monthly/Weekly." However, only one subject responded "No and Monthly/Weekly", which indicated the subject did not like to eat jerky, but that the subject bought jerky at least monthly or weekly. This response indicated conflicting behavior tendencies and although significant ($P < 0.05$), the validity of these responses are questioned for this particular demographic/behavior question.

Physical analyses

Jerky samples from the five experimental treatments and commercial jerky were analyzed for a_w and pH. Treatment 2: 8 h and treatment 1 achieved the same final a_w

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value, 0.74 (Table 5.7). After 10 h of drying, treatments 3 and 4 achieved the lowest a_w values, 0.63, while treatment 2: 10 h and the commercial jerky had a_w values of 0.68 and 0.72, respectively. The final pH values were highest for treatments 2: 8 and 10 h (5.76 and 5.72, respectively); treatments 3 and 4 had the lowest final pH values (5.23 and 5.20, respectively). In addition, treatment 1 and the commercial jerky had pH values of 5.30 and 5.58, respectively. The Code of Federal Regulations (USDHHS-FDA, 1999) states that acidified foods can have $a_w > 0.85$ when the finished equilibrium pH is 4.6 or below. However, in this study, all finished jerky products had pH values ranging from 5.20 to 5.76; thus, the pH was not low enough to classify these products as acidified foods. Therefore, the a_w must reach ≤ 0.68 in order for the jerky to be considered a shelf-stable product (Leistner, 1987).

CONCLUSIONS

This study evaluated consumer responses to beef jerky prepared following four pre-drying treatments found to result in a 4.3 to 5.8-log reduction of inoculated *E. coli* O157:H7 populations (Chapter IV). Commercial beef jerky served as a control in the sensory evaluation, since the traditional method of home drying beef jerky did not result in a 5 log reduction of inoculated *E. coli* O157:H7 cells (Chapter III).

Mean scores for commercial jerky were significantly ($P < 0.05$) higher (4.9 to 5.6) than mean scores for all the experimental products (3.4 to 5.5) for most sensory characteristics. Although differences among experimental products were slight, treatment 4 was preferred ($P < 0.05$) over treatment 2: 10 h, which was unfortunate since treatment 2 was the only pre-drying treatment found to achieve ≥ 5-log reduction of inoculated *E. coli* O157:H7 (Chapter IV). Part of the differences seen in acceptance

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Table 5.7 Commercial jerky and jerky prepared following four pre-drying treatments and dried for 8 (treatment 2 only) and 10 h at 62.5°C (air) in home type dehydrators by physical analyses (water activity/a_w and pH).

		Physical Analyses[a]	
Jerky treatment	Drying time (h)	a_w	pH
1[b]	10	0.74	5.58
2[c]	8	0.74	5.76
2[c]	10	0.68	5.72
3[d]	10	0.63	5.23
4[e]	10	0.63	5.20
Commercial[f]	N/A	0.72	5.30

[a] Means represent responses from n = 1
[b] Dipping in boiling water (95°C, 15 sec), followed by marinating (4°C, 24 h)
[c] Seasoning with pickling spices (4°C, 24 h), followed by dipping in hot pickling brine (78°C, 90 sec)
[d] Dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec), followed by marinating (4°C, 24 h)
[e] Marinating (4°C, 24 h), followed by dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec)
[f] *Oberto "Homestyle" Jerky*

between the commercial product and the experimental products could be attributed to physical differences. Although all samples were considered beef jerky, jerky prepared in the home-type dehydrators looked and tasted different than the commercial jerky used in the sensory test. Subjects who purchased commercial jerky most often or on a regular basis may not be accustomed to the properties of homemade jerky, and therefore may not be optimistic toward these products. Overall, results indicated that experimental products received scores between "slightly unacceptable" to "moderately acceptable" (3.4-5.5). This information is useful in forming recommendations to produce flavorful home-dried jerky, while minimizing the risk of *E. coli* O157:H7. Future work is needed to develop formulations that not only achieve acceptable bacterial log reductions of *E. coli* O157:H7 for processed fermented and semidry foods (Billy, 1997; Reed, 1995), but also to achieve acceptable taste panel scores.

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APPENDIX A

HUMAN RESEARCH COMMITTEE APPROVAL FORM AND INFORMED CONSENT FORM FOR SENSORY EVALUATION

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Office of Regulatory Compliance
Office of Vice President for Research
and Information Technology
Fort Collins, CO 80523-2046
(970) 491-1563
FAX: 491-2293

AMENDMENT APPROVAL

MEMORANDUM

To: John N. Sofos, Animal Sciences
From: Celia Walker, Administrator
Human Research Committee
Date: July 21, 1999
Re: Minimizing the Risk of Escherichia coli O157:H7 in Home Dried Food Systems, 98-188H
Request to amend

The Human Research Committee has approved your request to amend the above-referenced project in the following way(s):

- The number of participants has been increased to 175 for each of two years.
- Recruitment through personal contact at CSU may be made (please provide an approximate script).
- A flyer will be distriburted and has been revised.
- Participants will be excluded if they do not eat or like eating dried fruit.
- Research will be conducted in additional CSU locations.
- The evaluation card has been revised, for use on six samples, instread of the original nine.
- The duration of participation has been shortened, and the honorarium eliminated.
- Consent will be conducted at initial contact and with the introduction to the taste panel.
- The consent form is revised to reflect these changes and, in the Risk section, that the products will not be intentionally inoculated.

If you have questions, please contact me at 1-1563 or cwalker@research.colostate.edu.

Watch our web page at http://www.research.colostate.edu/regulatory/ for current information and forms for human research, now including both the H-100 Application for HRC Approval and the H-101 Renewal Form in both PDF and Word formats.

Animal Care and Use · Drug Review · Human Research · Institutional Biosafety · Material Transfer Agreements · Radiation Safety
410 University Services Center

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COLORADO STATE UNIVERSITY
INFORMED CONSENT TO PARTICIPATE IN A RESEARCH PROJECT

TITLE OF PROJECT: Minimizing the Risk of *Escherichia coli* O157:H7 in Home Dried Food Systems

NAME OF PRINCIPAL INVESTIGATOR: John N. Sofos, Ph.D.

NAME OF CO-INVESTIGATOR: Patricia A. Kendall, Ph.D., RD

CONTACT NAME AND PHONE NUMBER FOR QUESTIONS/PROBLEMS: Patricia A. Kendall
(970) 491-1945

SPONSOR OF PROJECT: Cooperative Extension and Experiment Station

PURPOSE OF THE RESEARCH:
This study involves research into minimizing the risk of the foodborne pathogen *E. coli* O157:H7 in home dried beef and game jerky and dried apple slices. In 1995, investigators concluded that homemade venison jerky was implicated in an outbreak of *E. coli* O157:H7. *Traditional drying methods were used in the preparation of the jerky.* This research study is designed to develop safe and consumer acceptable methods of drying foods at home.

PROCEDURES/METHODS TO BE USED:
You will taste food products that have been dried using procedures found to adequately destroy any potential disease causing pathogens that might be on the food. You will taste food products containing ingredients all found to be safe by the Food and Drug Administration. These products were prepared in a classroom-kitchen, used by Colorado State University. It is expected that you will evaluate the samples according to appearance, texture, moisture content, flavor, and overall acceptability. The sample testing should not take more than 30 minutes. You will not be videotaped or audiotaped during any tastings.

RISKS INHERENT IN THE PROCEDURES:
There are no known risks involved in this research study.
It is not possible to identify all potential risks in an experimental procedure, but the researcher(s) have taken reasonable safeguards to minimize any known and potential, but unknown, risks. The products to be tested will not be intentionally inoculated.

BENEFITS:
You will be able to taste and consume beef and game jerky or apple slice samples. In addition, you will further research to *find consumer acceptable methods of minimizing the risk of E. coli* O157:H7 in home dried food products.

CONFIDENTIALITY:
Strict confidentiality of information will be maintained by recording data using numbers to identify the participants. Resulting data will be reported in research materials in aggregate. Only the investigators and necessary personnel (doctoral student) will have access to the participant's identification.

LIABILITY:
The Colorado Governmental Immunity Act determines and may limit Colorado State University's legal responsibility if an injury happens because of this study. Claims against the University must be filed within 180 days of the injury.

Questions about subjects' rights may be directed to Celia S. Walker at (970) 491-1563.

PARTICIPATION:
Your participation in this research is voluntary. If you decide to participate in the study, you may withdraw your consent and stop participating at any time without penalty or loss of benefits to which you are otherwise entitled.

Your signature acknowledges that you have read the information stated and willingly sign this consent form. Your signature also acknowledges that you have received, on the date signed, a copy of this document containing _1__ page.

____
Participant name (printed)

____________________________ _______________
Participant signature Date

____________________________ _______________
Investigator or co-investigator signature Date

Page 1 of 1 Subject initials ______ Date ______

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APPENDIX B

SENSORY EVALUATION SCORECARD

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BEEF JERKY TASTE PANEL

Departments: Animal Sciences and Food Science & Human Nutrition

Consumer #: ___________

Circle the appropriate responses.

Gender : Male Female

Status : Faculty Staff Student Other

Age : Over 35 years old Under 35 years old

Have you ever made jerky? Yes No

Do you like to eat jerky? Yes No

How often do you eat jerky?

At least once/week At least once/month At least once/year Never

How often do you purchase jerky?

At least once/week At least once/month At least once/year Never

Beef Jerky Evaluation Directions:

1. Using the provided evaluation descriptions listed for each column, please fill-in the number that best describes your response to the appropriate attribute.
2. Start with the first sample listed and complete the entire evaluation for that sample. Then proceed to the next sample and follow the same procedure until finished.
3. Between samples, cleanse your palate with water and a bite of unsalted cracker.
4. If you have questions, please ask an available panel worker.

Beef Jerky Evaluation

	Evaluate before tasting sample		*Evaluate while tasting sample*		*Evaluate after tasting sample*	
Sample Number	**Appearance**	**Dryness**	**Texture**	**Moisture Content**	**Flavor**	**Overall Acceptability**
1						
2						
3						
4						
5						
6						
Evaluation Description Terms	7. Extremely acceptable 6. Very acceptable 5. Moderately acceptable 4. Slightly acceptable 3. Slightly unacceptable 2. Moderately unacceptable 1. Very unacceptable	7. Dry, fibrous when torn 6. Dry, bends like a green stick 5. Dry, bends like a dry stick 4. Dry, breaks like a dry stick 3. Dry, too tough to tear 2. Dry, too tough to bend 1. Dry, too tough to break	7. Chewy, tender 6. Chewy, somewhat tender 5. Chewy, tough 4. Chewy, very tough 3. Tough-brittle, dry 2. Crumbly, dry 1. Crumbly, very dry	7. Moist, not oily 6. Moist, somewhat oily 5. Moist, very oily 4. Somewhat moist, somewhat oily 3. Somewhat dry, oily 2. Dry, somewhat oily 1. Dry, not oily	7. Extremely acceptable 6. Very acceptable 5. Moderately acceptable 4. Slightly acceptable 3. Slightly unacceptable 2. Moderately unacceptable 1. Very unacceptable	7. Extremely acceptable 6. Very acceptable 5. Moderately acceptable 4. Slightly acceptable 3. Slightly unacceptable 2. Moderately unacceptable 1. Very unacceptable

Comments:

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APPENDIX C

DATA TABLES FROM CHAPTER III

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Table C. 3.1 Surface temperature of beef slices during drying of jerky prepared without marinade (62.5°C) and with marinade (62.5 and 68.3°C) (Used for Figure 3.2).

Time (h)	1[b]	2[c]	3[d]
15 min warm-up[a]	10.0 (0.0)	10.0 (0.0)	10.0 (0.0)
Placement of meat strips in dehydrator	31.0 (1.0)	31.0 (1.0)	34.3 (2.5)
0.5	37.7 (2.1)	40.3 (2.3)	44.7 (2.3)
1.0	47.0 (3.6)	44.7 (2.5)	55.7 (3.2)
1.5	49.0 (1.7)	49.7 (2.3)	55.0 (1.7)
2.0	54.7 (2.5)	52.0 (1.0)	58.0 (1.0)
2.5	54.7 (1.5)	54.3 (0.6)	62.3 (0.0)
3.0	57.7 (0.6)	55.3 (0.6)	63.3 (1.0)
3.5	58.3 (0.5)	57.5 (1.0)	64.3 (0.5)
4.0	58.9 (0.7)	57.6 (0.9)	64.0 (0.5)
4.5	61.7 (0.6)	57.7 (1.2)	66.7 (0.6)
5.0	60.7 (0.6)	59.0 (0.0)	67.0 (0.0)
5.5	61.3 (0.6)	60.0 (1.7)	67.0 (0.0)
6.0	61.7 (0.6)	60.0 (0.0)	68.0 (1.0)
6.5	61.3 (1.2)	60.0 (0.0)	68.7 (0.6)
7.0	62.0 (0.0)	61.0 (0.0)	69.0 (0.0)
7.5	62.0 (0.0)	61.7 (0.3)	69.0 (0.0)
8.0	62.0 (0.0)	62.2 (0.3)	69.0 (0.0)
8.5	62.7 (0.6)	62.3 (0.4)	69.0 (0.0)
9.0	62.7 (0.6)	62.3 (0.4)	69.0 (0.0)
9.5	62.7 (0.6)	62.4 (0.6)	69.0 (0.0)
10.0	62.7 (0.6)	62.0 (1.0)	68.7 (0.6)

[a] Means represent three values from one dehydrator: top, middle and bottom trays (n=3) (standard deviation)
[b] No marinade, dried at 62.5°C
[c] With marinade, dried at 62.5°C
[d] With marinade, dried at 68.3°C

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Table C. 3.2 Air temperature of dehydrator during drying of jerky prepared without marinade (62.5°C) and with marinade (62.5 and 68.3°C) (Used for Figure 3.1).

Time (h)	1[b]	2[c]	3[d]
15 min warm-up[a]	62.5 (0.4)	62.0 (0.0)	69.0 (0.7)
Placement of meat strips in dehydrator	49.0 (4.2)	48.5 (4.9)	49.5 (0.0)
0.5	55.3 (4.6)	49.5 (4.9)	55.0 (4.2)
1.0	59.3 (1.8)	56.5 (2.1)	62.0 (2.8)
1.5	60.3 (2.5)	58.8 (0.4)	65.8 (3.2)
2.0	61.3 (1.8)	60.0 0.0)	65.5 (2.1)
2.5	61.5 (1.4)	60.8 (0.4)	67.5 (0.7)
3.0	62.8 (1.8)	61.0 (0.7)	67.8 (0.4)
3.5	62.0 (0.7)	62.0 (0.0)	68.0 (0.0)
4.0	62.5 (0.0)	62.3 (0.4)	68.3 (0.4)
4.5	62.5 (0.0)	62.8 (0.4)	69.3 (1.1)
5.0	62.5 (0.0)	62.8 (0.4)	69.3 (1.1)
5.5	62.5 (0.0)	62.5 (0.0)	69.3 (1.1)
6.0	62.5 (0.0)	62.5 (0.0)	69.3 (1.1)
6.5	62.5 (0.0)	62.8 (0.4)	69.3 (1.1)
7.0	62.8 (0.4)	63.5 (1.4)	69.0 (0.7)
7.5	62.8 (0.4)	63.8 (1.8)	69.0 (0.7)
8.0	63.0 (0.7)	64.0 (2.1)	68.8(1.1)
8.5	63.3 (1.1)	63.5 (1.4)	68.8 (1.1)
9.0	63.5 (1.4)	63.5 (1.4)	69.0 (0.7)
9.5	63.5 (1.4)	63.5 (1.4)	68.8 (1.1)
10.0	63.5 (1.4)	65.0 (1.4)	69.0 (0.7)

[a] Means represent one value from two dehydrators (n=2) (standard deviation)
[b] No marinade, dried at 62.5°C
[c] With marinade, dried at 62.5°C
[d] With marinade, dried at 68.3°C

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Table C. 3.3 D-value and regression values for the effect of beef jerky preparation procedure (dried at 62.5°C), drying time (0-10 h) and culture media (tryptic soy agar--TSA and sorbitol MacConkey agar--SMAC) for bacterial enumeration on survival of inoculated *E. coli O*157:H7 (log CFU/cm^2) and natural bacterial flora for jerky prepared without marinade (62.5°C) and with marinade (62.5 and 68.3°C).

Jerky treatment	Culture Media	Slope	Y-intercept	Correlation coefficient	D-value: negative reciprocal of slope (h)
1[a]	SMAC	-0.678	6.048	0.64	1.47
	TSA	-0.782	6.794	0.81	1.28
2[b]	SMAC	-0.366	4.972	0.48	2.73
	TSA	-0.354	4.974	0.38	2.82
3[c]	SMAC	-0.643	5.943	0.39	1.56
	TSA	-0.911	6.871	0.59	1.10

[a] No marinade, dried at 62.5°C
[b] With marinade, dried at 62.5°C
[c] With marinade, dried at 68.3°C

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APPENDIX D

DATA TABLES FROM CHAPTER IV

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Table D. 4.1 Changes in bacterial counts (log CFU/cm^2) on beef jerky during pre-drying preparation. Treatments of inoculated meat slices included (A) Pre-drying step 1: dipping in boiling water (95°C, 15 sec), pre-drying step 2: marinating (4°C, 24 h); (B) Pre-drying step 1: seasoning with pickling spices (4°C, 24 h), pre-drying step 2: dipping in hot pickling brine (78°C, 90 sec); (C) Pre-drying step 1: dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec), pre-drying step 2: marinating (4°C, 24 h); (D) Pre-drying step 1: marinating (4°C, 24 h), pre-drying step 2: dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec). Counts were enumerated on tryptic soy agar (TSA) and sorbitol MacConkey agar (SMAC). Means are three values from two dehydrators (n=6) (Used for Figure 4.1).

Time (h)	1[b]	2[c]	3[d]	4[e]
15 min warm-up[a]	62.3 (0.4)	62.0 (0.0)	62.3 (0.4)	60.0 (2.8)
Placement of meat strips in dehydrator	52.3 (8.8)	49.0 (0.0)	50.8 (0.4)	49.3 (4.6)
0.5	61.3 (4.6)	54.3 (1.8)	55.5 (0.7)	52.8 (1.1)
1.0	64.3 (2.5)	59.0 (1.4)	57.8 (0.4)	58.0 (5.7)
1.5	63.0 (1.4)	61.0 (1.4)	60.0 (0.7)	61.0 (2.1)
2.0	63.0 (1.4)	62.0 (0.7)	62.3 (0.4)	61.5 (1.4)
2.5	64.0 (0.7)	62.3 (0.4)	62.5 (0.7)	62.3 (0.4)
3.0	64.3 (0.4)	62.8 (0.4)	62.8 (1.1)	63.0 (1.4)
3.5	64.5 (0.0)	63.3 (0.4)	63.0 (1.4)	63.0 (0.7)
4.0	64.3 (0.4)	64.0 (0.0)	63.0 (1.4)	63.0 (0.7)
4.5	65.0 (2.1)	64.3 (0.4)	62.5 (0.0)	62.5 (0.7)
5.0	63.8 (1.1)	64.5 (0.0)	63.0 (0.7)	63.3 (1.1)
5.5	64.0 (0.7)	64.5 (0.0)	62.5 (0.0)	63.0 (0.7)
6.0	63.8 (1.1)	64.5 (0.0)	62.8 (0.4)	63.0 (0.0)
6.5	64.3 (0.4)	64.5 (0.0)	63.0 (1.4)	63.5 (0.7)
7.0	64.3 (0.4)	64.5 (0.0)	63.0 (0.7)	63.3 (1.1)
7.5	64.3 (0.4)	64.5 (0.0)	62.5 (0.7)	63.0 (0.7)
8.0	64.3 (0.4)	64.5 (0.0)	63.0 (0.7)	62.5 (0.0)
8.5	64.0 (0.0)	64.0 (2.1)	64.0 (0.0)	62.5 (0.0)
9.0	63.8 (0.4)	63.3 (1.8)	63.5 (0.0)	62.5 (0.0)
9.5	63.8 (0.4)	64.0 (1.4)	63.8 (0.4)	62.3 (0.4)
10.0	64.0 (0.7)	64.0 (0.7)	63.8 (0.4)	62.5 (0.0)

[a] Means represent one value from two dehydrators (n=2) (standard deviation)

[b] Pre-drying step 1: dipping in boiling water (95°C, 15 sec); pre-drying step 2: marinating (4°C, 24 h)

[c] Pre-drying step 1: seasoning with pickling spices (4°C, 24 h); pre-drying step 2: dipping in pickling brine (78°C, 90 sec)

[d] Pre-drying step 1: dipping in acetic acid solution (57.5°C, 2.5%, 20 sec); pre-drying step 2: marinating (4°C, 24 h)

[e] Pre-drying step 1: marinating (4°C, 24 h); pre-drying step 2: dipping in acetic acid solution (57.5°C, 2.5%, 20 sec)

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Table D. 4.2 D-value and regression values for the effect of beef jerky preparation procedure (dried at 62.5°C), total preparation time (pre-drying steps and drying time), drying time (0-10 h) and culture media (tryptic soy agar--TSA and sorbitol MacConkey agar--SMAC) for bacterial enumeration on survival of inoculated *E. coli* O157:H7 (log CFU/cm^2) and natural bacterial flora.

Jerky treatment		Culture Media	Slope	Y-intercept	Correlation coefficient	D-value: negative reciprocal of slope (h)
1[a]	Total preparation time	SMAC	-0.736	5.324	0.81	1.36
		TSA	-0.588	5.300	0.65	1.70
	Drying time only	SMAC	-0.737	3.903	0.72	1.36
		TSA	-0.440	3.670	0.52	2.27
2[b]	Total preparation time	SMAC	-1.017	7.470	0.82	0.98
		TSA	-1.068	8.009	0.75	0.94
	Drying time only	SMAC	-0.499	3.581	0.52	2.00
		TSA	-0.316	3.159	0.47	3.16
3[c]	Total preparation time	SMAC	-0.958	8.139	0.78	1.04
		TSA	-1.013	8.611	0.86	0.99
	Drying time only	SMAC	-0.827	5.751	0.50	1.21
		TSA	-0.958	6.380	0.68	1.04
4[d]	Total preparation time	SMAC	-0.930	8.256	0.81	1.08
		TSA	-0.999	8.821	0.79	1.00
	Drying time only	SMAC	-0.779	5.827	0.57	1.28
		TSA	-0.865	6.307	0.55	1.16

[a] Pre-drying step 1: dipping in boiling water (95°C, 15 sec); pre-drying step 2: marinating (4°C, 24 h)

[b] Pre-drying step 1: seasoning with pickling spices (4°C, 24 h); pre-drying step 2: dipping in pickling brine (78°C, 90 sec)

[c] Pre-drying step 1: dipping in acetic acid solution (57.5°C, 2.5%, 20 sec); pre-drying step 2: marinating (4°C, 24 h)

[d] Pre-drying step 1: marinating (4°C, 24 h); pre-drying step 2: dipping in acetic acid solution (57.5°C, 2.5%, 20 sec)

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APPENDIX E

DATA TABLES FROM CHAPTER V

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Figure E. 5.1 Oberto Homestyle Jerky Label, Oberto Sausage Company, Seattle, WA

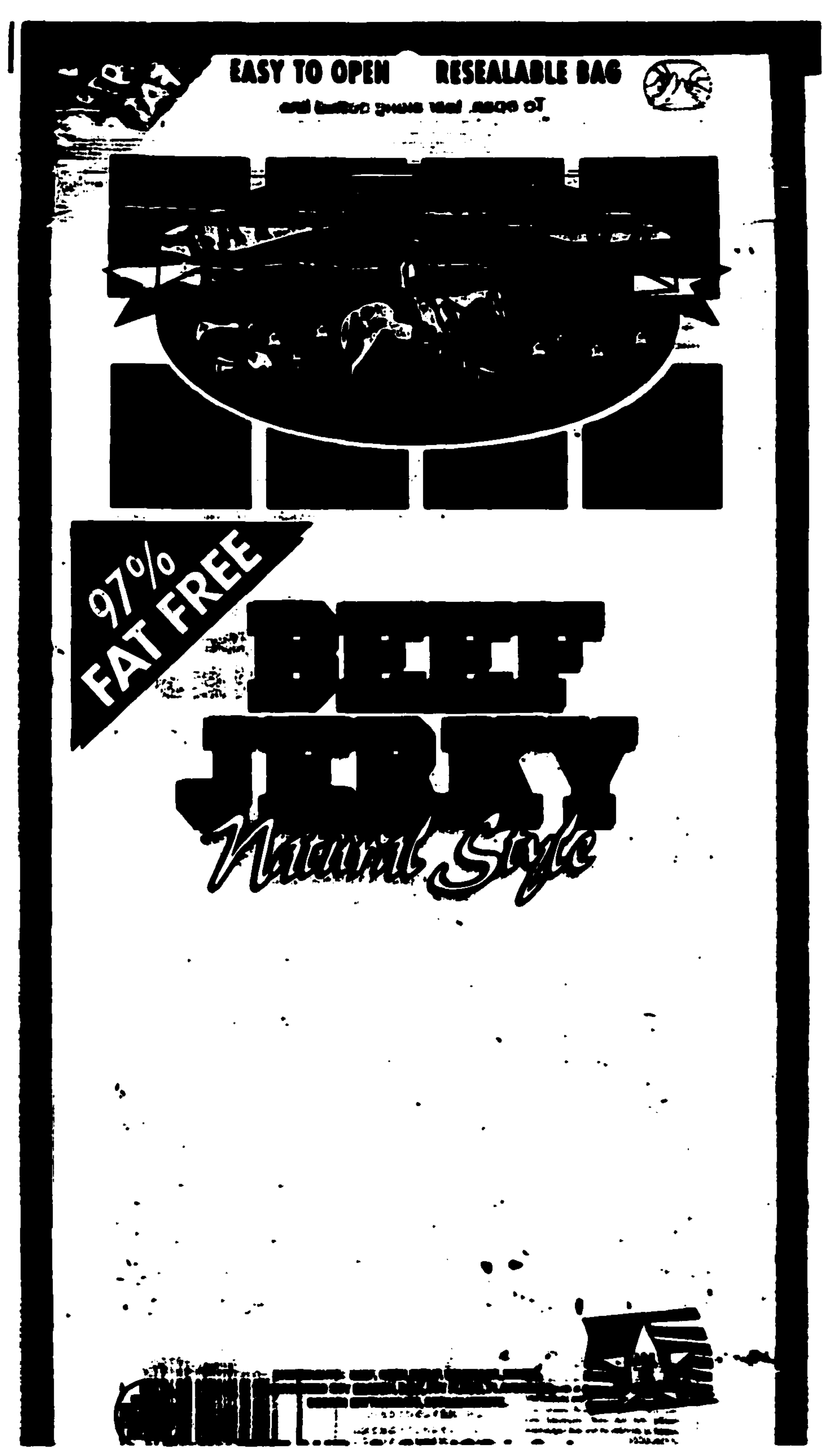

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Table E. 5.1 Comparison of Consumer Taste Panel (n=120) "Flavor" scores for commercial jerky and jerky prepared following four pre-drying treatments and dried for 8 (treatment 2 only) and 10 h at 62.5°C (air) in home-type dehydrators by gender and age.

		What is your gender: Male or Female & What is your age: Over 35 or Under 35			
		Flavor[a]			
Jerky treatment	Drying time (h)	Male		Female	
		Over 35	Under 35	Over 35	Under 35
		n = 21	n =30	n =19	n = 50
		0.6[g]	0.7	0.5	0.6
1[b]	10	3.8^{Ab}	3.7^{Abc}	4.3^{Aa}	4.4^{Abc}
2[c]	8	3.8^{Ab}	4.0^{Abc}	3.6^{Aab}	4.0^{Ac}
2[c]	10	3.9^{Ab}	3.6^{Ac}	3.5^{Ab}	3.8^{Ac}
3[d]	10	3.6^{Ab}	4.4^{Ab}	4.2^{Aab}	4.3^{Abc}
4[e]	10	4.2^{Ab}	4.3^{Ab}	4.0^{Aab}	4.5^{Ab}
Commercial[f]	N/A	5.3^{Aa}	5.1^{Aa}	3.5^{Bb}	5.3^{Aa}

[A-B] Means with different superscripts within rows between columns are significantly (P< 0.05) different
[a-c] Means with different superscripts within columns are significantly (P < 0.05) different
[a] Evaluation description terms were 7-1, with 7: Extremely acceptable and 1: Very unacceptable
[b] Dipping in boiling water (95°C, 15 sec), followed by marinating (4°C, 24 h)
[c] Seasoning with pickling spices (4°C, 24 h), followed by dipping in hot pickling brine (78°C, 90 sec)
[d] Dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec), followed by marinating (4°C, 24 h)
[e] Marinating (4°C, 24 h), followed by dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec)
[f] *Oberto "Homestyle" Jerky*
[g] Standard error of the mean

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Table E. 5.2 Comparison of Consumer Taste Panel (n=120) "Overall Acceptability" scores for commercial jerky and jerky prepared following four pre-drying treatments and dried for 8 (treatment 2 only) and 10 h at 62.5°C (air) in home-type dehydrators by whether subjects like jerky and by the frequency of jerky purchase.

		Do you like to eat jerky: Yes or No & How often do you eat jerky: Never, Yearly, or Monthly/Weekly					
		Overall Acceptability[a]					
		Yes			No		
Jerky treatment	Drying time (h)	Never n = 3	Yearly n = 82	Monthly/ Weekly n = 35	Never n = 11	Yearly n = 8	Monthly/ Weekly n = 1
		0.9[g]	0.2	0.3	0.4	0.5	1.5
1[b]	10	3.0[Aa]	3.7[Ab]	3.7[Ab]	3.1[ABab]	4.4[Aa]	2.0[Bc]
2[c]	8	2.0[Bb]	4.0[Ab]	3.5[ABb]	3.0[Aab]	3.1[Ab]	4.0[Ab]
2[c]	10	4.3[Aa]	3.6[Ab]	3.2[Ab]	2.1[Bb]	4.6[Aa]	3.0[ABb]
3[d]	10	3.7[Aa]	4.0[Ab]	3.7[Ab]	3.2[Aa]	3.8[Aab]	4.0[Ab]
4[e]	10	3.0[Aa]	4.1[Aab]	4.1[Aab]	2.9[Aab]	4.0[Ab]	3.0[Ab]
Commercial[f]	N/A	4.3[Aa]	5.1[Aa]	5.3[Aa]	3.0[Bab]	4.6[Aa]	5.0[Aa]

[A-B] Means with different superscripts within rows between columns are significantly ($P < 0.05$) different
[a-c] Means with different superscripts within columns are significantly ($P < 0.05$) different
[a] Evaluation description terms were 7-1, with 7: Extremely acceptable and 1: Very unacceptable
[b] Dipping in boiling water (95°C, 15 sec), followed by marinating (4°C, 24 h)
[c] Seasoning with pickling spices (4°C, 24 h), followed by dipping in hot pickling brine (78°C, 90 sec)
[d] Dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec), followed by marinating (4°C, 24 h)
[e] Marinating (4°C, 24 h), followed by dipping in warm acetic acid solution (57.5°C, 2.5%, 20 sec)
[f] *Oberto "Homestyle" Jerky*
[g] Standard error of the mean

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