FOOD SAFETY ISSUES AND PHYSICAL PROPERTIES ASSOCIATED

WITH HOME-STYLE BEEF JERKY

by

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(Under the Direction of Mark A. Harrison)

ABSTRACT

Home-style jerky has grown in popularity over the past years because it is easy to prepare, lightweight, low fat, and tasty. However, during the 1980's and 1990's, several foodborne outbreaks, associated with home-style and small scale jerky processors, led people to re-examine the safety of the jerky making process. This thesis, addressed food safety issues related to *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* spp. on preparation of home-style beef jerky. Antimicrobial effects on of sugar and salt marinades were compared. Sodium chloride levels on the jerky process were investigated as the antimicrobial effect of a low and a regular salt level marinade were compared. Whether acid adapted cells have a higher survival rate than nonadapted cells was investigated. The type of marinade did not have an effect on physical properties. The survival of the acid-adapted and nonadapted cells were not significantly different.

INDEX WORDS: Beef jerky, acid adaptation, *E. coli* O157:H7, *L. monocytogenes*, *Salmonella*, reduced salt, marination

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INTRODUCTION

According to the World Health Organization foodborne illness is the most

widespread health problem in the world (8). The Centers for Disease Control and

Prevention (CDC) estimate 76 million illnesses occur annually in the U.S. alone (5) with

7,000 deaths projected due to foodborne illnesses (8). Economic loss due to productivity

loss, medical cost, and food recalls amounts to $6.9 billion in just the U.S. (8). The four

major pathogens of concern, *Campylobacter, Escherichia coli* O157:H7, *Listeria*

monocytogenes, and *Salmonella,* account annually for an estimated 14 million illnesses,

60,000 hospitalizations, and 1,800 deaths in the U.S. (2). Consumers who prepare food

for their own consumption or for small-scale retail purposes often face food safety issues.

This is especially true for processes which may not reliably reduce the level of foodborne

pathogens to a desired level.

Dehydration used as a food preservative has been practiced for hundreds of years

(1). When properly done, this method of preservation has worked well in reducing the

number of both spoilage and pathogenic bacteria on food. However, occasionally

problems can occur. Beef jerky is a popular dehydrated food, but it was not until the

1980's and 1990's that the drying process for jerky was questioned when several illness

outbreaks and recalls occurred. Studies have found that dehydration alternatives can

provide safer jerky by having the internal temperature of the meat reach 71.1°C before or

after the drying step (6). Although this is safer, the question of consumer acceptability

needed to be addressed. Harrison et al. (4) found that a safer alternate method where the

jerky strips were marinated and dried in a 60°C dehydrator and then placed in a 135°C oven for10 min was as acceptable to consumers as the traditional method (drying at 60°C) of marination and dehydration. Due to outbreaks of foodborne illness in recent years linked to other pathogens such as *E. coli* O157:H7 and *L. monocytogenes,* the USDA meat and poultry hotline (3) suggests steaming or roasting meat to 71.1°C before drying. The U.S. Food Safety and Inspection Service (FSIS) recommends a 5-log reduction in *E. coli* O157:H7 on jerky to be considered safe (7). However, no instructions are provided as to the exact procedure to accomplish this. Another recent concern that has become a food safety issue is the possibility that pathogenic cells can become habituated or tolerant to acid which could enable them to survive in the acidic conditions encountered in the marinated food and the host body. Since marinades for jerky are acidic, this potential food safety hazard requires further investigation. This thesis addresses various food safety issues associated with preparation of home-style beef jerky. Before decisions can be made with confidence concerning the inactivation of foodborne pathogens subjected to processing techniques and preservation agents, it is critical to acquire data that is relevant to real life situations. This is especially important when one considers the critical control points that would be evaluated for a minimally processed food operation. Such an example is prepared home-style beef jerky.

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CHAPTER 1

LITERATURE REVIEW

Physical methods of food preservation use physical treatments to inhibit, destroy or remove undesirable microorganisms without introducing additives (Farkas, 1997). One way to accomplish this is to physically dehydrate the food. Dehydration is the oldest food preservation method and it works on the principle of limiting available water to unwanted microbes. Dehydrating reduces the water activity (a_w) which in turn reduces microbial growth and increases lag phase of bacteria resulting in biological forces being controlled (Farkas, 1997). As the temperature increases, the humidity decreases and the osmotic pressure increases. In 1795, the first dehydration room was invented (Desrosier, 1970) and over time people began to realize that all enzyme activity is arrested by dehydration (Rector, 1925). Dried foods are popular because they are nutritious, tasty and lightweight in addition to easy preparation and storage. Dried foods are high in fiber and low in fat which also adds to its appeal. Besides fruits and vegetables, meat has always been a popular food to dehydrate.

Bacteria can be introduced to red meat after removal of the hide through several routes (Jackson et al., 1997). The initial incision can introduce unwanted bacteria from the hide to the muscle. Contamination of workers' hands, aerosols and dust from hide removal, the evisceration process, and fabrication are other ways to introduce unwanted microflora onto red meats. Bacterial attachment to muscle surface involves two stages. The first one involves a loose, reversible sorption believed to involve van der Waals forces. The second stage is an irreversible attachment involving the production of glycocalyx, an extracellular polysaccharide layer. Surface type, growth phase, temperature, cell density and motility also play a role in attachment.

PREVIOUS BEEF JERKY STUDIES

Holley (1995, 1998) examined the effectiveness of drying procedures in eliminating the risk of foodborne illness from *Salmonella, Staphylococcus, Bacillus subtilis* and *Clostridium perfringens.* Smith et al. (1977) investigated the effect of various drying methods on *Salmonella* and *Staphylococcus*. Both Holly and Smith and co-workers determined that heated drying, higher than 53.9°C, was necessary to reduce the risk of foodborne illness. In a more recent study, a reduction of 5.5-6.0 log CFU/g of *E. coli* O157:H7, *L. monocytogenes,* and *S.* Typhimurium was noted after cooking whole meat beef jerky strips prior to drying samples for 10 h (Harrison and Harrison,1996). In uncooked samples, however, populations of the three organisms were reduced by only 3.3, 1.8 and 3.1 log, respectively, after 3 h of drying. In a separate study using ground beef jerky that either contained or lacked a nitrite cure mix, *E. coli* O157:H7, *L. monocytogenes,* and *S.* Typhimurium populations were only reduced by 2.5- 4.0 logs CFU/g after 8 h of drying in samples that lacked the cure mix (Harrison et al., 1997, 1998). When cure mix was added, the bacterial populations were reduced by at least 4 logs. Addition of the cure mix resulted in a greater reduction in *E. coli* O157:H7 and *S.* Typhimurium populations. Faith et al. (1998) and Buege and Luchansky (1999) have shown in previous jerky experiments that the fat content of the meat can influence the reduction of pathogens. Meat with higher percentages of fat provided extra protection for the pathogens that were present. A correlation between drying temperature and time also exists. The higher the temperature, generally the less time required for complete

dehydration. However, care should be taken to not use temperatures that are too high, or case hardening will result in a strip that is not fully dehydrated.

In evaluating the efficacy of traditional preservation procedures, it is critical to consider that bacterial cells injured by such processes as heating, freezing and drying may undergo repair when conditions become more favorable or suitable for growth (e.g., during a storage period). This potential to regain the ability to cause illness is a particular threat with pathogens such as *E. coli* O157:H7 that are believed to have a low infectious dose (Griffin, 1991). There is evidence that when exposed to nonlethal stresses, many foodborne pathogens can undergo changes that may provide them with some degree of resistance to conditions that are typically considered lethal. For example, *E. coli* O157:H7, *L. monocytogenes* and *S.* Typhimurium can become more acid resistant and possibly more resistant to other stresses (e.g., heat, osmotic pressure), if subjected to relatively mild acidity before exposure to more acidic conditions (Buchanan and Doyle, 1997; Leyer et al., 1995; O'Driscoll et al., 1996; Ravishanker and Harrison, 1999). Growth of *E. coli* in an acidogenic (TSB with 1.0 % glucose) broth produced cells that expressed an acid resistance response while cells grown in a non-acidogenic (TSB without glucose) broth did not undergo the response (Buchanan and Edelson, 1999). Acid resistance was increased in those grown in the acidogenic broth. Most marinades used in jerky preparation are acidic in nature. Since foodborne pathogens may be present on the raw product which is exposed to the marinade, there is the possibility that the

microorganisms could undergo an adaptation that might become more resistant to the environmental stresses that the muscle tissue would receive during jerky processing.

Recovery of *E. coli* O157:H7 on two media types was examined by Harrison et al. (1998). This study examined the efficacy of various jerky preparation methods in reducing *E. coli* O157:H7 populations in ground beef and compared the recovery rate of *E. coli* O157:H7 on two selective plating media, modified sorbitol MacConkey agar and modified eosin methylene blue agar (MEMB). A trend toward slightly higher recovery rates with MEMB was observed.

Effect of pre-treatments on E. coli *O157:H7 inoculated onto whole beef jerky strips*.

A study by Albright et al. (2003) investigated the effect of 4 different pre-treatments of whole strip jerky on *E. coli* O157:H7. After 10 h of drying, the treatment marinated for 24 h at 4°C followed by submersion in pickle brine at 78°Cfor 90 s was shown to have the largest overall reduction and the highest pre-pretreatment reduction (5.7-5.8 log CFU/cm^2). The other pre-treatments consisting of: (1) boiling at 94°C for 15 s plus marination at 4°C for 24 h; (2) immersing strips in a 1:1 vinegar and water solution for 20 s at 57.5°C plus marination at 4°C for 24 h and; (3) marination at 4°C for 24 h and then immersing in a 1:1 vinegar and water solution for 20 s at 57.5°C resulted in a 4.3- 4.5, 4.9-5.2 and 4.7- 4.8 log CFU/ cm^2 reduction, respectively (Albright et al., 2003). This research would be more valuable if sensory testing of the various pre-treatments was conducted so that the application of the safest method could be applied by the consumer.

Consumer acceptability and pathogen inactivation

Producing safe jerky that retains acceptable quality attributes is important.

Lethality of *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* as well as consumer

acceptability and sensory attributes of jerky prepared by four methods (traditional, boil

strips prior to drying, pre-cook strips to 71.1ºC in an oven heated to 163.0ºC prior to

drying, and heating strips to 71.1ºC oven after drying) were examined by Harrison et al.

(2001). Of the four treatments, consumers preferred heating strips in the oven after drying

even over the traditional method. The authors found that although the four treatments

were significantly different in color, saltiness and texture, only texture appeared to

influence overall consumer acceptability. Microbial challenge studies subjecting *E. coli*

O157:H7, *L. monocytogenes* and *Salmonella* spp. to the four treatments resulted in log

population reductions of 5.8, 3.9 and 4.6, respectively. Oven treatment of strips after

drying reduced the pathogen populations by an additional 2 logs. A safer, acceptable

home-dried beef jerky can be produced by oven-heating jerky strips after drying.

 Effect of various marinade treatments on acid and nonadapted L. monocytogenes.

Calicioglu et al. (2003a) tested 5 various marinade treatments on whole strip

jerky. All strips were dried at 60ºC for 10 h. Results showed that dipping the strips into

1% Tween 20 for 15 min. and then into 5% acetic acid for 10 min. followed by traditional

marination had the highest inactivation of *L. monocytogenes* (5.9-6.31 log CFU/cm^2) and,

the traditional marination had the least effect aside from the control strips which

contained no marinade. The authors concluded that acid-adapted cells may not increase

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resistance of microbes in jerky processing; thus their findings suggest no significant differences between acid-adapted and nonadapted cells. The practicality of using Tween 20 in a home-prepared marinade may be questionable.

Effect of various marinade treatments on acid-adapted and nonadapted L. monocytogenes and E. coli O157:H7 inoculated onto beef jerky strips after drying.

Calicioglu et al. (2002) used the same marinade treatmentsas they did in the previous study. This time however, the jerky strips were inoculated after drying to observe survival of *L. monocytogenes* during storage. The strips were stored at 25ºC for 60 d and sampled at various intervals. By the end of the storage period, there was no significant difference between acid-adapted and nonadapted cells. This same study was done by Calicioglu et al. (2002) using *E. coli* O157:H7. This time, populations decreased faster on jerky inoculated with acid-adapted rather than with nonadapted cells in all marinade treatments. Rather than acid-adaption enhancing survival, the acid adaptation *E. coli* O157:H7 enhanced inactivation during storage.

ESCHERICHIA COLI

Types of Pathogenic E. coli

There are six types of pathogenic *E. coli* O157:H7. Enteroinvasive (EIEC) *E. coli* causes nonbloody diarrhea, local tissue damage and is uncommon in the United States. Enteroaggregative *E. coli* (EAggEC) effects children of developing countries along with immuno-compromised individuals. "Traveler's diarrhea" is caused by enterotoxigenic *E. coli* (ETEC) where 2 enterotoxins are produced. Enterohemmorrhagic *E. coli* (EHEC)

causes hemorrhagic colitis and serotype O157:H7 is the dominate serotype in this group and causes bloody diarrhea (Doyle, 1997).

Description and history of E. coli *O157:H7*

In 1982, *E. coli* O157:H7 (EHEC) was recognized as a human pathogen (Buchanan and Doyle, 1997; Byrd, 1999; Cheville, 1996; Feng, 1995). More than 100 strains have been identified with the toxin gene (Acheson, 1999).   In 1987, the Shiga-like toxins of *E. coli* O157:H7 and *Shigella* were compared by Calderwood et al. (1987) and they found both bacteria shared the same receptor, structure, and mode of action. Some other disease causing serotypes include O26:H11, O13:H2, O11:H, and O113:H21 with a large outbreak occurring due to O11 in Australia (Acheson, 1999). More than 160 serotypes are known to cause disease in humans and over 200 in cattle. The bacterium is designated by its somatic O (polysaccharide) and flagellar H antigens (Buchanan and Doyle, 1997). According to Acheson (1999), *E. coli* O157:H7 obtained the Shiga-like toxin when it became infected with a bacteriophage that contained the Shiga toxin genes. The majority of EHEC cases are caused by contaminated food and water, but person to person transmission has been reported (Benjamin and Datta, 1995). Because person to person transmission occurs, it is believed the infectious dose is low, less than or equal to 1,000 cells (Chen and Kasper, 1998; Labudde, 2002). This pathogen has been isolated from retail ground beef, pork, poultry, lamb and fecal samples from calves and heifers (Padhye and Doyle, 1991).

Pathogenicity

The animal and human gut is an ideal place for bacterial growth for some types of microorganisms and there can be as many as one trillion cells/g of digesta (Cornell University, 1998). *E. coli* O157:H7 adheres to epithelial cells in the lining of the intestinal tract and there are several stages to this adhesion. As the bacteria approach the intestinal cell surface, a loose association between the bacteria and cell is formed. Next, an "attaching and effacing lesion" occurs where the bacteria make and secrete a number of proteins that affect underlying human gut cells. Intimin is made by the bacteria and is expressed on the bacterial cell surface. This transmembrane bacterial protein mediates actin (part of the epithelial cell cytoskeleton) aggregation in cells attached to the organism by acting as hooks and creating intimin docking sites or receptors on the human host cells. *Symptoms*

More than 20,000 infections from *E. coli* occur each year (Cornell University, 1998). An estimated 2 to 7% of persons infected with *E. coli* O157:H7 will develop HUS and have renal failure (Besser et al., 1993). The incidence of infection by *E. coli* O157:H7 is 1 out of 10,000 people (Web MD, 1999). Symptoms of this illness start as short lived abdominal pains, cramping, and fever. After 1 to 2 d, non-bloody and bloody diarrhea develops and lasts 4 to 10 d with an average duration of 8 d (Tarr, 1995). Next hemolytic uremic syndrom (HUS) may develop 1 week after onset of diarrhea along with intravascular destruction of red blood cells (hemolysis), depressed platelet counts, lack of urine formation (anurina), swelling, and acute renal failure (Buchanan and Doyle, 1997).

Ten-percent of infected children less than 10 years of age suffer from HUS (Tarr, 1995).

HUS is the leading cause of renal failure in U.S. children. In adults, thrombotic

thrombocytopenic purpura (TTP) can develop along with neurological abnormalities.

Symptoms and difficulties from TTP include seizures, coma, stroke, colonic perforation,

pancreatitis and hypertension.

Diagnosis

This pathogen does not ferment sorbitol, hence the use of sorbitol MacConkey

agar for distinction between it and generic *E. coli* by cultural methodology. Diagnosis of

E. coli O157:H7 involves plating a stool specimen on sorbitol MacConkey agar that

contains d-sorbitol instead of lactose as the carbon source. *E. coli* O157:H7, along with

most other hemorrhagic *E. coli* strains, cannot utilize the d-sorbitol and colonies will

remain colorless rather than the bright pink colonies characteristic of non-hemorrhagic

strains (Feng, 1995). There is no known association between virulence of *E. coli*

O157:H7 to its inability to ferment d-sorbitol (Padhye and Doyle, 1991; Tarr, 1995).

Outbreaks

Several hemorrhagic colitis outbreaks were recorded in the U.S. in 1982 and

surveys done in the late eighties showed that 3.5% of ground beef, 1.5% of pork, 1.5%

poultry and 2.0% lamb samples were contaminated with *E. coli* O157:H7, with

hamburger being the most common source of infection. Since its recognition as a human

pathogen in the 1980's, there have been more than a dozen outbreaks linked to *E. coli*

O157:H7 and some of these are listed in Table 1.1 (Feng, 1995).

Prevention

In addition to consumers taking on more responsibility for prevention of foodborne diseases, several relatively cost effective, simple steps can be done by the food industry. Avoidance of fecal contamination as the meat is processed and adding a steam pasteurization cabinet or organic acid rinses are methods that have been used in attempts to minimize pathogens (Labudde, 2002). Other methods which will reduce the number of *E. coli* O157:H7 in the slaughter house are reducing the number of cattle shedding the bacteria and reducing the magnitude of the shedding. Animals fasting for longer than 24 h prior to slaughter is not an option because of the Humane Slaughter Act of 1958 (Labudde, 2002). There is a debate concerning what to feed pre-slaughter animals. Some suggest switching the diet from grain to hay 5 d prior to slaughter (Orr, 2003). This is considered advantageous to some since the hay does not provide residual starch to be fermented in the colon thus preventing exposure of bacteria to the acid conditions conducive for ATR which may increase survivability. However, others have shown that switching the diet yielded more fecal shedding than if the diet remained the same. Also, hay fed cattle shed *E. coli* O157:H7 longer than those fed grain (Orr, 2003).

LISTERIA

Description and history of Listeria

Listeria is a gram positive, nonfastidious, motile, non-sporeforming, non-acid fast, mesophilic, catalase positive, oxidase negative, urease negative pathogen seen in mammals (37 species), birds (17 species), fish and shellfish (U.S. Food And Drug

Administration, 1992a). According to Leyer and Johnson (1993), 1-9% of the human

population has *L. monocytogenes* in their feces. This pathogen targets the elderly,

immuno-compromised individuals, pregnant women, fetuses and diabetics. *L.*

monocytogenes caused the first recorded case of human listeriosis in 1985 although it was

known for its illness in domestic animals since 1929 (Bremer et al., 1995). *L.*

monocytogenes was the only known species until 1985 when others, such as *L. ivanovii*

and *L. seeligeri*, were discovered. Three of the eight known strains cause human and

animal infection.

Listeria is unique in that it is ubiquitous and able to not only survive freezing

temperatures (-20°C), but also shows an increased virulence at low temperatures and is

able to reproduce at 3°C even though its optimal temperature range is 30-37°C. These

characteristics make *L. monocytogenes* a threat to ready-to-eat foods. It is also salt

tolerant. Human to human transmission also occurs.

Pathogenicity

There are three steps in listeriosis infection. First the host cell is penetrated by

Listeria followed by survival and multiplication of the pathogen. Finally, the target tissue

is invaded by the bacterium. The principal site of human infection is the liver.

Symptoms

Listeriosis is characterized by septicemia, encephalitis, spontaneous abortions,

stillbirths, flu-like symptoms, persistent fever, nausea, vomiting and diarrhea (U.S. Food

And Drug Administration, 1992a). Onset of disease is a few days to three weeks with

gastrointestinal symptoms appearing within 12 h.

Diagnosis

Listeriosis can only be positively diagnosed by culturing the organism from blood,

cerebrospinal fluid, or stool. Once the diagnosis is made, the treatment is usually

penicillin or ampicillin. Gentamycin has been recommended by some experts, but its

efficiency is questionable. Both septra and erythromycin have been shown to be effective

in the treatment of *Listeria* (U.S. Food and Drug Administration, 1992a).

Outbreaks

The foods that can harbor *L. monocytogenes* include pasteurized milk, raw milk,

soft cheese, coleslaw and pate (Centers for Disease Control and Prevention, 2002a). The

majority of the cases are due to consumption of contaminated dairy and plant foods

(Samelis and Metaxopoulos, 1999). Meat is also a culprit but less frequently. Table 1.2

highlights listeriosis outbreaks since 1980. The oral dose able to cause infection is

unknown but believed to be low (fewer than 1,000 cells in susceptible individuals)

(Samelis and Metaxopoulos, 1999; U.S. Food And Drug Administration,1992a).

Because of the seriousness of listerosis and low infectious dose, many countries,

including the U.S. have implemented a zero tolerance for *Listeria* in ready-to-eat foods.

While this may be the case, food producers or processors are not required to test for

Listeria except for ready-to-eat foods and being an ubiquitous organism, *Listeria* would

probably be found in many of the samples albeit in low numbers.

Prevention

Prevention consists of thoroughly cooking raw food from animal sources, such as beef, pork or poultry (U.S. Food And Drug Administration,1992a). Raw vegetables should be washed thoroughly before eating. Uncooked meats should be kept separate from vegetables and from cooked foods and ready-to-eat foods. Consumption of unpasteurized milk or foods made from unpasteurized milk should be avoided. Washing hands, knives, and cutting boards after handling uncooked foods should be practiced.

SALMONELLA

Description and history of Salmonella

Salmonella has been known to cause illness for over 100 years (Centers for Disease Control and Prevention, 2002b). An American scientist named Salmon discovered this bacterium and hence the name *Salmonella* (D'Aoust, 1997). More than 2,000 serotypes of human disease causing *Salmonella* have been identified (Doyle and Cliver, 1990) with serotypes Typhimurium and Enteritidis being among the most common ones encountered in the U.S. (Centers for Disease Control and Prevention, 2002b). This rod shaped, gram negative, non-spore-forming, facultative anaerobic, motile bacterium can be found in or on environmental sources that include water, soil, factory surfaces, kitchen surfaces, animal feces, raw meats, raw poultry, and raw seafood (Foster, 1993; U.S. Food And Drug Administration, 1992b).

Pathogenicity

Salmonella serotypes typically cause one of three diseases in humans: gastroenteritis (e.g., *S*. Typhimurium, *S*. Enteritidis, etc.), enteric fever (*S*. Typhi or S. Paratyphi) or an invasive systemic disease (*S*. Choleraesuis). The most common illness encountered in the U.S. is the gastroenteritis salmonellosis (duration 1- 4 d) which is caused by 150 different serotypes (Doyle and Cliver, 1990). Less than 2.5% of human salmonellosis cases in the U.S. are due to *S*. typhi which causes typhoid fever. Enteric fever is also infrequent (< 0.5%) in the U.S. Enteric and typhoid fever are caused by *S.* Paratyphi A, *S*. Schottmuelleri and *S*. Hirschfeldii and also share food vectors such as raw milk, raw salad, eggs and shellfish.

One source estimates that 40,000 cases of salmonellosis are annually reported in the U.S. and approximately 1,000 people die due it its effects (Centers for Disease Control and Prevention, 2002b). Another source estimates as many as 2- 4 million cases per year occur (U.S. Food And Drug Administration, 1992b).   The majority of the outbreaks are caused by consuming undercooked eggs, raw meat and poultry, unpasteurized milk, cross-contamination, and improper handling. Foods that have harbored *Salmonella* include frog legs, coconut, sauces and salad dressings, cake mixes, cream-filled desserts and toppings, dried gelatin, peanut butter, cocoa, and chocolate (U.S. Food and Drug Administration, 1992b). Most domestic pets including cats, dogs, reptiles, pigs and ducks can be carriers of *Salmonella*. Humans have also been carriers

for several days to months and this is especially critical when the carrier is a food handler employed in nursing homes and child-care centers.

To become infectious, the bacteria must pass from the gut lumen and penetrate into the epithelium of the small intestines where inflammation then occurs (U.S. Food and Drug Administration, 1992b). Evidence even suggests that an enterotoxin may be produced, but this has not yet been proven. Most people with the disease can rid themselves of the bacteria without any treatment. Onset of chronic related diseases like Reiter's syndrome requires the bacterium to infect mucosal surfaces. While infectious dose is difficult to measure, several factors, such as type of food and strain, play a role. For a healthy individual the infectious dose is thought to be 10^8 or 10^9 cells (Cliver) but for immuno-compromised individuals the infectious dose may be as low as 1-10 cells. (D'Aoust, 1997).

Symptoms

Salmonellosis results in vomiting, diarrhea, nausea, headache, fever and cramping within 12-72 h after consumption of contaminated food (Centers for Disease Control and Prevention, 2002b). Salmonellosis may develop in anyone but as with *Listeria*, children and elderly are more susceptible. Long term problems can develop from this infection. Postenteritis reactive arthritis and Reiter's syndrome characterized by painful joints, irritation of the eyes and painful urination may appear after 3 weeks of infection and may persist for months to years after disease onset (U.S. Food and Drug Administration, 1992b). This can even lead to chronic arthritis (Centers for Disease Control and

Prevention, 2002b). Antibiotic treatment, such as ampicillin, gentamicin,

trimethoprim/sulfamethoxazole or ciprofloxcin, is used when the disease has spread into

the bloodstream. Some *Salmonella,* like *Salmonella* Typhimurium DT 104, have

developed a resistance to antibiotics possibly due to the heavy use of antibiotics in animal

feed (Centers for Disease Control and Prevention, 2002b).

Diagnosis

Diagnosis, made with a stool sample, requires several days for results. Once the

presence of *Salmonella* is known, additional tests determine which serotype is involved

and thus the proper antibiotic can be given, if necessary. Rapid methods are now

available to food companies that give results for the presence of *Salmonella* within 48 h

(U.S. Food and Drug Administration, 1992b).

Outbreaks

As seen in Table 1.3, many foods have been associated with salmonellosis. The

majority of the outbreaks have been from eggs and plant foods (e.g., sprouts). The variety

of food carriers makes testing and detection difficult.

Prevention

Preventative measures include: (1) washing hands well after using the restroom;

(2) washing hands well after handling raw meat or poultry; (3) not eating raw or

undercooked eggs, meat, or poultry; (4) not drinking unpasteurized milk; (5) disposing of

dirty diapers properly; (6) washing hands well after changing diapers; (7) refrigerating

food promptly; (8) thawing foods properly and; (9) avoid cross contamination, especially among raw and cooked foods.

PHYSICAL PROPERTIES

Intrinsic factors are ones that are inherent to the food itself and examples of such factors include water activity, pH, oxidation-reduction potential, moisture content and nutrient content (Jay, 2000). Some of these factors will be defined and their roles in food spoilage and/or pathogen survival in food will be discussed.

Water Activity, (a_w)

Water activity is the ratio of vapor pressure of water in a material to the vapor pressure of pure water at the same temperature (Fontana, 2001). Perhaps the best definition is the "energy state of water in the food" and its "potential to act as a solvent and participate in chemical and physical reactions and growth of microorganisms" (Fontana, 2001). Pure water has an a_w of 1.00 since all of the water that is present is available for chemical reactions and microbial growth. As the water becomes bound in chemical bonds or is used by microorganisms, the vapor pressure of the solution decreases as does the calculated a_w. The mathematical equation for a_w is p/p_o where p = vapor pressure of the solution (in a closed system) and p_o = vapor pressure of pure water (in a closed system). Controlling water activity in food for preservation purposes has been used for thousands of years despite the lack of scientific reasoning why it works. Sugar, salt, dehydration, and freezing are a few of the ways to alter the water activity of a food. Lowering the water activity is used to stabilize and protect the foods in regard to

microbiological reactions, chemical and physical properties, and rate of deteriorative

reactions (Fontana, 2001). Reducing a_w results in an increased lag phase in microbial

cells which will in turn decrease the growth rate (Jay, 2000). Several factors control

water activity and can be categorized as either osmotic or matric effects (Fontana, 2001).

Colligative effects, capillary effects and surface interactions are such examples.

Colligative effects include the dissolved salt or sugar and interaction with the water via

dipole-dipole, ionic and hydrogen bonds. Capillary effects involve changes in the

hydrogen bonding between water molecules. Surface interactions occur when water

interacts directly with chemical groups on undissolved ingredients (starch and proteins)

via dipole-dipole forces, ionic bonds (H_3O^+ or OH^-), van der Waals forces and hydrogen

bonds (Fontana, 2001). Water activity, not water content, determines the lower limit of

available water for microbial growth (Fontana, 2001). The lowest a_w in which the

majority of food spoilage bacteria can grow is 0.90 (Fontana, 2001). In general, gram

negative bacteria require a higher a_w than gram positive bacteria due to cell wall

differences (Jay, 2000).

Water activity is temperature dependent and some products have an increased

water activity with increasing temperature and vice versa. Foods with high water

activities show negligible variation in water activity when compared at varying

temperatures. Water activity can be measured using either chilled mirror dew point

technology or relative humidity in combination with change in electrical resistance or

capacitance. Both methods vary in accuracy, repeatability, speed of measurement, stability in calibration and convenience of use (Fontana, 2001).

The importance of water activity related to the safety and shelf life of foods is evident by the fact that regulations by the U.S. Food and Drug Administration and U.S. Department of Agriculture references water activity in Good Manufacturing Practices and Hazardous Analysis and Critical Control Point plans.   Studies have shown that at a_w levels below 0.60 the available water is tightly bound so it is unavailable to most organisms.

In general, bacteria require a higher a_w for growth compared to yeasts and molds. The a_w for most fresh foods is 0.99 or greater. Water activity of dry foods is known to affect survival and thermal inactivation of *Salmonella*. Mattick et al. (2000) have shown that proliferation of *Salmonella* is inhibited at a_w less than 0.93. Bearson et al. (1996) found that the a_w of cereal would inhibit growth of *Salmonella*, but if the bacterium was already established on it, it would be able to survive.

Moisture Content

Moisture content is different from a_w since it includes both bound and unbound water where as a_w refers to unbound water only. There are several methods used to determine moisture content of a sample: forced oven draft, vacuum oven, microwave oven and infrared drying. Another method to determine the water content of a food product consists of weighing the food, drying it in a 105ºC oven overnight, and then weighing the dried food. The AOAC Method 935.47 is yet another procedure to obtain

moisture content (AOAC, 1995). For oven-drying methodologies, the following equation

is used to determine percent moisture on a wt./wt. basis.

% moisture = <u>(wt. of wet sample - wt. of dry sample)</u> x100
                                wt. of wet sample

Color Measurements

Color can be scientifically defined. In 1905, A.H. Munsell developed a system

that measures color of an object in terms of hue, value, and chroma. While the system

has been revised over time, it is still in use today. Others methods include the XYZ

tristimulus values, Yxy color space, L*C*h, Hunter Lab color space, and L*a*b. The

present CIE color space, also called L*a*b, is the most common color space for

measuring an object based on the principle of color sensing by the human eye. This

system was developed from the XYZ tristimulus values and Yxy color space systems.

The "L" value reflects lightness and "a" and "b" values are chromiticity (vividness)

coordinates. A positive "a" value reflects red and a negative "a" value reflects green

color, while a positive and negative b value reflects yellow and blue, respectively

(DeMan, 1990).

pH

When discussing pH, words like acid, base, hydronium ion (H_3O^+) and hydroxide

ion (OH^-) are often used. Acids are substances that increase hydronium ion

concentration when added to water and bases are ones that increase hydroxide ion

concentration (Ketchum, 1984). A scale was developed to measure pH which is defined

as the negative logarithm of the hydrogen ion concentration (pH= - log [H^+]).

Undissociated acid acts as an antimicrobial agent (Adams and Hall, 1988).

Lipophilic undissociated acid molecules penetrate the bacterium's plasma membrane. In

high pH cytoplasm, the acid dissociates to release protons and conjugate bases which in

turn disrupt the membrane's proton motive force disabling the energy yield and transport

on which it depends. This is why pKa, and not solely pH play a role in the destructive

nature of acids on bacteria. Acid resistance, acid habituation and acid tolerance response

are three distinct conditions cells may undergo when exposed to acidic conditions.

CELLULAR RESPONSES TO ACIDIC ENVIRONMENTAL STRESS

In order to survive in the host, pathogenic bacteria must be able to overcome

stresses such as the acidic stomach, physical barriers of epithelial cells that line the

gastrointestinal tract and various immune defenses like the onslaught of macrophages.

Beginning in the mouth, bacteria are exposed to digestive enzymes followed by exposure

to low pH, volatile fatty acids, bile, and low oxygen in the small intestines (Gahan and

Hill, 1999). Enterics thrive at a homeostasis of pH 7.6-7.8 and as long as the pH is within

1 unit in either direction, homeostasis is responsible for survival of the cell (Montville,

1997). pH homeostasis is dependent on how permeable the bacterial cell membrane is to

the protons in the acid. Internal pumps either remove or introduce protons into the cell

depending on whether they are exposed to acidic or alkaline conditions (Montville, 1997).

Activated in acidic environments, the K-proton antiporters and Na-proton antiporters act

as cellular pumps. Evidence supports that induction to acid tolerance depends on

hydrogen ions crossing the outer membrane to activate a sensor in the periplasma or on

the periplasmic face of the cytoplasmic membrane with passage of protons being most

probable via the pho E porin.   Protons cross the outer membrane using this pho E system

(Pinedo et al., 1987; Rowbury et al., 1992). In bacterial cells, the cytoplasmic membrane

creates a barrier between external environment and cellular cytoplasm which regulates

what enters and leaves the cell. These actions permit homeostasis of the cytoplasm.

Whenever the cell's homeostasis system cannot function to maintain a neutral pH, other

systems within the cell are activated.

Acid resistance

Acid resistance (AR) response mechanism applies to cells in stationary phase that

are grown in minimal media and acid challenged. Challenge pH values typical range

from pH 2.0-2.5. There are three types of AR responses (Montville, 1997). The first

response is activated by a brief exposure to glutamate prior to pH challenge. The second

type of AR response requires extracellular glutamate during the challenge pH challenge.

The alternate sigma factor controls this process. No induction is needed for this response,

but several hours at low pH are required. For the third AR response, arginine is required

during the pH challenge.

Acid Habituation

Acid habituation is often associated with log phase *E. coli* cells. If acid build up

is gradual, then the cells may habituate. Buchanan and Edelson define acid habituation as

exposure to moderate acidic conditions (pH 5.0) leading to withstanding more acidic pH

values (<2.5) (Buchanan and Edelson, 1999). Habituation can occur rather rapidly. For

instance, at pH 5.0 organisms habituate within 7-10 min at 37ºC (Rowbury et al., 1992;

Rowbury and Hussain, 1987). Foster (2000) uses habituation to describe proliferation of

the bacterium in nutrient broth that is exposed for a short period of time (7 min) to pH 3.

Acid Tolerance

Acid tolerance response (ATR) protects log phase cells during long term exposure

to low pH. This reaction involves several steps. Media containing exponential phase

cells is acidified to a moderate pH (near 5.5) for several hours and then exposed to a

lethal pH (< 4.0). Two types of ATR have been described. Transiently induced ATR

requires the Fe regulator, Fur. Once the proteins are activated, they remain unstable if not

engaged at pH 3.3 for 20-30 min. The second type of ATR is referred to as sustained

ATR which is dependent on rpoS. Growth in media with pH 7, followed by acidification

at pH 4 achieves sustained ATR. Virulent bacterial strains exhibit sustained ATR and

this process can occur rapidly, within 20 min. ATR can be explained in part by the cell's

ability to repair damaged DNA caused by high H^+ concentration. ATR is growth phase

dependent and requires the stress-specific sigma factor rpoS for full induction (Rowbury,

1995).

It is unclear what triggers induction of the rpoS but some believe it may be due to

the slowing of cell division because of the encountered stress (Baik et al., 1996). Growth

rate and phase of cells determine the expression of rpoS controlled regulon which aids in

stressful situations (Jordan et al., 1999). There is both an exponential (log) and stationary

phase ATR. Cells in stationary phase exhibit poor pH homeostasis but exhibit the highest

ATR (Jordan et al., 1999). In *E. coli* O157:H7, fifty acid shock proteins are induced in exponential phase cells (Baik et al., 1996). Of these proteins, 8 require the alternative sigma factor rpoS which is required for sustained ATR in *Salmonella*. Starved cells and stationary cells are more acid resistant than exponential cells. As with *E. coli* O157:H7, rpoS is responsible for ATR in *Salmonella*. The concentration of the acid, pH of the environment and dissociation constant of the chemical are factors that influence ATR. The unionized protonated form of the acid is more permeable to the cytoplasmic membrane than the ionized form (Baik et al., 1996). Exponential phase ATR occurs in both minimal and complex media. Cells are grown to mid-exponential phase and then the pH of the media is changed to pH 5. The "adapted" cells are then challenged at pH 2.5-3.5 for 1-4 h. This type of ATR is present in *E. coli, Listeria* and *Salmonella* but not in *Shigella flexneria*.

Acid shock

Acid shock is encountered when the cell goes straight from a neutral pH to one that is acidic (e.g.,<4.0). Cells undergoing ATR have phenotypical responses that aid in survival of bacterial cells exposed to extreme acidity (Brown et al., 1997). This coping method involves a two-stage process: adaptive period where the cells are exposed to a mild pH (5.0-6.0) followed by an acid challenge or shock exposure to pH below 4.0 (Garren et. al, 1997). Adapted cells resist acid damage to DNA better than unadapted (Rowbury, 1995).

Table 1.1. Selected pathogenic *E. coli* outbreaks, location of outbreak, source of outbreak, and number of confirmed cases per outbreak since 1982.

Year	Place	Source of outbreak	Number of Cases	Source
1982	Oregon	Hamburger at fast food	26	Doyle et al., 1997
1989-90	Montana	Drinking water	243	Doyle et al., 1997
1991	Oregon	Swimming water	21	Doyle et al., 1997
1991-92	Massachusetts	Unpasteurized apple cider-dropped apples	26	Keene et al., 1997
1993	Multi-state	Raw hamburger	731	Doyle et al., 1997
1994	Washington & California	Pre-sliced dry fermented salami	23, 2 HUS	Tilden et al.,1996; Faith et al., 1998
1994	Virginia	Undercooked ground beef	20	Centers for Disease Control and Prevention, 1995a
1995	Oregon	Home-style jerky	5	Keene, et al., 1997
2000	Multi-state	Conagra	~ 30	Labudde, 2002

Table 1.2. Selected Listeria monocytogenes outbreaks, location of outbreak, source of outbreak, and number of confirmed cases since 1981.

Year	Place	Source	Number of Cases	Source
1981	Canada-1st North American	Coleslaw	41	Bahk and Marth, 1990
1983	Massachusetts	Pasteurized Milk	49	Bahk and Marth, 1990
1985	California	Mexican-style Cheese	100+	Bahk and Marth, 1990
1985	United States	Pasteurized milk	49	Bockserman, 1998
1989-1990	United Kingdom	Pate	300	Rocourt and Cossart, 1997
2000	Multi-state	Deli Turkey	47	Centers for Disease Control and Prevention, 2002c
2002	United States	Turkey	46	Anonymous, 2003

Table 1.3. Selected *Salmonella* outbreaks, location place of outbreak, source of outbreak, and number of confirmed cases since 1974.

Year	Place	Source	Number of Cases	Source
1974	Navajo Reservation	Egg in potato salad	est 3,400	D'Aoust, 1997
1984	Canada	Cheese	est 2,700	D'Aoust, 1997
1985	U.S.	Possibly milk	16, 284	D'Aoust, 1997
1985	Georgia	Turkey salad	100	Centers for Disease Control and Prevention, 1985
1991	Japan	Egg	10,000 +	D'Aoust, 1997
1991	U.S. & Canada	Cantaloupe	400	Centers for Disease Control and Prevention, 1991
1995	Multi-state	Ice cream products	80	Centers for Disease Control and Prevention, 1994
1995	New Mexico	Beef jerky	93	Centers for Disease Control and Prevention, 1995a
1995	Nevada	Turkey & dressing	7	Centers for Disease Control and Prevention, 1995b
1998	Midwest/East	Toasted oats	209	Centers for Disease Control and Prevention, 1998b
2002	Florida	Tomatoes	141	Centers for Disease Control and Prevention, 2002b

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CHAPTER 2

EFFECT OF VARIOUS PROCESSING PARAMETERS ON PATHOGEN

REDUCTION OF HOME-STYLE BEEF JERKY[1]

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ABSTRACT

Beef jerky is a popular dehydrated food. Due to foodborne illness cases linked to home prepared jerky during the 1980's and 1990's (4,5), questions arose concerning the safety of the product. There are numerous variations on how home-style beef jerky can be prepared. The objectives of this paper were to address several different safety concerns and aspects of home-style beef jerky. When applied to jerky strips, both sugar and salt marinades significantly increased the lethality of the drying process on inactivation of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* spp. There was no significant difference in the reduction of *E. coli* O157:H7 populations inoculated on beef strips regardless of whether the cells were in direct or indirect contact with the liquid marinade during marination. The effectiveness of vertical air flow dehydration versus horizontal air flow dehydration on reducing the populations of *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* spp. was determined along with the comparison of the final product's physical properties. Among the physical properties measured, horizontal air flow reduced the water activity and moisture level of jerky more than vertical air flow. The water activity and percent moisture for strips dried with horizontal and vertical air flow were 0.679 and 0.735 and 20.04% and 23.06%, respectively. Lethality of moist versus dry heat applied as a post-dehydration step to inactivate *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* spp. jerky strips was determined. Using dry heat is as effective as moist heat in the post-dehydration step. Factors that affect the outcome of the physical properties of jerky as well as the safety

aspects were revealed during this study and all of the steps that result in a safer jerky can

be applied by a consumer with minimal effort for these scenarios.

INTRODUCTION

Dehydration, one of the oldest food preservation methods, reduces the water activity (a_w) of the food, which in turn reduces microbial growth and increases lag phase of bacteria. Synergistic antimicrobial effects can occur during beef jerky preparation due to the interaction between reduced product a_w, increased air temperature used for drying, and the variety of ingredients used in marination of jerky.

Several beef jerky studies have been done to help clarify the importance and significance of certain steps in the jerky process. Alternative, safer preparation methods were examined for home-style jerky preparation. However, producing safe jerky that also retains acceptable quality attributes is important. Lethality of *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* as well as consumer acceptability and sensory attributes of jerky prepared by four methods (traditional, boil strips prior to drying, pre-cook strips to 71.1°C in an oven prior to drying, and heating strips to 71.1°C after drying) were examined. Of the four treatments, consumers preferred heating strips in the oven after drying even over the traditional method (10). The authors found that although the four treatments were significantly different in color, saltiness and texture, only texture appeared to influence overall consumer acceptability. Microbial challenge studies subjecting *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* spp. to the four treatments resulted in log population reductions of 5.8, 3.9 and 4.6, respectively. Oven treatment of strips after

drying reduced the pathogen populations by an additional 2 logs. A safer acceptable home-dried beef jerky can be produced by heating jerky strips to 71.1℃ after drying.

A study by Albright et al. (1) investigated 4 different pre-treatments of whole strip jerky on *E. coli*. After 10 h of drying, the treatment seasoned for 24 h at 4℃ followed by submersion in pickle brine at 78℃ for 90 s was shown to have the largest overall reduction and the highest pretreatment reduction (5.7-5.8 log CFU/cm^2). The other pre-treatments consisting of: (1) boiling at 94℃ for 15 s with marination at 4℃ for 24 h; (2) immersing strips in a 1:1 vinegar and water solution for 20 s at 57.5℃ with marination at 4℃ for 24 h and; (3) marination at 4℃ for 24 h and then immersing in a 1:1 vinegar and water solution for 20 s at 57.5℃, showed a 4.3-4.5 , 4.9-5.2 and 4.7-4.8 log CFU/ cm^2 reduction, respectively (1). This research would be more valuable if sensory testing of the various pre-treatments were conducted so that the application of the safest method could be applied by the consumer.

In a separate study using ground beef jerky that either contained or lacked a nitrite cure mix, *E. coli* O157:H7, *L. monocytogenes, S.* Typhimurium populations were reduced by 2.5- 4.0 logs CFU/g after 8 h of drying in samples that lacked the cure mix (11,12). When cure mix was added, the populations were reduced by at least 4 logs. Faith et al. (8) and Buege and Luchansky (3) have shown in previous jerky experiments that the fat content of the meat can influence the reduction of pathogens. Meat with higher percentages of fat provided extra protection for the pathogens that were present.

The interactions between all variables associated with home-style whole beef jerky lead to many questions related to safety and consumer friendly aspects that need to be investigated. When marinating, often only one side of the meat strip is submerged in the marinade. No previously reported studies determined the effect this has on safety of the finished product. Suggested recommendations have been made to incorporate a post-dehydration step using heat to further enhance pathogen reduction on jerky. For consumer acceptance, the physical properties of beef jerky made with safer recommendations should be similar to jerky made the more traditional way or similar to commercially available products. All of these concerns were addressed with the intention to apply what was learned to the consumer so a safer, quality jerky is produced.

MATERIALS AND METHODS

Bacterial strains and inoculum preparation. *E. coli* O157:H7 932 (clinical), E009 (beef), 204 P (pork), E0019 (cattle feces), and 380-94 (salami) and *L. monocytogenes* (Brie cheese isolate), Scott A (clinical isolate), LCDC #81-861 (coleslaw outbreak isolate), V7 (milk isolate) and 301 (cheddar cheese isolate) were obtained from the Center for Food Safety, The University of Georgia, Griffin, GA. *Salmonella* (*S*. Typhimurium 654, *S*. Typhimurium DT 104 H3380, *S*. Typhimurium DT 104 H3402, *S*. California and *S*. Enteritidis) were obtained from USDA/ARS, Athens, GA. All strains were preserved on Microbank ™ beads (Pro-Lab Diagnostic, Austin, TX) frozen at -20ºC. Each strain was activated in 9 ml portions of tryptic soy broth (TSB; Difco Labs, Division of Becton Dickinson and Co., Sparks, MD) at 37ºC (statically) for 20-24 h. Each culture was

centrifuged for 20 min at 2,500 x g and the pellet re-suspended in 10 ml 0.1% peptone

water (Bacto peptone, Difco Labs). The five strains of each bacterial type were pooled

just prior to inoculation. One side of each beef strip was inoculated.   One-hundred µl of

each pool was inoculated on separate 1/3 portions of the strip surface to prevent the

pathogen types from overlapping. Adhesion time was 30 min at room temperature under

a laminar air flow hood unless noted otherwise. One ml of each pool was placed in 9 ml

tubes containing 0.1% peptone and serially diluted to determine initial inoculum levels

for each bacterial pool.

Bacterial enumeration and enrichment. For experiments requiring bacterial

enumeration, sampling was done by placing a jerky strip in a sterile stomacher bag with

225 ml 0.1% peptone. It was then pummeled in a stomacher (TekMar model 400,

Cincinnati, OH) for 2 min on high speed. Serial dilutions were made using 0.1% peptone

buffer. Portions were spiral plated (Autoplate 4000; Exotech, Gaithersburg, MD) on

bismuth sulfite agar (BSA; Difco Labs), *Listeria* selective agar (LSA; Oxoid;

Basingstoke, Hampshire, England), and sorbitol MacConkey agar (SMAC; Oxoid) for

Salmonella, L. monocytogenes and *E. coli* O157:H7 enumeration, respectively. The

plates were incubated for 24 h at 37ºC before colony forming units were counted and then

re-incubated and re-examined after an additional 24 h.

For the marination versus non-marination strips, subcultures of pummeled

samples were enriched in the event that the populations of the pathogens were reduced to

levels not detectable by direct plating. Enrichment consisted of inoculating 1 ml from the

stomacher bag into 9 ml portions of lactose broth (Difco Labs), UVM *Listeria* enrichment broth base (Oxoid), and modified tryptic soy broth (modified TSB; Difco Labs) (10.0 g casamino acids, 1.5 g bile salts No.3, 6.0 g dibasic, anhydrous sodium phosphate and 1.35 g potassium phosphate per liter of TSB) for *Salmonella*, *L. monocytogenes* and *E. coli* O157:H7 enrichment, respectively. All three enrichment broths were incubated at 37°C for 18-24 h. After incubation, portions of the modified TSB cultures were streak plated onto SMAC plates. Plates were incubated at 37°C for 24 h and examined for the presence of representative colonies. Subcultures were also made from the lactose broth into selenite cystine (Difco Labs) and TT broth Hajna (Difco Labs) and from UVM *Listeria* enrichment broth into Fraser broth (Difco Labs) and then incubated at 37°C for 24 h. After incubation of the broths, portions were streak plated onto BSA, XLD (Difco Labs) and brilliant green agar (BGA; Difco Labs) for possible *Salmonella* spp. isolates and onto LSA for possible *L. monocytogenes* isolates. Plates were incubated at 37°C for 24 h and examined for the presence of representative colonies.

The identification of representative, presumptive isolates from the enrichment steps above were tested. Presumptive *Salmonella* spp. and *E. coli* O157:H7 isolates were identified using the Micro-ID™ identification system for Enterobacteriaceae (Remel, Lenexa, KS) as per manufacturer's instructions. *Salmonella* isolates were serotyped with *Salmonella* O-antisera and *E. coli* isolates were serotyped with *E. coli* O157 and H7 antisera. (Difco Labs). *Listeria* isolates were identified using the Micro-ID™ *Listeria* system (Remel, Lenexa, KS) as per manufacturer's instructions.

Beef strip preparation. Bottom round steak was purchased from a local Athens, GA supermarket. The edges of the beef were trimmed of visible fat and then sliced into 6.0 x 1.5 x 1.5 cm size strips.

Marinade composition. The marinated strips were stored for 16 h at 4°C in either a typical salt level marinade consisting of 60 ml (1/4 cup) soy sauce, 15 ml (1 Tbs.) Worcestershire sauce, 0.6 g (1/4 tsp.) black pepper, 1.25 g (1/4 tsp.) garlic powder, 4.35 g (1 tsp.) hickory smoked flavor salt and 1.5 g (½ tsp.) onion powder/ 454 g (1lb.) meat or a sugar based marinade containing 237 ml (1 cup) lite soy sauce, 5.86 g (1 tsp.) salt, 2.86 g (2 tsp.) hot pepper flakes, 2.66 g (1 tsp.) paprika, 9.04 g (2 tsp.) minced garlic and 44.4 ml (3 Tbs.) maple syrup/ 454 g (1 lb.) meat.

Dehydrators. Two home-style vertical air flow food dehydrators (model # 1000, American Harvest, Inc., Chaska, MN) were used to dehydrate both strip types. The dehydrators were preheated to 60°C prior to drying the strips. The internal air temperature and internal temperature of a strip for each dehydrator were monitored and recorded by a data recorder (model RD106 A, Omega, Stamford, CT) equipped with copper/constantan thermocouples (5TC-TT-T, Omega, Stamford, CT).

Physical properties. Water activity (a_w) was measured at $25 \pm 2^\circ C$ with a model CX2

Aqua Lab water activity meter (Decagon Devices, Inc., Pullman, WA). pH was measured

by the surface method using a surface electrode and a Corning model 340 pH meter

(Corning, Inc., Corning, NY). Percent moisture was determined by the AOAC Method

935.47 (AOAC International, 1995) and Quantab® titrator strips (0.5-1.% Cl-) were used

to measure percent sodium chloride (Environmental Test Systems, Inc., Elkhart, IN).

However, modification to the instructions were required because the salt content in the

jerky samples were higher than the strips could measure. As per the manufacturer's

instructions, a 10 g finely chopped sample was added to 90 ml of boiling water and stirred

vigorously for 30 s, allowed to stand for 1 min and then stirred vigorously for another 30

s. The Quantab® reading was multiplied by 10 to calculate actual salt concentration in

the sample. Maximum load and toughness (amount of energy necessary to break the

sample) were assessed using a Warner-Bratzler shearing device until the total break of the

sample. These force-deformation curves were measured by a texture analyzer model TA-

XTZ (Texture Technologies Corp., Scarsdale, NY). The strips were placed inside the

Warner-Bratzler shear blade and sheared perpendicular to the muscle. For each sample,

two strips were sheared and each strip was sheared twice. The average force (N) of the

four values was calculated. The area A Minolta chroma meter (model CR-310) was used

to measure L*a*b values.

Dehydrators. The American Harvest, Inc. model # FD40 dehydrator (Chaska, MN) was used as a vertical air flow drier and the Equiflow model #7010 (B & J Industries, Marysville, WA) was used to obtain horizontal air flow.

Marination versus non-marination. Half of the beef strips used for this study were marinated with a salt based marinade, while the rest were not marinated. A vertical air flow dehydrator set at 60°C was used to dry the meat. Four different methods of preparation for marinated strips were examined: (1) dehydrated; (2) dehydrated and then placed in a 135°C oven (mechanical convection oven, model 18 EM; Precision Oven, Chicago, IL) for 10 min; (3) boiled for 5 min in marinade and dehydrated and; (4) placed in 135°C oven for 10 min and then dehydrated. Non-marinated strips were treated in the same fashion. For the non-marinated strips that were boiled, water was used in lieu of marinade. Strips were sampled after each step in the jerky making process. This was done in triplicate.

Effect of direct versus indirect contact of marinade on *E. coli* O157:H7. Four strips of meat were surface inoculated with the 5 strain pool of *E. coli* O157:H7 as described previously. Two of the strips were placed face down in a shallow pan containing the salt based marinade and two strips were placed face up in the pan. The pans were placed in a 4°C refrigerator for 8 h ($\pm$ 3 h) prior to enumeration of surviving *E. coli* O157:H7 populations on plate count agar (PCA, Difco Labs) and SMAC as described previously. This was done in triplicate.

Comparison of a sugar and salt marinade on physical properties of jerky prepared in a vertical and horizontal air flow dehydrator during various storage conditions. Sugar and salt marinated jerky strips dried in either a 60ºC horizontal or vertical air flow food dehydrator were prepared. Percent moisture, a_w and pH were measured for raw beef, beef strips after marination and jerky after dehydration for both marination types. After dehydration, jerky strips were divided into the following groups for a storage study: (1) sugar marinated strips in ziplock® type bags stored at room temperature in a desiccator; (2) salt marinated strips stored in a ziplock® bag at room temperature in a desiccator; (3) sugar marinated strips stored in a Cryovac® bag placed inside a desiccator and stored at room temperature; (4) salt marinated strips stored in a Cryovac® bag placed inside a desiccator and stored at room temperature; (5) sugar marinated strips stored at 4.0ºC in a Cryovac® bag; and (6) salt marinated strips stored at 4.0ºC in a ziplock® bag. All samples were stored for 4 weeks prior to measuring a_w.

Effect of vertical vs. horizontal air flow on physical properties of beef jerky dried at various temperatures and comparison of various post-drying treatments. Beef strips inoculated with *E. coli* O157:H7 strains were dried in either a horizontal or vertical air flow food dehydrator with an internal temperature of either 54 or 60ºC. Post-drying treatments resulted in an internal temperature of 71.1ºC under moist or dry heat conditions. To examine the effect of dry versus moist heat for reduction of *E. coli* O157:H7 populations on beef jerky strips, the strips were post-inoculated in the same manner as described previously. The strips were then placed on a baking sheet. The

strips were exposed to one of three treatments. The control strips were placed on a

baking sheet and placed directly into the oven. The second treatment had the strips and

pan wrapped in aluminum foil. The last treatment consisted of the strips on the pan being

wrapped in foil and 1 cup of water was placed in the foil with caution taken not to wet the

strips. The strips were than placed in either a 93.3°C oven for 30 min. or a 135.0°C oven

for 10 min. (mechanical convection oven, model 18 EM, Precision Oven, Chicago, IL).

Samples were plated on PCA and SMAC at the following intervals; before marination,

after marination, after dehydration, and after oven treatment. This experiment was done in

triplicate.

Physical properties of commercial brands of whole beef jerky. Five commercially

available beef jerky brands (A, B, C, D, E) were purchased from the Internet and a local

Athens, GA grocery store to compare product a_w, pH, percent moisture, percent NaCl, and

color (L*a*b). The brands were compared among themselves and with home-style whole

strip beef jerky.

Statistical analysis. For the study comparing vertical and horizontal air flow, the average

bacterial counts of three separate replications were transformed using the logarithmic

transformation. Comparison was made using SAS which compared the log reduction of

vertical versus horizontal air flow. The two dehydrator temperatures (54 or 60°C) were

also compared among and between air flow types. The oven conditions (dry and moist)

were also compared within and between air flow types. The physical properties measured

for the five commercial brands of jerky were also analyzed with SAS (16). Analyses

were conducted using the PROC GLM procedure for SAS for both home-made and commercially available jerky.

<div align="center">RESULTS</div>

Marination versus non-marination. There was a larger log population reduction for strips that were marinated compared to the non-marinated strips. The log bacterial population reduction for *E. coli, L. monocytogenes*, and *Salmonella* were 5.8, 4.1, and 4.6 for marinated strips and 4.0, 2.8, and 3.5 for the non-marinated strips, respectively (Tables 2.1, 2.2, 2.3). Marination in a salt-based marinade increased the lethal effect of drying for all three pathogens. For the strips that were boiled for 5 min. prior to drying, bacterial population of pathogens reached undetectable levels of *E. coli* O157:H7 and *L. monocytogenes* by direct plating (4.0×10^2) for both marinated and non-marinated strips (Tables 2.1 and 2.2). The bacterial log populations of *E. coli* and *L. monocytogenes* were reduced to undetectable levels (4.0×10^2) for both pre-heating in an oven at 163°C for 10 min and post-heating at 135°C in an oven for 10 min for marinated and non-marinated jerky strips (Tables 2.1 and 2.2). *Salmonella* populations were reduced on marinated strips that were treated in an oven to undetectable levels for both heat treated strips (Table 2.3).

Detectable *Salmonella* were still present for both pre-heating in an oven at 163˚C for 10 min and post-heating at 135ºC in an oven for 10 min for non-marinated strips (Table 2.3).

Effect of direct versus indirect contact of marinade on *E. coli* O157:H7. The average mesophilic aerobic populations for the strips that were placed in the marinade so the inoculated surface was not submerged in the marinade and plated on PCA was 1.87 x10^9 CFU/strip. For those strips where the inoculated surface was in direct contact with the marinade, the populations were 1.62 x 10^9/strip. *E. coli* O157:H7 populations detected on the SMAC plates for strips that were not submerged and submerged in the marinade were 1.1 x 10^7 and 2.17 x 10^6, respectively. Strips exposed to direct contact with marinade resulted in *E. coli* O157:H7 CFU/strip populations that were ~ 1 log lower than strips with indirect contact. As would be expected, the non-selective PCA yielded higher populations than the SMAC plates.

Comparison of a sugar and salt marinade on physical properties of jerky prepared in a vertical and horizontal air flow dehydrator during various storage conditions. Sugar marinated jerky had an a_w of 0.65 and a pH of 5.27. Salt marinated jerky had an a_w of 0.75 and a pH of 5.64. The drying time for the sugar marinated jerky to reach the same visual endpoint was approximately 3 h longer than that for the salt marinated jerky.

For the comparison of air flow direction and various storage conditions, there was no noticeable difference in the a_w of sugar or salt marinated samples stored under various conditions. The various storage conditions for 4 weeks and their respective water activities are presented in Table 2.4.

Effect of vertical vs. horizontal air flow on physical properties of whole beef jerky dried at various temperatures and comparison of various post-drying treatments. Dehydrator temperature (54 or 60ºC) had a significant effect (p<0.05) on pH and percent moisture of the final product. Airflow direction significantly (p<0.05) affected water activity of the final product, with strips dried at 54.4ºC using horizontal air flow producing a product with a lower water activity (0.679) as compared to the vertical flow (0.735). For strips dried at 60ºC, the water activity of strips dried with horizontal and vertical air flow were 0.665 and 0.706, respectively. Air flow type also significantly affected moisture levels (p<0.05) of the product, with strips dried at 54.4ºC using horizontal air flow resulting in a lower moisture level (20.04%) versus vertical flow (23.06%) (Table 2.6). This same trend was observed in strips dried at 60ºC with horizontally dried strips yielding a 16.03% moisture and vertical yielding 20.24% moisture. Strips dried at 60ºC had less available water than those dried at 54ºC (Table 2.7).

Comparison of *E. coli* O157:H7 population reduction and physical properties for dry and moist heat on post-dehydrated jerky strips. Results for the comparison of moist versus dry heat on reduction of *E. coli* O157:H7 showed that the average log population reduction determined on SMAC plates for jerky strips exposed to dry or moist heat after dehydration was 5.73 for both treatments (Table 2.5). Tables 2.6, 2.7, and 2.8 summarize the physical properties for the various air flows, dehydrator temperatures, oven temperatures, and heating types. Tables 2.9 and 2.10 contain the texture analysis

results for the air flow and various post treatments. For strips dried at 54.4°C using

vertical air and heated in a 93.3°C oven, more force (kg) was used to sheer the samples

compared with the strips that were placed in the 135°C oven. For strips dried at 54.4°C,

time required to sheer strips for both air flow directions, despite post oven temperature,

were within 8.53 and 11.43 s. The area(kg.s) under the force-deformation curve was not

noticeably different for all strips dried at 54.4°C and 60.0°C with values ranging from

46.10-62.84 kg.s and 39.01-59.65 for strips dried at 54.4°C and 60.0°C, respectively.

Comparison of commercial brands of whole beef jerky. Table 2.8 shows the values of

the physical properties for both the commercial and home-style jerky. For all sampled

jerky, there was no significant difference seen for the physical property (p>0.05).

<div align="center">DISCUSSION</div>

There are numerous variations on preparation methods used for home-style beef

jerky. A number of factors involved, if done marginally, raise food safety concerns. In

addition there have been several jerky outbreaks since the early 1980's (4,5). This paper

addresses several different safety aspects of home-style jerky. The effects of marination

on the survival of *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* spp. on whole beef

jerky strips were determined. The salt in the marinade aids in reducing a_w of the product,

which in turn aids in maintaining safety and shelf life of jerky (15).

Soy sauce, fermented soybeans or wheat, consists of 5% ethanol, sodium

benzoate, and lactic acid or acetic acid and has a pH of 4.5 (14). It is often the main

ingredient in many jerky marinades and the antimicrobial effects of the lactic or acetic

<div align="center">57</div>

acid help reduce pathogen populations on whole jerky strips. During the home-style

marination process, often the meat is not fully submerged in the marinade. To determine

if direct contact with the marinade is required for pathogen inhibition or population

reduction, a study was done that compared *E. coli* O157:H7 populations on strips that

were not submerged and submerged in the marinade during marination. Results from

non-submerged and submerged strips were not noticeably different. The findings from

this experiment support the current recommendations such as those found in food

preservation guides such as *So Easy to Preserve* (2) for jerky marination in which the

marinade may not cover the entire strip. The use of more marinade to entirely cover the

strip does not enhance the effort to reduce *E. coli* populations on the product.

In a comparison of effect a salt-based marinate or a sugar-based marinate had on

physical attributes of the beef jerky, the salt marinated product had a higher a_w than the

sugar marinated product. The sugar marinated beef strips required a longer drying time (3

h longer) compared to the salt marinated strips. A recipe calling for a sugar based

marinade may need more drying time to achieve the same level of safety as the salt

marinated strips. Jerky is a product with a long shelf life assuming the storage conditions

do not introduce moisture into the dehydrated meat. A storage study was conducted

involving both sugar and salt marinated jerky where the final products were stored for 4

weeks under various conditions. The different combinations of storage temperatures, bag

types and storage conditions examined in this study showed there was no difference in the

water activity (a_w) over time related to the type of marinade used to prepare the product.

An experiment was designed to compare the physical properties of jerky prepared in either a vertical or horizontal dehydrator dried at either 54 or 60°C. Horizontal air flow resulted in a lower water activity and lower percent moisture level than vertical flow. As the temperature increases, the humidity decreases and the osmotic pressure increases (7,9). Dehydration at the higher temperature resulted in lower water activity.

Strips were post-contaminated with *E. coli* O157:H7 and effect of dry versus moist heat on the pathogen inactivation was recorded. Some studies have shown that moist heat is more detrimental than dry heat due to the faster rate of protein denaturation that occurs with moist heat (13). Dry heat is less lethal and relies on dehydration and oxidation to kill the cells. Dry heat also requires higher temperatures and a longer heating time compared to wet heat (9). For this product and preparation process, data does not support the notion that moist heat has a greater lethal effect on *E. coli* O157:H7 when heated in an oven.

A comparison of the physical properties of commercially available whole strip beef jerky was made within the brands and with home-style whole strip beef jerky. Statistical analysis showed no significant difference ($p > 0.05$) for a_w, percent moisture, percent sodium chloride, pH and color measurements (L*a*b).

Many factors contribute to the safety of home-style beef jerky. While one factor alone may not be significant for safety, the synergistic effects of the various factors contribute to the overall safety of the product. As new methods of jerky marination, preparation, dehydration and storage are introduced to consumers, the acceptability by the

consumer is an important aspect. Both the consumer acceptability and safety must be

considered when evaluating current and alternative methods for home-style jerky.

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Table 2.1. Populations of *E. coli* O157:H7 (log CFU/strip) on marinated and non-marinated beef jerky strips for four different preparation treatments.

Treatment	Marinated					Non-marinated			
	Pre-treatment	After marinating	After boiling	After oven	After drying	Pre-treatment	After boiling	After oven	After drying
1[a]	6.39	5.89	na[b]	na[b]	< 0.60[c]	6.37	na[b]	na[b]	3.18
2[d]	6.39	5.89	< 0.60[c]	na[b]	< 0.60[c]	6.37	< 0.60[c]	na[b]	< 0.60[c]
3[e]	6.39	5.89	na[b]	< 0.60[c]	< 0.60[c]	6.37	na[b]	3.06	< 0.60[c]
4[f]	6.39	5.89	na[b]	< 0.60[c]	< 0.60[c]	6.37	na[b]	2.95	< 0.60[c]

[a] Strips dehydrated at 60°C.

[b] This step of the jerky processing was not performed for this treatment.

[c] Populations were below detectable level (4.0×10^2).

[d] Strips boiled for 5 min prior to drying at 60°C.

[e] Strips pre-heated in a 163°C oven for 10 min prior to drying at 60°C.

[f] Strips dehydrated at 60°C followed by heating in a 135°C oven for 10 min.

Table 2.2. Populations of *L. monocytogenes* (log CFU/strip) on marinated and non-marinated beef jerky strips for four different preparation treatments.

Treatment	Marinated					Non-marinated			
	Pre-treatment	After marinating	After boiling	After oven	After drying	Pre-treatment	After boiling	After oven	After drying
1[a]	4.65	2.93	na[b]	na[b]	< 0.60[c]	4.51	na[b]	na[b]	1.69
2[c]	4.65	2.93	< 0.60[c]	na[b]	< 0.60[c]	4.51	< 0.60[c]	na[b]	< 0.60[c]
3[e]	4.65	2.93	na[b]	< 0.60	< 0.60[c]	4.51	na[b]	1.44	< 0.60[c]
4[f]	4.65	2.93	na[b]	< 0.60	< 0.60[c]	4.51	na[b]	0.60	0.60

[a] Strips dehydrated at 60°C.

[b] This step of the jerky processing was not performed for this treatment.

[c] Populations were below detectable level (4.0×10^2).

[d] Strips boiled for 5 min prior to drying at 60°C.

[e] Strips pre-heated in a 163°C oven for 10 min prior to drying at 60°C.

[f] Strips dehydrated at 60°C followed by heating in a 135°C oven for 10 min.

Table 2.3. Populations of *Salmonella* (log CFU/strip) on marinated and non-marinated beef jerky strips for four different preparation treatments.

Treatment	Marinated					Non-marinated			
	Pre-treatment	After marinating	After boiling	After oven	After drying	Pre-treatment	After boiling	After oven	After drying
1[a]	6.42	5.33	na[b]	na[b]	1.84	6.24	na[b]	na[b]	2.76
2[c]	6.42	5.33	< 0.60[d]	na[b]	< 0.60[d]	6.24	< 0.60[d]	na[b]	< 0.60[d]
3[e]	6.42	5.33	na[b]	< 0.60[d]	< 0.60[d]	6.24	na[b]	2.28	1.85
4[f]	6.42	5.33	na[b]	< 0.60[d]	< 0.60[d]	6.24	na[b]	2.84	2.05

[a] Strips dehydrated at 60ºC.

[b] This step of the jerky processing was not performed for this treatment.

[c] Strips boiled for 5 min prior to drying at 60ºC.

[d] Populations were below detectable level (4.0×10^2).

[e] Strips pre-heated in a 163ºC oven for 10 min prior to drying at 60ºC.

[f] Strips dehydrated at 60ºC followed by heating in a 135ºC oven for 10 min.

Table 2.4. Average a_w after 4 weeks of storage of whole strip beef jerky made with either a sugar or salt-based marinade.

Storage Treatments	Sugar Marinated strips	Salt Marinated strips
Ziplock®/ Room temperature/ Desiccator	0.656	0.634
Cryovac®/ Room temperature/ Desiccator	0.682	0.570
Cryovac®/ Room temperature	0.656	0.670
Ziplock®/ 4°C	0.621	0.594
Ziplock®/ Room temperature	ns[a]	0.638

[a] This storage treatment was not done for sugar marinated strips.

Table 2.5. Average log populations and log reduction for *E. coli* O157:H7 on beef jerky strips that were post-treated with dry or moist heat applied.

Sample time	Log population	Log population reduction
Before marinade	7.80	-
After marinade[a]	7.47	0.33
After dehydration[b]	2.74	5.06
After dehydration and oven heating[c]	2.01	5.79
After dehydration and oven heating using dry heat[d]	2.08	5.72
After dehydration and oven heating using moist heat[e]	2.08	5.72

[a] After marination for 16 ($\pm$ 2 h) at 4°C.
[b] Strips dehydrated at 60°C.
[c] Dehydrated strips heated in a 163°C oven for 10 min.
[d] Dehydrated strips wrapped in aluminum foil and heated in a 163°C oven for 10 min.
[e] Dehydrated strips wrapped in aluminum foil with water added to the package and heated in a 163°C oven for 10 min.

Table 2.6. Comparison of air flow direction, post-dry oven temperatures and heating conditions on properties of whole beef strips dehydrated at 54.4 °C.

Air flow direction	Post-dry oven temperature (°C)	Post-dry heating conditions	a_w	pH	Moisture (%)	NaCl (%)	Color[a]		
							L	a	b
Vertical	na[b]	na[b]	0.735	5.47	23.06	5.70	25.11	3.43	5.26
Vertical	93.3	Dry	0.696	5.65	21.31	6.30	25.22	4.01	5.50
Vertical	93.3	Moist	0.715	5.53	21.96	5.73	24.99	3.79	5.45
Vertical	135.0	Dry	0.714	5.57	22.50	5.70	25.84	3.90	6.13
Vertical	135.0	Moist	0.736	5.78	22.93	5.17	23.96	3.60	4.98
Horizontal	na[b]	na[b]	0.679	5.55	20.04	6.36	26.07	4.52	6.42
Horizontal	93.3	Dry	0.657	5.48	19.59	7.92	26.72	4.05	6.28
Horizontal	93.3	Moist	0.681	5.58	21.07	5.37	25.15	3.54	5.03
Horizontal	135.0	Dry	0.661	5.65	18.66	8.88	25.84	4.26	5.94
Horizontal	135.0	Moist	0.687	5.60	21.24	4.73	24.92	3.81	5.47

[a] Color measurements include lightness (L) values of 0 to100, redness (a) values of -50 to +50 and yellowness (b) values of -50 to +50.
[b] This treatment was not post-treated.

Table 2.7. Comparison of air flow direction, post-oven temperatures and heating conditions on properties of whole beef strips dehydrated at 60.0°C.

Air flow direction	Post-dry oven temperature (°C)	Post-dry heating conditions	a_w	pH	Moisture (%)	NaCl (%)	Color[a]		
							L	a	b
Vertical	na[b]	na[b]	0.706	5.68	20.24	6.33	25.10	3.86	5.58
Vertical	93.3	Dry	0.717	5.70	20.96	5.49	25.50	3.83	5.74
Vertical	93.3	Moist	0.714	5.92	20.99	5.96	25.11	3.64	6.66
Vertical	135.0	Dry	0.695	5.74	21.39	4.77	25.51	3.63	5.61
Vertical	135.0	Moist	0.691	5.89	21.93	5.34	28.27	3.21	6.05
Horizontal	na[b]	na[b]	0.665	5.58	16.03	7.45	24.96	4.14	5.66
Horizontal	93.3	Dry	0.661	5.62	17.65	7.60	23.72	3.77	4.80
Horizontal	93.3	Moist	0.681	5.75	18.22	5.74	24.60	3.40	5.14
Horizontal	135.0	Dry	0.668	5.68	17.58	7.82	25.03	4.24	5.79
Horizontal	135.0	Moist	0.670	5.62	17.53	5.62	25.34	3.49	4.87

[a] Color measurements include lightness (L) values of 0 to100, redness (a) values of -50 to +50 and yellowness (b) values of -50 to +50.
[b] This treatment was not post-treated.

Table 2.8. Comparison of air flow direction, post-oven temperatures and heating conditions on properties of whole beef strips dehydrated at 60.0°C and commercially available beef jerky.

	Post-dry oven temperature (°C)	Post-dry heating conditions	a_w	pH	Moisture (%)	NaCl (%)	Color[a]		
							L	a	b
Home-style, vertical	na[b]	na	0.706	5.68	20.24	6.33	25.10	3.86	5.58
Home-style, vertical	93.3	Dry	0.717	5.70	20.96	5.49	25.50	3.83	5.74
Home-style, vertical	93.3	Moist	0.714	5.92	20.99	5.96	25.11	3.64	6.66
Home-style, vertical	135.0	Dry	0.695	5.74	21.39	4.77	25.51	3.63	5.61
Home-style, vertical	135.0	Moist	0.691	5.89	21.93	5.34	28.27	3.21	6.05
Home-style, horizontal	na[b]	na	0.665	5.58	16.03	7.45	24.96	4.14	5.66
Home-style, horizontal	93.3	Dry	0.661	5.62	17.65	7.60	23.72	3.77	4.80
Home-style, horizontal	93.3	Moist	0.681	5.75	18.22	5.74	24.60	3.40	5.14
Home-style, horizontal	135.0	Dry	0.668	5.68	17.58	7.82	25.03	4.24	5.79
Home-style, horizontal	135.0	Moist	0.670	5.62	17.53	5.62	25.34	3.49	4.87
Commercial brand A	na	na	0.790	5.88	29.49	5.28	26.67	13.68	4.78
Commercial brand B	na	na	0.753	5.54	28.58	6.57	23.99	6.95	2.72
Commercial brand C	na	na	0.787	5.67	29.27	6.07	27.30	11.59	4.23

	Post-dry oven temperature (ºC)	Post-dry heating conditions	a_w	pH	Moisture (%)	NaCl (%)	Color[a]		
							L	a	b
Commercial brand D	na	na	0.629	5.62	16.44	5.85	29.63	20.57	6.64
Commercial brand E	na	na	0.584	5.60	16.47	7.32	27.35	5.26	8.15

[a] Color measurements include lightness (L) values of 0 to100, redness (a) values of -50 to +50 and yellowness (b) values of -50 to +50.
[b] For commercially bought jerky, these parameters do not apply. For home-style jerky, this treatment was not post-treated.

Table 2.9. Textural analysis using the Warner-Bratzler method for jerky strips dehydrated at 54.4ºC with either vertical or horizontal air and post treated with dry heat or moist heat at either 93.3 or 135ºC.

	Vertical					Horizontal				
	No post treatment	Dry		Moist		No post treatment.	Dry		Moist	
		93.3ºC	135ºC	93.3ºC	135ºC		93.3ºC	135ºC	93.3ºC	135ºC
Force (kg)	19.33[a] ± 2.60	20.62 ± 1.68	17.27 ± 2.13	20.26 ± 5.08	17.21 ± 4.48	20.66 ± 3.10	19.43 ± 2.77	25.90 ± 3.21	22.39 ± 5.21	21.55 ± 1.64
Time (s)	8.39 ± 0.02	11.43 ± 4.35	8.73 ± 0.19	8.70 ± 0.25	8.88 ± 0.12	8.69 ± 0.02	8.89 ± 0.26	8.56 ± 0.41	8.99 ± 0.25	8.53 ± 0.43
Area (kg.s)	46.10 ± 5.76	53.05 ± 23.10	49.98 ± 12.67	50.80 ± 16.75	55.59 ± 21.57	56.45 ± 16.96	54.76 ± 2.78	62.84 ± 15.72	55.02 ± 22.13	60.30 ± 14.32

[a] Means ± standard deviation.

Table 2.10. Textural analysis using the Warner-Bratzler method for jerky strips dehydrated at 60.0°C with either vertical or horizontal air and post treated with dry heat or moist heat at either 93.3 or 135°C.

	Vertical					Horizontal				
	No post treatment	Dry		Moist		No post treatment.	Dry		Moist	
		93.3°C	135°C	93.3°C	135°C		93.3°C	135°C	93.3°C	135°C
Force (kg)	21.64[a] ± 2.75	20.25 ± 5.68	18.48 ± 3.08	23.69 ± 3.94	16.87 ± 8.20	21.42 ± 1.27	19.08 ± 4.01	17.29 ± 3.83	17.24 ± 2.86	19.95 ± 1.76
Time (s)	9.58 ± 1.58	9.89 ±1.76	9.79 ± 2.02	10.13 ± 2.10	9.81 ± 2.05	8.91 ± 0.07	9.04 ± 0.18	8.83 ± 0.13	9.05 ± 0.4	9.00 ± 0.16
Area (kg.s)	45.93 ± 12.68	53.55 ± 28.35	39.01 ± 6.51	54.21 ± 19.55	47.79 ± 27.90	59.65 ± 11.24	54.24 ± 18.38	46.10 ± 13.34	45.09 ± 13.19	45.37 ± 9.76

[a] Means ± standard deviation.

CHAPTER 3

FATE OF *ESCHERICHIA COLI* O157:H7, *LISTERIA MONOCYTOGENES* AND

SALMONELLA SPP. IN REDUCED SODIUM BEEF JERKY[1]

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ABSTRACT

Interest in low-sodium food products necessitates re-examination of home preservation processes relying in part on salt for antimicrobial effects. The fates of *Escherichia coli* O157:H7, *Listeria monocytogenes,* and *Salmonella* spp. in reduced sodium home-style beef jerky were determined. Ground or whole beef strips, with different salt levels, were inoculated with the pathogens. Samples were either dried in a 60°C dehydrator or heated to an internal temperature of 71.1°C prior to drying in a 60°C dehydrator. Populations were determined at time 0 and 2 h intervals until dry. Reductions of pathogens were greater in ground beef jerky with non-reduced salt levels compared to that with reduced salt levels, and in most cases, greater reduction (1.0-1.5 logs) was observed for ground beef strips heated prior to drying. However, at time intervals during the drying stage for whole and ground beef jerky, there were differences in the number of viable microorganisms when salt levels were compared, with less lethality in the reduced salt samples. Ground beef strips made with reduced sodium resulted in less pathogen reduction. Therefore, preparing ground beef jerky with reduced salt is a potential food safety hazard since the lower salt level did not reduce the pathogen population as much as the regular salt marinade. For dried whole jerky strips, there generally were no significant differences ($p > 0.05$) in pathogen populations between the non-reduced and reduced salt marinade in the end product. The results from this study support the importance of the antimicrobial effect of sodium chloride in particular products on the pathogens used in this experiment.

INTRODUCTION

The use of salt (sodium chloride) in the preservation of meat products has been a widely accepted preservation method throughout recorded history. The antimicrobial properties of salt are well established, and it is a widely used ingredient in the preparation of beef jerky. The Surgeon General's *Dietary Guidelines for Americans* (9) recommends a moderate intake of sodium. An abundance of low- or no-sodium products in the marketplace attracts consumers who are searching for products and new methods of preparation to help them reduce their intake of sodium. The desire to make low- or no-sodium products at home is an outgrowth of this movement. Home processes that were once considered to be safe due to antimicrobial effects of salt may need to be examined due to the growing popularity of low- and no-sodium recipes for home processed foods.

Outbreaks of foodborne illness due to *Salmonella* in beef jerky (3) and *E. coli* O157:H7 in venison jerky (7), coupled with the fact that a variety of preparation methods and drying procedures abound, raises concern over the safety of processed meat products made in the home. Discrepancies in temperatures and amounts of ingredients with preservative effects indicate the need for investigation. The inactivation of selected pathogens in beef jerky prepared with salt concentrations that were typically used have been evaluated previously (5, 6).

The objective of this investigation was to determine the fate of *E. coli* O157:H7, *L. monocytogenes,* and *Salmonella* spp. on home-style ground and whole beef jerky strips prepared with non-reduced and reduced salt levels.

MATERIALS AND METHODS

The investigation examined the effect of two drying methods (drying uncooked samples and drying pre-cooked samples that had reached an internal temperature of 71.1°C prior to drying) and two salt levels (non-reduced and reduced salt) on three different organisms (*E. coli* O157:H7, *L. monocytogenes,* and *Salmonella* spp.) for each type of meat (whole and ground beef strips). The trials for the ground beef strips and whole beef jerky were conducted separately with three replications on three different days for each type of meat. The meat for the three replications came from the same batch of meat for both product types.

Bacterial strains and inoculum preparation. *L. monocytogenes* Brie (cheese isolate), Scott A (clinical isolate), LCDC #81-861 (coleslaw outbreak isolate), V7 (milk isolate) and 301 (cheddar cheese isolate) and *E. coli* O157:H7 932 (clinical), E009 (beef), 204 P (pork), E0019 (cattle feces), and 380-94 (salami) were obtained from the Center for Food Safety, The University of Georgia, Griffin, GA. *Salmonella* (*S*. Typhimurium 654, *S*. Typhimurium DT 104 H3380, *S*. Typhimurium DT 104 H3402, *S*. California and *S*. Enteritidis) were obtained from USDA/ARS, Athens, GA. All strains were preserved on Microbank ™ beads (Pro-Lab Diagnostics, Austin, TX) frozen at -20°C. Each strain was activated in 9 ml portions of tryptic soy broth (TSB; Difco Labs, Division of Becton

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Dickinson and Co., Sparks, MD) at 37°C for 20-24 h, followed by transferring 1 ml of culture to 120 ml TSB that were incubated at 32°C for 18-24 h. To wash each culture, they were centrifuged for 20 min at 2,500 x g, spent TSB decanted, the pellet was re-suspended in 20 ml Butterfield's phosphate buffer (0.1M, pH 7.0) and then re-centrifuged. After decanting the buffer, cultures were suspended in separate sterile tubes containing 10 ml Butterfield's phosphate buffer and then serially diluted to approximately 10^8 CFU/ml. The five strains of each bacterial type were pooled just prior to inoculation. One ml of each pool was placed in 9 ml tubes containing 0.1% peptone and serially diluted to determine initial inoculum levels for each bacterial pool.

Whole strip beef jerky preparation and analysis. Beef, loin cut, was obtained from the Department of Food Science and Technology's food processing laboratory and stored at -20°C. Prior to inoculation, the beef was placed in a 4°C incubator for approximately 12 h to slightly thaw. The edges of the beef were trimmed of visible fat and then sliced to approximately 15.0 x 1.5 x 1.5 cm strips. One side of each strip was inoculated with 100 μl of each pool on 1/3 portions of the strip to prevent the pathogen types from overlapping. Samples were incubated at 4°C overnight (16 h $\pm$ 2 h) to allow for microbial adhesion to the beef. The marinade used to represent one with regular levels of sodium consisted of 118.5 ml soy sauce, 29.6 ml Worcestershire sauce, 9.8 g garlic salt, 9.8 g black pepper and 9.8 ml liquid smoke/1,820 g of meat. The reduced sodium marinade contained 118.5 ml low-salt soy sauce, 29.6 ml 65% less sodium Worcestershire sauce,

9.8 g garlic powder in lieu of garlic salt, 9.8 g black pepper and 9.8 ml liquid smoke/1,820 g of meat.

When sampled, each strip was placed in a sterile stomacher bag containing 100 ml of Butterfield's phosphate buffer and stomached (TekMar model 400, Cincinnati, OH) for 30 s on regular speed. Portions were spread plated on bismuth sulfite agar (BSA; Difco Labs), *Listeria* selective agar (LSA) (Oxoid; Basingstoke, Hampshire, England), and sorbitol MacConkey agar (SMAC, Oxoid) for *Salmonella, Listeria* and *E. coli* O157:H7, respectively and incubated at 32ºC for 24 h before counting colony forming units. The plates were then re-incubated and were re-examined after an additional 24 h incubation.

Ground beef jerky preparation and analysis. Lean ground beef (90% lean, pH 5.83) obtained from the Department of Food Science and Technology's food processing laboratory was frozen immediately upon grinding and stored at -20ºC. Prior to inoculation, meat was placed in a 4ºC incubator for approximately 18 h to thaw. The spices and seasonings were added to1,820 g (4 lb.) ground beef at the following levels:19.6 g seasoning salt, 9.8 g salt, 4.9 g garlic salt, 2.5 g red pepper, 9.8 ml liquid smoke, 19.6 ml Worcestershire and 19.6 ml soy sauce. The reduced sodium mixture contained 19.6 g salt-free seasoning, 4.9 g garlic powder, 2.5 g red pepper, 9.8 ml liquid smoke, 19.6 ml Worcestershire sauce (having 65% less sodium than regular Worcestershire sauce) and 19.6 ml low-salt soy sauce. The regular salt mixture contained 6,490 mg of sodium while the reduced salt mixture contained 207 mg of sodium.

The ground beef was inoculated with 1 ml of each bacterial strain and mixed for 1 min using a mixer set on high speed (model K5-A, Hobart Corporation, Troy, OH). Jerky strips (33.5 x 2.5 x 0.75 cm) were made using The Beef Jerky Works™ (BJW#1P, American Harvest, Inc., Chaska MN). To determine initial microbial load, 25 g portions of beef were weighed, placed in a sterile stomacher bag with 100 ml Butterfield's phosphate buffer and stomached (TekMar model 400, Cincinnati, OH) for 30 s on normal speed. Serial dilutions of the samples were prepared using Butterfield's phosphate buffer. Portions were spread plated on BSA, LSA, and SMAC for *Salmonella, Listeria* and *E. coli* O157:H7, respectively and incubated at 32°C for 24 h before counting colony forming units. They were re-examined after an additional 24 h of incubation.

Subcultures of all pummeled samples in this study were enriched in the event that the populations of the three pathogens were reduced to undetectable levels (8.0×10^1) by direct plating. One ml portions of each sample were inoculated into 9 ml portions of TSB (Difco Labs). After incubation at 32°C for 24 h, the appropriate selective media (as listed previously) were streaked in order to detect presumptive viable *Salmonella*, *E. coli* O157:H7 or *L. monocytogenes*. The plates were incubated at 32°C for 24 h before observing for typical colony growth for the three pathogens used in this study.

The identification of representative, presumptive isolates from the enrichment steps above were tested. Presumptive *Salmonella* spp. and *E. coli* O157:H7 isolates were identified using the Micro-ID™ identification system for Enterobacteriaceae (Remel,

Lenexa, KS) as per manufacturer's instructions. *Salmonella* isolates were serotyped with

Salmonella O-antisera and *E. coli* isolates were serotyped with *E. coli* O157 and H7

antisera. (Difco Labs). *Listeria* isolates were identified using the Micro-ID™ *Listeria*

system (Remel, Lenexa, KS) as per manufacturer's instructions.

Dehydration and heat treatments. Inoculated samples were either placed directly into a

60°C pre-heated horizontal air flow food dehydrator (model #7010; B&J Industries,

Marysville, WA) or heated in a convection oven (GCA mechanical convection oven,

model 18 EM; Precision Oven, Chicago, IL) pre-heated to 93°C until the internal

temperature of the strips reached 71.1°C. Internal temperature of the samples was

monitored continuously during cooking with thermocouples and a Speedomax strip chart

recorder (Type T-68, Leeds & Northup, North Wales, PA). After cooking, strips were

placed in the pre-heated dehydrator and sampled at 0, 2, 4, and 6 h of drying at 60°C.

Sampling intervals for the uncooked strips placed directly in the dehydrator were at 0, 2,

4, 6 and 8 h of drying. Two strips were analyzed at each sample time with the bacterial

counts being averaged over both strips.

Percent moisture and sodium chloride analysis. Moisture content of jerky was

determined by the AOAC Method 935.47 (1). Quantab® titrator strips (0.5-1.% Cl-)

were used to measure percent sodium chloride of the product (Environmental Test

Systems, Inc., Elkhart, IN).

Statistical analysis. The experimental design was a complete block for each type of

meat consisting of a factorial arrangement of the different combinations of drying

methods, salt levels and organisms. The sources of variation included the effects for

drying method, salt level, organism and the interaction terms. The bacterial counts were

transformed using the logarithmic transformation. Regression analysis was utilized to

evaluate the rate of reduction in log bacterial counts over time. Due to the fact that the

sampling periods for the uncooked and pre-cooked strips were not directly comparable,

analysis of variances were conducted separately for the initial and final sampling periods.

All analyses were conducted using the PROC GLM procedure in SAS (8).

RESULTS

Meat types dried under similar conditions had similar moisture content in the

finished product (Tables 3.1 and 3.2). In general, salt concentration was higher in whole

beef jerky than in ground beef jerky, both initially and throughout the drying period. This

difference is partially due to the fact that the recipes for the two jerky types are not

identical. As salt concentration increased, moisture content decreased (Tables 3.1 and

3.2).

The average log population for *E. coli*, *L. monocytogenes*, and *Salmonella* on

cooked and uncooked ground beef jerky strips made with non-reduced and reduced salt

levels are in Tables 3.3, 3.4, and 3.5, respectively. The average log population for *E. coli*,

L. monocytogenes, and *Salmonella* on cooked and uncooked whole beef jerky strips made

with low and regular salt levels are in Tables 3.3, 3.4, and 3.5, respectively. The analysis

of variance for each meat type indicated no significant differences (p>0.05) among the

main effects or interactions for the initial starting conditions.

The preliminary analysis of variance results of the bacterial population for the final sampling times for the ground beef strips indicated highly significant main effects for drying method (p=0.0001), organism (p=0.0057), and salt level (p=0.0017) with all the interactions being non-significant (p>0.05). The results for the whole beef strips indicated a highly significant effect for drying method (p=0.0001). In regressing the rate of reduction in log bacterial populations, it was found to be predominantly significantly linear (p<0.05) trends over time for each salt level within each drying method and type of meat. The R^2 values ranged from 0.29 to 0.77.

The primary focus of this research investigation was to determine the effect of salt level on bacterial survival at the final sampling time. Due to the fact that it was expected to have highly significant differences between drying methods on bacterial survival and that the residual mean square errors between drying types within the meat types were not homogenous, separate analyses were conducted for each drying method within each meat type. The observed significance levels for differences between the salt levels for each drying method within meat type were: uncooked ground beef strips p=0.0514, pre-cooked ground beef strips p=0.015, uncooked whole beef strips p=0.133 and pre-cooked whole strips p=0.810. No significant differences between salt levels for the whole beef strips were detected. The differences among the types of organisms revealed significant differences only for the uncooked ground beef strips.

DISCUSSION

E coli O157:H7 was more easily inactivated than *L. mononcytogenes* and *Salmonella* spp. All organisms were more easily inactivated on whole strips where contamination is usually confined to the surface unlike the ground strips with contamination spread throughout. Pre-cooking whole strips prior to drying resulted in a decrease in *E. coli* O157:H7 populations of 0.5-1.5 logs and *Salmonella* populations decreased by 2 logs more than uncooked strips. The log reductions were similar for *Listeria* regardless of whole strips being cooked or not. Regardless of the salt levels, there was no difference in population reductions of *Listeria* and *Salmonella* in whole beef jerky strips. *E. coli* populations were slightly lower in whole strips that contained non-reduced salt levels. From this study, it is apparent that reducing the salt level for the ground beef samples resulted in less log bacterial population reduction. Uncooked samples had higher log bacterial populations compared to pre-cooked strips, especially for the ground beef strips. Uncooked ground beef strips made with regular salt levels had final log populations of 2.5, 4.86, and 3.09 for *E. coli*, *L. monocytogenes*, and *Salmonella,* respectively. The uncooked strips containing reduced salt levels had final log populations of 4.10, 4.96, and 4.44 for *E. coli*, *L. monocytogenes*, and *Salmonella,* respectively. Due to the lack of pathogen inactivation, it would be unadvisable for preparation of ground beef jerky unless the strips were pre-cooked, regardless of salt level. Salt contributes to the lethality of the drying process for ground beef jerky.

Hurdle technology is often used as a combined effort to control unwanted microorganisms. However, not all studies have shown that combinations of antimicrobial methods have a positive result. A study done by Casey and Condon (2) showed how the combination of sodium chloride and acid pH was less effective then acid pH alone on population reductions of *E. coli* O157:H45 . The presence of sodium chloride reduced the bactericidal effect of lactic acid on exponential cells when grown in pH 4.2 media with 4% sodium chloride (simulating the salt concentration in fermented sausage) resulting in a 10^3 fold higher survival rate when sodium chloride and acidic pH were used together. The sodium chloride is believed to counteract the acidification by organic acids (2).

Previously, Clavero and Beuchat (4) conducted a similar experiment but their conclusions did not support the concept of sodium chloride providing a protective effect on the cells exposed to low acid. This discrepancy in results may be due to the fact that Clavero and Beuchat used higher percent sodium chloride and incubation occurred at 5, 20, 30, and 37°C whereas Casey and Condon used 4% sodium chloride and incubated at 37°C only.

The safety of jerky relies on many factors. Salt plays a vital role in reducing water activity and lowering percent moisture which aids in the safety of the final product. In some cases the salt has shown to enhance survival of certain pathogens. The reduction of sodium in jerky marinades may contribute to pathogen survival so this practice should be avoided.

ACKNOWLEDGMENTS

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Table 3.1. Percent moisture and percent sodium chloride content of regular and reduced salt ground beef jerky during preparation by either drying at 60°C or by preheating jerky to 71.1°C before drying at 60°C.

| Time (h) | Dehydrated strips | | | | Pre-cooked and dehydrated strips | | | |
| | Regular | | Reduced | | Regular | | Reduced | |
	% Moisture	% NaCl	% Moisture	% NaCl	% Moisture	% NaCl	% Moisture	% NaCl
Pre-cook	ns[a]	ns	ns	ns	73.98	1.82	71.40	0.50
0	68.99	1.12	70.07	0.45	48.85	ns	53.01	ns
2	55.28	ns	54.60	ns	34.76	ns	35.81	ns
4	38.78	ns	36.02	ns	26.23	ns	18.57	ns
6	32.46	ns	32.80	ns	16.62	5.23	17.45	1.07
8	22.78	3.89	21.02	0.46	ns	ns	ns	ns

[a] ns: not sampled

Table 3.2. Percent moisture and percent sodium chloride content of regular and reduced salt whole beef jerky during preparation by either drying at 60°C or by preheating jerky to 71.1°C before drying at 60°C.

| | Dehydrated strips | | | | Pre-cooked and dehydrated strips | | | |
| | Regular | | Reduced | | Regular | | Reduced | |
Time	% Moisture	% NaCl	% Moisture	% NaCl	% Moisture	% NaCl	% Moisture	% NaCl
Pre-cook	ns[a]	ns	ns	ns	68.34	2.11	68.13	0.82
0	70.74	2.67	70.15	1.10	41.10	ns	44.50	ns
2	42.65	ns	42.68	ns	24.62	ns	37.31	ns
4	20.45	ns	25.36	ns	18.12	ns	15.93	ns
6	17.20	ns	14.99	ns	13.97	4.01	15.47	2.14
8	13.10	6.35	14.59	3.88	ns	ns	ns	ns

[a] ns: not sampled.

Table 3.3. The average log population of *E. coli* O157:H7 for the initial and final sampling time for ground and whole beef strips during preparation by either drying at 60°C or by preheating jerky to 71.1°C before drying at 60°C.

| Population | Ground Beef Strips | | | | Whole Beef Strips | | | |
| | Dehydrate Only | | Pre-cook & Dehydrate | | Dehydrate Only | | Pre-cook & Dehydrate | |
	Regular	Reduced	Regular	Reduced	Regular	Reduced	Regular	Reduced
Initial	8.12	7.87	7.35 (5.36)[a]	7.42 (6.80)[a]	6.70	6.61	5.06 (3.65)[a]	6.80 (4.15)[a]
Final	2.50	4.10	1.40	2.07	3.23	3.45	2.00[b]	2.00[b]

[a] Values in parentheses are the population log count immediately after preheating strips to 71.1°C for 10 min.
[b] Indicates below detection level

Table 3.4. The average log population of *L. monocytogenes* for the initial and final sampling time for ground and whole beef strips during preparation by either drying at 60ºC or by preheating jerky to 71.1ºC before drying at 60ºC.

| | Ground Beef Strips | | | | Whole Beef Strips | | | |
| | Dehydrate Only | | Pre-cook & Dehydrate | | Dehydrate Only | | Pre-cook & Dehydrate | |
Population	Regular	Reduced	Regular	Reduced	Regular	Reduced	Regular	Reduced
Initial	7.20	7.10	7.21 (5.17)[a]	7.12 (6.42)[a]	5.50	6.32	5.49 (2.38)[a]	4.66 (2.84)[a]
Final	4.86	4.96	2.13	2.95	1.88	2.94	2.00[b]	2.00[b]

[a] Values in parentheses are the population log count immediately after preheating strips to 71.1ºC for 10 min.
[b] Indicates below detection level

Table 3.5. The average log population of *Salmonella* for the initial and final sampling time for ground and whole beef strips during preparation by either drying at 60°C or by preheating jerky to 71.1°C before drying at 60°C.

| Population | Ground Beef Strips | | | | Whole Beef Strips | | | |
| | Dehydrate Only | | Pre-cook & Dehydrate | | Dehydrate Only | | Pre-cook & Dehydrate | |
	Regular	Reduced	Regular	Reduced	Regular	Reduced	Regular	Reduced
Initial	7.23	7.65	7.00 (3.78)[a]	7.50 (6.33)	6.00	6.21	5.70 (2.86)	3.77 (2.69)[a]
Final	3.77	3.09	1.40	2.77	3.34	4.17	2.10	2.14

[a] Values in parentheses are the population log count immediately after preheating strips to 71.1°C for 10 min.

CHAPTER 4

SURVIVAL OF ACID-ADAPTED AND NONADAPTED *ESCHERICHIA COLI*,

LISTERIA MONOCYTOGENES AND *SALMONELLA* SPP. ON GROUND OR

WHOLE BEEF JERKY[1]

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ABSTRACT

The objective of this paper was to monitor and compare the survival of acid-adapted and nonadapted *Escherichia coli* O157:H7, *Listeria monocytogenes,* and *Salmonella* spp. on ground and whole beef jerky strips during the home-style jerky process. Each organism and meat type was compared separately and analyzed using a split-plot type experimental design. Samples were taken at time 0, 2, 4, 6, and 10 h for ground beef strips. Maximum log reduction was first observed after 6 h for all three pathogens in ground beef strips. For ground beef strips, the maximum mean log reduction over the entire 10 h drying process was higher for acid-adapted *E. coli* (6.22 logs) and *Salmonella* (4.73) compared to nonadapted *E. coli* (5.30 logs) and *Salmonella* (3.96). However, only the treatments for *E. coli* populations were significantly ($p<0.05$) different. In ground beef strips, the maximum log reduction for *L. monocytogenes* was greater in the nonadaptive strains (4.51) as compared to acid-adapted (4.28) strains after 10 h of drying. Sampling of whole beef strips was done at the following intervals: after inoculation, after marination (T:0), 4, 8, 12, and 14 h.   Maximum log reduction was first observed after 8 h for whole strips. For whole beef strips, the log reduction was almost identical for strips inoculated with *Salmonella*. Log reduction for acid-adapted and nonadapted *E. coli* O157:H7 were 5.25 and 5.13, respectively. For *L. monocytogenes,* log populations declined by 4.81 logs for acid-adapted and 4.87 logs for nonadapted cells. The results from this study show there is no significant ($p>0.05$) difference in survival of *L. monocytogenes* and *Salmonella* acid-adapted and nonadapted cells on ground or whole beef home-style jerky. However, there was a significant difference for ground beef strips

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inoculated with *E. coli* O157:H7 with acid-adapted populations having a greater reduction

after 10 h of drying.

INTRODUCTION

Exposure of foodborne bacteria to mild or severe acidic conditions frequently occurs as a result of the acids that are either found in or artificially added to foods. Whenever the cell's pH homeostasis system cannot function to maintain a neutral pH, other systems within the cell are activated (9). These alternate systems are very complex and are growth dependent. Each system functions uniquely. Acid resistance (AR), acid habituation (AH) and acid tolerance response (ATR) are systems that allow survival by some bacteria under acidic conditions (1, 2, 3, 7, 13, 14). A recent concern that has become a food safety issue is the possibility that pathogenic cells can become tolerant or adapted to acid which may enhance survival upon exposure to acidic foods and defenses of the human body (e.g., stomach acidity and digestive enzymes). Acid adaptation in *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* has been demonstrated in some laboratory studies (1, 2, 3, 7, 8, 11, 13, 14) and in food systems such as beef jerky and juices (4, 5, 6, 14).

Beef jerky is a popular dehydrated food. During home-style preparation, the meat is often marinated in an acidic marinade which may allow any bacteria that are present on the meat to become adapted to the acidic environment. This adaptation may enhance survival of pathogenic bacteria, especially on a food product like jerky that typically does not have a cooking step to inactivate unwanted bacteria. The objective of this paper was to monitor and compare the survival of acid-adapted and nonadapted *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* spp. on ground and whole beef jerky strips during the home-style jerky process.

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MATERIALS AND METHODS

Bacterial strains and inoculum preparation. *E. coli* O157:H7 932 (clinical), E009

(beef), 204 P (pork), E0019 (cattle feces), and 380-94 (salami) and *L. monocytogenes*

(Brie, Scott A, LCDC, V7, 301) were obtained from the Center for Food Safety, The

University of Georgia, Griffin, GA. *Salmonella* (*S*. Typhimurium 654, *S*. Typhimurium

DT 104 H3380, *S*. Typhimurium DT 104 H3402, *S*. California and *S*. Enteritidis) were

obtained from USDA/ARS, Athens, GA. All strains were preserved on Microbank ™

beads (Pro-Lab Diagnostics, Austin, TX) frozen at -20°C. Each strain was activated in 9

ml portions of tryptic soy broth (TSB; Difco Labs, Division of Becton Dickinson and Co.,

Sparks, MD) at 37°C for 20-24 h. The method of Buchanan and Edelson (2) was used to

obtain acid-adapted and nonadapted bacterial populations. To obtain acid-adapted

cultures, one ml of each strain was added to 120 ml TSB with 1% (wt/vol) dextrose (TSB

plus dextrose) incubated without agitation at 37°C for 18-24 h. To achieve nonadapted

cultures, one ml of each strain was added to 120 ml TSB without dextrose (TSB-G,

Difco Labs) and incubated without agitation at 37°C for 18-24 h. Each culture was

centrifuged for 20 min at 2,500 x g, and the spent TSB was decanted and the bacterial

pellet was re-suspended in 10 ml 0.1% peptone buffer (Bacto peptone, Difco Labs). The

five strains of each bacterial type were pooled just prior to inoculation. Pooled inocula for

each species was prepared by combining 3 ml of each strain. One ml of each pool was

placed in 9 ml tubes containing 0.1% peptone and serially diluted to determine initial

inoculum levels for each bacterial pool.

Ground beef strip preparation. Ground sirloin (98% fat free) was bought from a local grocery store. The bacterial cultures were prepared as previously described the same day as the meat was dehydrated. A commercial beef jerky spice mix (Original Flavor by American Harvest, Inc.; Chaska, MN) and cure mix were added to the meat at a ratio of one packet of spice mix and one packet cure mix per 454 g meat, according to the manufacturer's instructions. Separate batches were inoculated with either 15 ml 0.1% peptone (control), 15 ml of acid-adapted bacterial strains, or 15 ml of nonadapted bacterial strains. To prevent possible cross-contamination, the control was mixed first, the nonadapted batch second and the acid-adapted third. Between the batches, the mixing bowl and mixer were cleaned with bleach, soap, and water. Each batch was mixed for 2 min at medium speed (model K5-A, Hobart Corporation, Troy, OH). Jerky strips (33.5 x 2.5 x 0.75 cm) were made using The Beef Jerky Works[TM] (BJW#1P, American Harvest, Inc.; Chaska, MN). Samples were taken during drying at time 0, 2, 4, 6, and 10 h.

Whole beef strip preparation.   Bottom round steak was purchased from a local grocery store and used for the whole beef jerky strips. The edges of the beef were trimmed of visible fat and then sliced to approximately 9.0 x 1.5 x 1.5 cm strips. Strips were inoculated in a laminar air flow hood with 50 µl of each pool on separate one-third portions of each strip ensuring that the different pathogens did not overlap. The strips remained under the hood for 30 min to allow bacterial adhesion. The marinade (118.5 ml soy sauce, 29.6 ml Worcestershire sauce, 9.8 g garlic salt, 9.8 g black pepper and 9.8 ml liquid smoke/1820 g of meat) was added to the strips which were stored in ziplock bags at 4°C for overnight (16 h $\pm$ 2 h). The strips were blotted to remove visible liquid before

being placed in a 60ºC pre-heated vertical air flow food dehydrator (Garden Master

Model # 1000, American Harvest, Inc.; Chaska, MN). Sampling for whole beef strips

was done at the following intervals: after inoculation, after marination (T:0), and during

drying at 4, 8, 12, 14 h.

pH measurements. pH was measured by the surface method using a surface electrode

and a Corning model 340 pH meter (Corning, Inc., Corning, NY).

Microbial analysis and enrichment. To determine initial microbial load on ground beef

strips, 25 g portions of beef were weighed, placed in a sterile stomacher bag with 225 ml

0.1% peptone buffer and pummeled (TekMar model 400; TekMar Co., Cincinnati, OH)

for 30 s on normal speed. To determine initial microbial load on whole jerky strips, one

strip was placed in a sterile stomacher bag with 225 ml 0.1% peptone buffer and

pummeled (TekMar model 400, TekMar Co; Cincinnati, OH) for 30 s on normal speed.

For both jerky types, serial dilutions of the samples were prepared using 0.1%

peptone. Portions of the stomached sample for both jerky types were spirally plated on

bismuth sulfite agar (BSA; Difco Labs), *Listeria* selective agar (LSA) (Oxoid;

Basingstoke, Hampshire, England), and sorbitol MacConkey agar (Oxoid) for

Salmonella, Listeria and *E. coli* O157:H7, respectively. The plates were incubated for 24

h at 37ºC before colony forming units were counted, and the plates were re-incubated and

re-examined after an additional 24 h.

Subcultures of all pummeled samples in this study were enriched in the event that

the populations of the pathogens were reduced to levels not detectable (4.0×10^1) by

direct plating. Enrichment consisted of inoculating 1 ml from the stomacher bag into 9

ml portions of lactose broth (Difco Labs), UVM *Listeria* enrichment broth base (Oxoid), and modified tryptic soy broth (modified TSB; Difco Labs) (10 g casamino acids, 1.5 g bile salts No.3, 6.0 g dibasic, anhydrous sodium phosphate and 1.35 g potassium phosphate per liter of TSB) for *Salmonella*, *Listeria* and *E. coli* O157:H7, respectively. All three enrichment broths were incubated at 37°C for 18-24 h. After incubation, portions of the modified TSB cultures were streaked onto SMAC. Plates were incubated at 37°C for 24 h and examined for the presence of representative colonies. Subcultures were also made from the lactose broth into selenite cystine (Difco Labs) and TT broth Hajna (Difco Labs) and from UVM *Listeria* enrichment broth into Fraser broth (Difco Labs) and then incubated at 37°C for 24 h. After incubation of the broths, portions were streak plated onto bismuth sulfite agar (BSA; Difco Labs), XLD agar (Difco Labs) and brilliant green agar (BGA; Difco Labs) for possible *Salmonella* spp. isolates and onto LSA for possible *L. monocytogenes* isolates. Plates were incubated at 37°C for 24 h and examined for the presence of representative colonies.

Presumptive *Salmonella* spp. and *E. coli* O157:H7 isolates, from the enrichment steps were identified using the Micro-ID[TM] identification system for Enterobacteriaceae (Remel, Lenexa, KS) as per manufacturer's instructions. Presumptive *Listeria* isolates were identified using the Micro-ID[TM] *Listeria* system (Remel, Lenexa, KS) as per manufacturer's instructions.

Dehydrators. Two home-style vertical air flow food dehydrators (model # 1000, American Harvest, Inc.; Chaska, MN) were used to dehydrate both strip types. The dehydrators were preheated to 60°C prior to drying the strips. The internal air

temperature and internal temperature of an acid-adapted and nonadapted strip were

monitored and recorded by a data recorder (model RD106 A, Omega, Stamford, CT)

equipped with copper/constantan thermocouples (5TC-TT-T, Omega, Stamford, CT).

Statistical analysis. A split-plot type experimental design was used. Each experiment

was replicated three times and the results analyzed using the GLM (general linear models)

procedure in SAS (12). Acid-adapted and nonadapted treatments were compared

separately for each organism (*E. coli*, *L. monocytogenes* and *Salmonella* spp.) for ground

or whole beef strips. Plate counts were converted to $\log_{10}$ counts and analyzed at each

sampling time for significant differences ($p < 0.05$). Dehydrator number (1 or 2) and

replication were main plot effects and subplot effects of treatment (acid-adapted or

nonadapted) and interaction of dehydrator and treatment were also analyzed.

<div align="center">RESULTS</div>

Analysis for *E. coli* and *Salmonella* spp. showed there was no significant

difference ($p< 0.05$) in log reduction between the two dehydrators. There was also no

interaction between dehydrator and treatment, for these two organisms. The dehydrator

used significantly affected log reduction of *L. monocytogenes* ($p<0.05$) in whole strips at

time 4 h, with dehydrator 2 resulting in a higher mean log reduction (4.33) as compared

with dehydrator 1 (4.02) but this does not occur at the other sampling times. There was a

significant interaction ($p<0.05$) between the dehydrator and treatment (acid-adapted and

nonadapted) for whole strips inoculated with *L. monocytogenes* at sample time 4 h. For

acid-adapted *E. coli* O157:H7 inoculated in ground beef, the maximum mean log

reduction over the entire10 h drying process was 6.22 as compared to 5.30 log reduction

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for nonadapted (Table 4.1). For whole jerky strips, this same trend resulting in a larger log reduction for acid-adapted (5.32) versus nonadapted *E. coli* O157:H7 (5.17) occurred at sampling time 8 h (Table 4.1). For both treatment types of *L. monocytogenes* and *Salmonella*, in ground beef strips, the populations achieved the largest log reduction at 10 h with nonadapted *L. monocytogenes* having a larger log reduction (4.51) than acid-adapted (4.28) and acid-adapted *Salmonella* resulting in a larger log reduction (4.73) than nonadapted (3.96) (Table 4.1). For whole jerky strips, *L. mononcytogenes* had a mean log reduction of 4.94 as compared to nonadapted with a mean log reduction of 4.87. The log reduction was almost identical for whole strips inoculated with *Salmonella* (Table 4.1). The average pH for the acid-adapted and nonadapted cultures incubated overnight in broth was 4.88 and the nonadapted was 6.97, respectively.

DISCUSSION

Enterics thrive at pH 7.6-7.8 and as long as the pH is within 1 unit in either direction, pH homeostasis is responsible for survival of the cell (10). pH homeostasis is dependent on how permeable the bacterial cell membrane is to the protons in the acid. Whenever the cell's pH homeostasis system cannot function to maintain a neutral pH, other systems within the cell are activated (9).

Acid tolerance response (ATR) protects log phase cells during long term exposure to low pH. Several steps are required to achieve ATR in a laboratory setting. Medium containing exponential phase cells is acidified to a moderate pH (near 5.5) for several h and then exposed to a lethal pH (< 4.0). This phenomenon may also result from bacteria being exposed to acidic conditions via acidic foods. Virulent bacterial strains exhibit

sustained ATR and this process can occur rapidly, within 20 min. ATR can be explained in part by the cell's ability to repair damaged DNA caused by high H^+ concentration.

The role of various intrinsic and extrinsic factors, strain, storage temperature, acidulent, and growth phase interact and influence the cell's survival under adverse conditions (1, 8, 14). Acid-adaptation studies involving bacterial growth in media have been conducted (2, 3, 7, 14). Garren et al. (7) found that acid-adapted *E. coli* O157:H7 isolate 932 had a higher sodium chloride tolerance as compared to nonadapted and acid shocked cells. Polyphosphates and phosphates inhibit the acid response at pH 4.5-5.8 by competitively preventing protons form crossing the osmotic membrane. ATR is growth phase dependent and requires the stress-specific sigma factor rpoS for full induction (11). ATR broth studies involving enterohemorrhagic *E. coli* O157:H7 have shown the highest acid tolerance in late stationary phase. Similar patterns were seen in *Shigella flexneria* and *Salmonella* (1). In one study, cell survival was greater in apple juice than synthetic gastric fluid illustrating the necessity to study effects of food substrates on bacteria rather than in broth (14).

Several studies involving acid-adapted and nonadapted pathogens on beef jerky have been done recently and several of the studies reinforce the findings of the present research which concludes acid-adapted bacterial strains do not enhance survival of *L. monocytogenes* or *Salmonella.* Calicioglu et al. (6) examined the effect of storage on the proliferation of *Salmonella* on whole jerky strips that were inoculated after drying. The results showed that acid-adapted *Salmonella* decreased quicker than nonadapted cells for all treatments. The synergistic effects of modified marinades (e.g. dipping in Tween 20

or acetic acid for 10 min) and low water activity of the final product do provide antimicrobial effects against post-contamination. A similar study examined the inactivation of *L. monocytogenes* during drying and storage of whole beef strips. Again, acid-adapted cells did not enhance survival. This study did conclude that modifications in the marinade (e.g.dipping in Tween 20 or acetic acid for 10 min) did have an effect on pathogen survival (4). However, another study by Calicioglu et al. (5) compared the survival of acid-adapted and nonadapted *E. coli* O157:H7 on post-dried beef jerky and found that acid-adapted *E. coli* O157:H7 enhanced inactivation during storage. While this study did show that survival was better for acid-adapted *E. coli*, this experiment inoculated the beef strips after the strips were dried so the effects of heat and other stress factors encountered during drying did not factor into the inactivation of the cells.

The present experiment examined and compared population reduction of acid-adapted and nonadapted *E. coli* O157:H7, *L. monocytogenes,* and *Salmonella* in two types of beef jerky. The results showed that statistically there was no difference (p>0.05) between the reductions of the populations of acid-adapted and nonadapted cells for *L. monocytogenes* and *Salmonella* for both ground and whole beef jerky. There was a significant difference (p= 0.0446) in the decrease of population types for ground strips inoculated with *E. coli* O157:H7 unlike the whole strips which showed no difference.

The interaction and cross-protection of many systems within the bacteria enhance survival (7, 9, 10, 13). For example, man attempts to keep this cross-protection to a minimum by using hurdle technology in an attempt to inactivate and reduce pathogen populations in a food. These results, like those of other similar studies, emphasize the

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complexity of bacterial survival in adverse conditions and the need for more research

with real food systems.

ACKNOWLEDGMENTS

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Table 4.1. Mean log reductions between sample times for acid-adapted and nonadapted *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* spp. for home-style ground and whole beef jerky strips.

| | Time | *E.coli* O157:H7 | | *L. monocytogenes* | | *Salmonella* | |
		Acid-adapted	Nonadapted	Acid-adapted	Nonadapted	Acid-adapted	Nonadapted
Ground	2	1.08	1.42	0.03 [b]	1.12[a]	0.87	1.83
	4	4.79	4.47	1.36	3.24	3.41	2.81
	6	5.89	5.30	2.35[b]	3.06[a]	4.30	3.57
	10	6.22[a]	5.30[b]	4.28	4.51	4.73	3.96
Whole	0[c]	1.14	1.18	2.11	2.09	2.10	1.88
	4	5.09	4.86	3.90	4.46	4.66	4.12
	8	5.32	5.17	4.87	4.86	4.85	4.56
	12	5.19	5.13	4.94	4.74	4.85	4.87
	14	5.25	5.13	4.81	4.87	4.85	4.82

[a, b] Values with different letters are significantly different ($p < 0.05$). The p-values compared are between acid-adapted and nonadapted for each time interval and for each organism.
[c] Time 0 is after overnight marination at 4°C and before dehydration.

CONCLUSIONS

Jerky, solely relying on dehydration, may not be as safe as previously thought. Experimental data has shown that a heat step, in which the meat reaches an internal 71.1℃, is both safer from food pathogens and accepted by consumers. Salt plays in important safety and preservative role for jerky and reduced salt marinades may not produce a safe jerky product. The data from these experiments showed that bacterial cells, with the potential to acid adapt while being in the acidic marinade, do not have enhanced survival compared to nonadapted *E. coli, L. monocytogenes* and *Salmonella* spp.