

THE EFFECTS OF FOUR PACKAGING SYSTEMS AND STORAGE TIMES ON THE
SURVIVAL OF LISTERIA MONOCYTOGENES IN SHELF-STABLE SMOKED PORK
AND BEEF SAUSAGE STICKS AND WHOLE MUSCLE TURKEY JERKY

by

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Abstract

To validate how packaging and storage reduces *Listeria monocytogenes* (*Lm*) on whole muscle turkey jerky and smoked sausage sticks, four packaging systems, including heat seal (HS), heat seal with oxygen scavenger (HSOS), nitrogen flushed with oxygen scavenger (NFOS), and vacuum (VAC), and four ambient temperature storage times were evaluated. Commercially available whole turkey jerky and pork and beef smoke sausage sticks were inoculated with *Lm* using a dipping or hand-massaging method, respectively. Commercially available whole turkey jerky and pork and beef smoked sausage sticks were inoculated with *Lm* using a dipping or hand-massaging method, respectively. There was no interaction on packaging and storage time on *Lm* reduction on smoked sausage sticks and an *Lm* log reduction of >2.0 log CFU/cm² was achieved in smoked sausage sticks packaged in HS, HSOS, and VAC. A >2.0 log CFU/cm² reduction was achieved after 24 h of ambient temperature storage, regardless of package type. NFOS was less effective in reducing *Lm* by more than 0.5 log CFU/cm² compared to HS, HSOS or VAC. After 30 d of ambient storage, *Lm* had been reduced by 3.3 log CFU/cm² for all packaging environments. In turkey jerky, *Lm* reduction was affected by the interaction of packaging and storage time. HS, HSOS, NFOS, or VAC in combination with 24, 48, or 72 h ambient temperature storage achieved <1.0 log CFU/cm². After 30 d at ambient temperature storage, *Lm* was reduced by >2.0 log CFU/cm² in HS and VAC, and could serve as a post-lethality treatment. Alternatively, processors could package turkey jerky in HSOS or NFOS in combination with 30 d ambient storage period as an antimicrobial process. Very little data has been published describing how packaging atmospheres affects *Lm* survival in RTE meat. The mechanism for *Lm* reduction under these conditions is not fully understood and additional research is needed.

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CHAPTER 1 - Introduction

The presence of *Listeria monocytogenes* (*Lm*) in ready-to-eat (RTE) cooked meat is a major safety concern and it is strictly regulated in the U.S. In 2003, the U.S. Department of Agriculture (USDA) released a final rule addressing control of *Lm* in RTE products due to the severity of listeriosis and the ability of *Lm* to grow at refrigeration temperatures. In 2002, the Centers for Disease Control and Prevention (CDC) reported that there are about 2,500 cases annually from listeriosis, including 500 deaths and nearly around \$200 million in monetary loss in the U.S. (CDC 2002). Between 1990 and 1999, Levine and others 2001 reported *Lm* contamination in nine different meat product categories including jerky and small-diameter cooked sausages. They found jerky and small diameter cooked sausage to account for 0.52% and 3.56% *Lm* prevalence. The CDC (1999) reported that a multistate outbreak of *Lm* in 1998 and 1999 was attributed to the consumption of frankfurters and deli meats, resulting in 101 cases and 21 deaths. In addition, the CDC also reported several other multistate outbreaks of *Lm* including the 2002 outbreak in the Northeastern U.S. where 12.5 million kg of fresh and frozen RTE poultry products were recalled and attributed to consumption of sliceable turkey deli meat. There were 46 confirmed cases, 7 deaths, and 3 stillbirths or miscarriages associated with this outbreak. Due to these outbreaks, *Lm* contamination was associated with RTE meat and poultry products. Zhu and others (2005) noted that *Lm* contamination of cured and non-cured RTE meat is a major safety concern because: (1) RTE meats have a long shelf-life and are consumed without further heating; (2) *Lm* can grow to a threatening level during refrigerated storage because of its ability to grow in the presence of curing salt at refrigerated temperatures (Lou and Yousef 1999), and (3) the emergence of multiple resistance in *Listeria* spp. due to acquisition of a replicon from staphylococci (Lemaitre and others 1998).

In efforts to prevent *Lm* contamination in RTE products, the USDA Food Safety Inspection Service (FSIS) mandated that establishments employ one of three alternatives to control *Lm*. Under alternative 1, an establishment must employ both a post-lethality treatment and an antimicrobial agent or process to control *Lm* on RTE products. Under alternative 2, an

establishment must employ either an antimicrobial agent or process or a post-lethality treatment. Lastly, under alternative 3, an establishment relies only on sanitation to control *Lm*.

Several published studies have described antilisterial interventions for RTE meat manufacturing establishments. Interventions can be the addition of antimicrobial agents against *Lm* or in the form of post-process intervention. These interventions include in-package thermal pasteurization, chemical antimicrobials, biopreservatives, high pressure processing, and hurdle technology (Zhu and others 2005). McCormick and others (2003) reported a *Lm* reduction by >6.0 log in packaged low-fat turkey bologna after exposure to 85°C water bath for 10 sec and <6.0 *Lm* log reduction after exposure to 61°C water bath for 10 min. Use of chemical preservatives such as lactate and diacetate were found to inhibit the growth of pathogenic microorganisms in meat products (Glass and others 2002; Mbandi and Shelef 2002; Samelis and others 2002; Stekenburg 2003). The growth of *Lm* in cooked meat products could be prevented by using sodium lactate in combination with a low pH. Studies have found the growth of *Lm* was effectively controlled in artificially contaminated vacuum-packaged sausages formulated with 1.8% to 2% sodium lactate and 0.25% sodium acetate, or glucono-delta-lactone during refrigerated storage (Qvist and others 1994; Blom and others 1997; Samelis and others 2002). Biopreservation with various strains of lactic acid bacteria may be a suitable alternative to chemical preservatives (Jacobsen and others 2003). Bacteriocin-producing *Lactobacillus plantarum* has been reported to exhibit antimicrobial properties against *Lm* in naturally and artificially contaminated salami (Campanini and others 1993).

Lm contamination of RTE meat or poultry products occurs post-processing since *Lm* is easily killed by heating or cooking (Zhu and others 2005). Thus, antilisterial intervention should not only exhibit inhibitory activity on *Lm*, but should also exhibit bacteriocidal activity against *Lm* at post-lethality and throughout the product shelf-life. USDA FSIS (2006) defines a post-lethality treatment as an antimicrobial process that reduces *Lm* by >1.0 log. Hurdles frequently applied in RTE meat products are low pH, low a_w , preservatives such as nitrite, sorbate, and sulfite, in-package thermal processing, refrigerated storage, and vacuum packaging (Zhu and others 2005). An antimicrobial is defined as a process that can inhibit *Lm* from growing by >2.0 log (USDA FSIS 2006).

Ingham and others (2004) evaluated 15 RTE meat products made using drying, fermentation, and/or smoking and found that a one-week period of post-packaging room-

temperature storage prior to distribution can serve as a post-lethality treatment for most of these products. Establishments that manufacture these products could operate under alternative 1. There is a lack of information in evaluating effectiveness of a post-lethality treatment that will allow products to be held for a shorter time period. Combining modified atmosphere packaging, an antimicrobial process and using short-term storage prior to distribution could be an effective antimicrobial process that would also act as a post-lethality treatment. In order for a lethality treatment to be used, USDA FSIS requires that it must be scientifically validated to provide evidence that will show the effectiveness of the antimicrobial process.

The objective of this study was to validate how packaging and storage reduces *Lm* on whole muscle turkey jerky or shelf-stable smoked sausage sticks using four packaging systems and four ambient temperature storage times.

CHAPTER 2 - Review of Literature

Listeria monocytogenes

History

Listeria monocytogenes (*Lm*) causes listeriosis and was discovered more than 70 years ago by E. G. D. Murray and James Pirie (Murray and others 1926). Cases of listeriosis in the human population had been overshadowed by other foodborne illnesses such as salmonellosis or campylobacteriosis, and substantiated foodborne outbreaks of listeriosis were rare (Bell and Kyriakides 2005). It was in the early 1980's when listeriosis demonstrated its severe nature and high mortality rate in immune-compromised populations, such as pregnant women, immune-compromised adults, and other at-risk individuals. It became a major foodborne illness after a foodborne listeriosis outbreak in Nova Scotia, Canada in 1981 due to contaminated coleslaw. Swaminathan and others (2007) explained that the complex interactions between various factors reflecting changes in social patterns was the cause of the emergence of increased cases of listeriosis over time. For example, improvements in medicine, public health, sanitation, and nutrition during the past 50 years have resulted in increased life expectancy, particularly in developed countries. The ongoing acquired immune deficiency syndrome (AIDS) epidemic, as well as immunosuppressive medications for the treatment of malignancies and management of organ transplantations, have greatly expanded the immunocompromised population at increased risk for listeriosis. Changes in food production practices, particularly the high degree of centralization and consolidation of food production and processing, the expanding national and international distribution of foods, and increased use of refrigeration as a primary means of food preservation are factors leading to increased cases of listeriosis. Changes in food habits such as increased consumer demand for convenience food that has a fresh-cooked taste, that can be purchased ready-to-eat (RTE), refrigerated, or frozen and be prepared rapidly, and that requires essentially little cooking before consumption and changes in handling and preparation practices have increased the risk of listeriosis due to *Lm* contamination in the post-processing environment.

Biological Nature

Listeria monocytogenes is a gram positive, short (0.4-0.5 x 0.5-2.0 μm) rod-shaped, catalase positive, non-sporulating, motile by flagella bacterium (Bell and Kyriakides 2005). It is one of the six species in the genus *Listeria*, and is considered the only pathogen among the species together with *L. ivanovii*. It is a versatile organism that can grow almost anywhere because it is nutritionally undemanding. Carbohydrates such as glucose are essential for its multiplication and that is why it favors food as an agent of transmission (Ryser and Marth 1991). It reaches stationary phase after 12 h in brain heart infusion (BHI) at 30°C and motility can be observed for cultures maintained at 20 and 25°C, but is non-motile at 37°C.

Listeria monocytogenes can survive under harsh environmental conditions such as extreme temperatures, pH, and the presence of a high salt level. It can also survive and grow in a wide range of food types that have relatively high water activities ($a_w > 0.92$). Although it cannot survive pasteurization, *Lm* can tolerate 10% NaCl and 200 ppm of NaNO₂ (Wagner and McLauchlin 2008) that makes processed meats a favorable substrate for its survival if present in the post-processing environment.

Factors Affecting Growth and Stress Adaptation Mechanisms

Temperature

Listeria monocytogenes can be psychrotrophic to mesophilic in nature and can survive and grow in a wide range of temperatures (-0.15- 45°C), with slower growth occurring at lower temperatures. Ryser and Marth (1991) reported that *Lm* in dairy products stored at 4°C had a generation time ranging from 1-2 d. This range of generation time for *Lm* agreed with the values reported by Hudson and Mott (1993) who evaluated *Lm* in vacuum packaged beef stored at 4°C. When stored at -15°C, Hudson and Mott (1993) found the generation time of *Lm* in vacuum packaged beef to be 100 h. Barbosa and others (1994) reported that the average generation time for 39 *Lm* strains was 43, 6.6, and 1.1 h at 4, 10, and 37°C, respectively. Temperatures below 0°C are bacteriostatic or moderately prevent *Lm* growth and the organism will die when exposed to temperatures above 50°C (Swaminathan and others 2007).

Exposure to low temperatures causes less stress to *Lm*, thus, heat stress is a more sensitive hurdle for the organism. Being a heat sensitive organism, *Lm* does not withstand pasteurization of milk, and D values in various foods vary from 16.7 to 1.3 min at 60°C and 0.2

to 0.06 min at 70°C (Bell and Kyriakides 2005). In addition, cooking, blanching, and sterilization are also lethal to the organism. Time of exposure to heat is an important factor. When time of exposure is insufficient, only sublethal heat stress is achieved. Whether it will be lethal or sublethal heat stress, the bacterium has mechanisms to resist such stress before it reaches the human host's cell. Miller and others (1972) and Earnshaw and others (1995) explained that in general, the basic heat resistance of microorganisms is due to the inherent stability of its cell membrane and consequently its intrinsic macromolecules such as ribosomes, DNA, enzymes, and intracellular proteins. The so-called "heat shock response" is a mechanism used by many organisms, including *Lm*, in order to resist both lethal and sublethal heat stress (Stack and others 2008).

The heat shock response is a highly conserved cellular defense mechanism characterized by the increased expression and accumulation of heat shock proteins (Hsps), whose expression enhances the survival of microorganisms at elevated temperatures (Yura and Nakahigashi 1999). Most Hsps belong to either molecular chaperones or adenosine triphosphate (ATP)-dependent proteases (ATPases) (Stack and others 2008).

pH

Based on the reports of Hill and others (1995) and Abee and Wouters (1999), *Lm* is a neutrophile, growing at an optimum pH of 6 to 7, similar to many other microorganisms. Swaminathan and others (2007) noted that *Lm* can grow in a pH range of 5.6 to 9.6, however, it can start to grow as low as pH 4.4 in laboratory media. Its growth in a high acid environment is attributed to the incubation temperature and the type of acid. Farber and others (1989) evaluated the effect of different acidulants on the survival of *Lm* in BHI broth. They observed that BHI broth acidified by acetic acid demonstrated an inhibitory effect on *Lm* growth at pH<5.6 and pH<5.0, incubated at 4 and 30°C, respectively. These values fall within the pH range where a larger proportion of acetic acid would be in an undissociated form, which is the form where the acid exhibits its antimicrobial action (ICMSF, 1980). On the other hand, BHI acidified with hydrochloric acid demonstrated inhibitory activity at pH 3.0 and pH 5.0 at 30 and 5°C, respectively. Furthermore, Swaminathan and others (2007) noted that, experimentally, inhibition of growth of *Lm* in tryptose broth increased as incubation temperature decreased in the presence of acetic, citric, and lactic acids up to a concentration level of 0.1%. Moreover, at the same pH,

the antimicrobial activity of these acids against *Lm* was related to their degree of dissociation, with citric and lactic acid being less detrimental for the pathogen.

Most *Lm* strains are not able to grow at a pH below 4.3 or at an acetic acid concentration above 0.4% (Vermeulen and others 2007). Thus, maintaining the cytoplasmic pH at a value close to neutrality despite fluctuations in pH in the environment is crucial to its survival. Increased in tolerance after adaptation to stressful environments, the organism may become highly resistant to even extremely acidic conditions. This is caused by a phenomenon called stress hardening (Lou and Yousef, 1997). Thus, in order to adapt and survive in acidic environments, *Lm* has developed several cellular mechanisms in order to maintain pH stability while continually adjusting to conditions that are optimal for survival.

The acid tolerance response involves acquisition of acid tolerance following brief exposure to mild acidic conditions resulting in an altered pattern of protein synthesis. This creates a situation in which the organism can produce acid shock proteins when subjected to a more challenging or even normally lethal pH (Foster 1991; Foster 1994; Kroll and Patchett 1992; O'Discroll and others 1997).

Water activity

Water activity (a_w) is defined as the amount of water available for microorganisms needed for its growth and survival (Jay and others 2005; Farkas 2007). This parameter is described by the ratio of the water vapor of water in food substrate to the vapor pressure of pure water at the same temperature (Jay 2005; Farkas 2005). It is an important factor for the microbiological stability of food. Under normal conditions, if the a_w of food is less than the requirement of the microorganism, growth of this organism will be inhibited.

The optimum a_w requirement for *Lm* is approximately >0.97 with the minimum a_w being from 0.90 for some strains, and 0.93 for most strains (Swaminathan and others 2007). This range of values supports the findings of Vermeulen and others (2007) that most *Lm* strains were not able to grow at $a_w < 0.93$. Shahalamat and others (1980) reported that *Lm* can, however, grow at a_w values as low as 0.83. An inverse relationship exists between the thermal resistance of *Lm* and the a_w of the medium in which it is suspended (Sunner and others 1991). *Listeria monocytogenes* can tolerate 10-12% NaCl and can grow to a high population in 6.5% NaCl (Swaminathan and others 2007).

In one study, Petran and Zottola (1989) reported growth of *Lm* in a 39.4% sucrose solution with an a_w of 0.92. *Lm* did not grow, but was observed to survive at 4°C in hard salami at an a_w of 0.79-0.86 (Johnson and others 1988). The a_w of most fresh meat products is above 0.99 (Jay 2000).

Control of *Listeria monocytogenes*

Use of sodium lactate

The USDA FSIS (2006) permits the use of sodium lactate up to concentrations of 4.8% in the final product formulation. This antimicrobial has been shown to prolong shelf life by lowering the a_w of foods (Chirife and Fontan, 1980). The ability of sodium lactate to lower a_w is not its only mechanism of inhibition. Other possible mechanisms include cytoplasmic acidification, specific anionic effect, and chelating action. De Wit and Rombouts (1989) demonstrated that adverse growing conditions, particularly sub-optimum temperatures, increased the antimicrobial effects of sodium lactate. This aspect of sodium lactate could be very useful in prohibiting the growth of *Lm* at refrigeration temperatures.

Sodium lactate's potential for inhibiting *Lm* has been demonstrated in a variety of food products. Sodium lactate (6.0%) was reported to delay the growth of *Clostridium botulinum* toxin in vacuum packaged uncured turkey products for >18 d at 28°C (Miller and others 1992). In a study using smoked salmon, Pelroy and others (1993) discovered that 3% sodium lactate prevented any increase in *Lm* during 40-50 d of storage at 5°C and 10°C. Wederquist and others (1994) reported that *Lm* growth was inhibited in turkey bologna using 2% sodium lactate at 4°C. Sodium lactate (1.8%) was shown to reduce *Lm* populations when compared to the control in cooked ground beef stored at 4°C (Harmayani and others 1992). Although the same results did not occur in raw ground beef as in cooked ground beef, it was shown that sodium lactate inhibits *Lm* more than other additives such as kappa-carrageenan, sodium erythorbate, or a combination of sodium alginate, lactic acid, and calcium carbonate. Additionally, *Lm* is greatly reduced by using 2.4% and 4.8% sodium lactate in conjunction with decreasing the temperature in minced beef products (McMahon and others 1999). Sodium lactate (4%) also suppressed *Lm* growth in sterile comminuted chicken and beef when incubated at 5, 20, or 35°C (Shelef and Yang 1991). Inhibition of *Lm* by sodium lactate increases with increasing fat content and decreasing temperature (Hu and Shelef, 1996).

A combination treatment involving sodium lactate and an additional antimicrobial has been studied to enhance the safety of foods. The addition of 2.5% sodium lactate and 0.25% sodium acetate to sliced and spreadable vacuum-packaged beef and pork cervelat sausage and cooked ham were found to inhibit *Lm* in the products for 4-6 wks when stored at 4°C by not allowing > 2.0 log CFU/g of *Lm* growth (Blom and others 1997).

Sodium diacetate

Sodium diacetate is approved as a flavoring agent in meat and poultry products at a level up to 0.25% by weight of the total formulation (USDA FSIS 2006). The use of sodium diacetate to inhibit the growth of *Lm* is also approved up to a level of 0.25% by weight of the total formulation. Sodium diacetate, which contains 40% acetic acid and sodium acetate, was first proven useful in the food industry as a mold inhibitor in baked products, and then later in mixed poultry feed, ensiled whole kernel corn, and corn silage (Glabe and Maryanski 1981). They found 90% growth inhibition of six species of molds on potato dextrose agar using 0.05-0.2% sodium diacetate at pH 3.5, and 0.15-0.5% sodium diacetate at pH 4.5 (Glabe and Maryanski 1981). At a concentration of 32mM, this antimicrobial was also shown to completely inhibit the growth of *Lm* in BHI broth at 20° and 5°C (Shelef and Addala 1993). Inhibition was observed to increase with decreasing temperature.

More recent studies have shown the usefulness of sodium diacetate in meat and poultry products, and the effects of sodium diacetate combined with other antimicrobials to control the growth of *Lm*. The addition of 0.5% sodium diacetate alone to turkey slurries exhibited a listericidal effect. However, a combination of 0.5% sodium diacetate and pediocin or sodium lactate showed even greater listericidal ability (Schlyter and others 1993b). It was also reported that 0.5% sodium diacetate caused only slight inhibition of *Lm* in turkey slurries, yet the combination of 0.5% sodium diacetate and 0.75% ALTA 2341 (a fermented corn syrup product) showed greater inhibition (Schlyter and others 1993a). The addition of sodium diacetate alone delays the growth of *Lm*, however multiple barriers may provide additional protection from food-related listeriosis.

Sodium acetate

Sodium acetate has been known to exhibit antilisterial effects (Weaver and Shelef 1993). Sodium acetate is currently approved as a flavoring agent in meat and poultry products at a level

up to 0.25% by weight of the total formulation (USDA FSIS 2006). Sodium acetate has proven useful for controlling pathogens in a variety of meat and poultry products. An uncured turkey product remained free of *C. botulinum* neurotoxin for over 18 d at 28°C when treated with 6% sodium acetate (Miller and others 1992). In vacuum packaged turkey bologna, sodium acetate was determined to significantly reduce the growth of *Lm* at 4°C by only allowing a maximum growth of 1.33 log CFU/g after 70 d of storage (Wederquist and others 1994).

Recent studies have shown the effects of sodium acetate combined with other antimicrobial agents to inhibit *Lm*. A combination of sodium acetate, ethylenediaminetetraacetic acid (EDTA), and ascorbic acid at 4°C and pH 4.0-4.5 was shown to increase the inactivation of *Lm* in BHI broth compared to the use of either antimicrobial alone, and at temperatures 4-28°C (Golden and others 1994). This combination of sodium acetate with EDTA and ascorbic acid demonstrates the usefulness of antimicrobial agent combinations at suppressing *Lm* growth. The use of 2.5% sodium lactate and 0.25% sodium acetate to inhibit *Lm* in vacuum packaged pork sausage and sliced, cooked ham is another example of inhibition by combination treatments (Blom and others 1997).

Three alternatives to control *Lm*

The ubiquitous nature of *Lm* makes it a potential environmental contaminant for any food product, including meat products and it is frequently isolated from the food-processing environment (Jacquet and others 1993; Hood and Zottola 1995; Rorvik and others 1995; Destro and others 1996; Gravani 1999; Johansson and others 1999; Miettinen and others 1999). Presence of *Lm* in RTE meat and poultry products can be eliminated through cooking because of its heat sensitivity (Stack and others 2008). Thus, *Lm* contamination of RTE meat and poultry products results primarily from post-lethality contamination. To effectively control *Lm* contamination in RTE meat and poultry products, USDA FSIS established three strategies establishments must use to control *Lm* (USDA FSIS 2003a). The use of a growth inhibitor and a post-lethality treatment must be employed by the establishment for it to operate under alternative one. An establishment operating under this alternative is subjected to USDA FSIS verification activities that focus on post-lethality treatment effectiveness. The use of either a growth inhibitor or a post-lethality treatment must be employed by the establishment for it to operate under alternative two. Establishments operating under this alternative are subjected to more USDA FSIS verification

activities than establishments operating under alternative one. Lastly, establishments that operate under alternative three rely on sanitation measures to control *Lm* contamination. Establishments operating under alternative three are subjected to the most frequent USDA FSIS verification activities.

To control *Lm* post-processing contamination, post lethality treatments such as steam pasteurization, hot water pasteurization, radiant heating, and high pressure processing have been developed (USDA FSIS, 2006). In addition, USDA FSIS requires establishments to include post-lethality treatment and antimicrobial agents in their HACCP plans if they control *Lm* using alternative one. Verification of post-lethality treatment and antimicrobial agents is necessary and its result should be made available to FSIS personnel upon request. The effectiveness in eliminating *Lm* to an undetectable level following post-lethality treatment must be validated and the validation should specify the log reduction achieved by the post-lethality treatment (CFR 2000). USDA FSIS requires the establishment to demonstrate that the post-lethality treatment is adequate to reduce or eliminate *Lm* to an undetectable level in its HACCP documentation.

Published research studies can be used as scientific documentation by an establishment for their post-lethality treatment validation as long as these studies use the product type or size, the type of equipment, time, temperature, pressure, or other variables used in the study in order to achieve the equivalent level of *Lm* reduction (USDA FSIS, 2006). According to USDA FSIS (2006), if an establishment uses products or variables other than those used in the referenced studies, it must perform its own validation studies to determine the effective reduction of *Lm* as a result of post-lethality treatment or antimicrobial agent applied to the products. In order to show that a product is safe, it is necessary for the establishment to specify the level of reduction achieved by the post lethality treatment or antimicrobial agent applied in its validation.

Unpublished studies may be used in the absence of published peer-reviewed paper that would contain the information needed for validation. However, there must be supporting documentation showing that the data and analysis of the results of these unpublished studies demonstrate that the specific level of application of the post-lethality treatment or antimicrobial agent on specified products is effective to produce a safe product. In addition, testing for *Lm* is necessary for verification of effectiveness of post-lethality treatment and antimicrobial agent (USDA FSIS 2006).

There are some antimicrobial processes that can also act as a post-lethality treatment to eliminate or control *Lm* growth according to USDA FSIS (2006). Any antimicrobial process that can provide a RTE product microbiological stability throughout shelf-life storage can also serve as a post-lethality treatment serving to prevent *Lm* growth in a post-lethality environment. Processing of shelf-stable products usually involves addition of salt, acids, nitrites, other additives, or cooking or drying to achieve a desired level of a_w and pH that will reduce *Lm*. The antimicrobial process that acts as a post-lethality treatment may exhibit a bacteriostatic and bacteriocidal effect in the product. In effect, the product would not support growth of *Lm* or other pathogens throughout the shelf-life of the product at ambient temperatures (USDA FSIS, 2006), however, pathogens could still survive.

USDA FSIS (2006) provided some conditions in order for a antimicrobial process to also serve as a post-lethality treatment. For example, processed meat products having an $a_w < 0.85$ will not support *Lm* growth. If there is a bacteriocidal effect in the product, and with supporting documentation such as a challenge study or published report that shows the effectiveness of the post-lethality treatment throughout shelf-life of the product prior to distribution, FSIS will consider this antimicrobial process as a post-lethality treatment. The establishment could operate under alternative one if requirements for this alternative are satisfied.

Jerky

USDA FSIS (2007) define meat or poultry jerky as a shelf-stable RTE product that has been processed through slicing (whole muscle) or restructuring the meat or poultry, marinating, cooking, and then drying. A meat or poultry jerky product is considered shelf-stable if it has an a_w of 0.85 or lower after drying. The finished product must also meet the moisture protein ratio (MPR) product standard of 0.75:1 (USDA FSIS 2003).

The “Compliance Guideline for Meat and Poultry Jerky” (USDA FSIS 2007) describes two important steps in jerky processing: cooking and drying. The cooking step serves as a lethality treatment to kill or reduce the numbers of microorganisms, especially pathogens. On the other hand, drying jerky makes the product shelf-stable and controls the growth of microorganisms, especially *Staphylococcus aureus*. However, it is critical that processors control *Salmonella* and *Escherichia coli* O157:H7 during jerky production by using validated research documenting humidity parameters during cooking. If the lethality compliance guidelines in

“Time-Temperature Tables for Cooking Ready-to-Eat Poultry Products” (USDA FSIS 1999) are used as supporting documentation, a humidity parameter of 90% must be used throughout the cooking step for jerky. The combination of temperature and humidity is critical in lethality treatment of jerky. Moist heat is more lethal to microorganisms than dry heat because of the greater efficiency of steam to deliver lethal thermal energy to the cells resulting in the denaturation of protein components of the cell (Carlberg 2004). Reduction of a_w by addition of salt, sugar and other substances will increase heat resistance of pathogens such as *Salmonella* spp. in the product.

The general jerky processing steps commonly used by manufacturers have been described by USDA FSIS (2007). These steps include strip preparation, marination, antimicrobial interventions, lethality treatment, drying, post-drying heat step, and handling. According to USDA FSIS (2006), antimicrobial interventions can increase the level of pathogen reduction beyond that achieved by heating alone. Application or addition of antimicrobials is usually done before or after marination. It is during the lethality step that pathogens such as *Salmonella* spp. and *Lm* are reduced or killed.

For poultry jerky, producers need to achieve a minimum internal temperature of 71°C for uncured poultry or 68°C for cured and smoked poultry (USDA FSIS 1999). The required reduction of *Salmonella* also can be achieved by using one of the time-temperature combinations listed in the “Time-Temperature Tables for Cooking Ready-to-Eat Poultry Products” (USDA FSIS 2007).

Shelf-Stable Sausage

Non-refrigerated semidry shelf-stable sausage must have an MPR of ≤ 3.1 and a pH value of ≤ 5.0 ; or alternately, must have a pH of ≤ 4.5 (or 4.6 with an $a_w \leq 0.91$), an internal brine concentration of $\leq 5\%$, and must be intact (or vacuum packaged if sliced), cured, and smoked (USDA FSIS 2005).

Packaging

The primary purpose of packaging is to maintain the product at a suitable quality, frequently defined as freshness, and also to assure the safety of the product to the consumer at the point of ultimate consumption (Kropf 2004). There are three major functions of packaging. First, packaging protects the product from environmental variables such as temperature, moisture or humidity, oxygen, airborne particles and light. Second, it protects the product against biological contamination such as microorganisms, rodents, and insects. Lastly, it serves as a marketing tool since it must provide space for a label that contains the information such as product identification, ingredients, nutritional information, net weight, verification of inspection, cooking instructions, promotional material, company name and location, and instructions on how to contact the processor.

Modified Atmosphere Packaging

Chilling slows the deterioration of stored foods but if the atmosphere surrounding the product is modified to reduce the oxygen concentration, product shelf-life generally increases considerably because of further reduction in the rate of chemical oxidation by oxygen and reduction in the growth of aerobic microorganisms (Parry 1993). Modifying packaging atmosphere through oxygen reduction is beneficial for shelf-stable RTE products with low a_w because reduced oxygen concentration can inhibit growth of some molds (Jay and others 2005). The technique used to reduce oxygen or altering food package or chamber atmosphere by flushing different gas mixtures of CO₂, N₂, and/or O₂ is known as modified atmosphere packaging (MAP) (Phillips 1996; Jay and others 2005). MAP includes vacuum packaging, controlled atmosphere packaging (CAP), and MAP systems or ‘dynamic MAP’ (Table 1) (Kropf 2004; Phillips 1996).

Packaging materials

Phillips (1996) described some of the packaging materials used for MAP, including polyvinylchloride (PVC), polyethylene terephthalate (PET), polyethylene (PE), and polypropylene (PP). Barrier properties, machine capability, sealing reliability, anti-fog properties, and special characteristics such as easy peel seals and high heat resistant packaging

are some of the factors that should be considered in choosing a packaging film (Smith 1993). Packaging materials must be flexible so as to form closely to the shape of the product being packaged, and must have the ability to shrink and be heat sealed. It must also be strong and puncture-resistant (Kropf 2004).

The type of the material used for packaging and the initial gas mixture used affects the atmosphere within the package. During storage some materials allow diffusion of gases in and/or out of the package. However, if the film is fully permeable, the atmosphere within the packaging gradually becomes the same as the air outside. On the other hand, if the film is semi-permeable, equilibrium modified atmosphere results. (Phillips 1996).

For a vacuum package, film requirements include good barrier properties for both moisture and gas. Films with very low oxygen permeability are used for cured meats because nitrosohaemochrome pigment is vulnerable to oxidative deterioration. Processed meat is frequently packaged in heat shrinkable and heat stable bags with three layers, such as ethylene-vinyl acetate or nylon, ethylene-vinyl alcohol, and ionomer coextrusions. Ionomers with zinc or sodium have very good heat-sealing layers (Kropf 2004).

Vacuum

Vacuum packaging is a type of packaging in which all air has been removed prior to sealing. It is a form of MAP because the normal air composition has been altered. Vacuum packaging of meat products offers a lot of benefits because of the extended shelf-life, reduced weight or moisture loss through the control of evaporation, aids in controlling oxidative rancidity, prevents growth of normal spoilage bacteria, and preservation of color. An increase in CO₂ occurs upon storage of a vacuum packaged food product (Jay 2005). This is a result of both fresh meat and microbial respiration where O₂ is consumed by enzymes of both microbes and meat and CO₂ is released in equal volumes (Kropf 2004; Jay 2005). The resulting atmospheric condition will severely inhibit aerobic microorganisms but will allow anaerobic bacteria such as lactic acid bacteria and *C. botulinum* to predominate if other environmental conditions such as pH, a_w, and temperature are at an optimum level.

Gas packaging/true MAP

The main gases that are used in MAP are O₂, N₂, and CO₂. These gases are used in different amounts and combinations depending on the nature of the product and the needs of the

processors and consumers (Phillips 1996). The choice is influenced by the microflora capable of growing on the product, the sensitivity of the product to O₂ and CO₂, and the color stability requirements. Church (1994) had suggested other gases such as nitrous and nitric oxides, carbon monoxide, sulphur dioxide, ethene and chlorine. However, most of these gases have not been developed for safety, consumer response, legal aspects, and cost reasons.

Oxygen (O₂)

O₂ has several effects on food. The presence of O₂ maintains the myoglobin in meat in oxygenated form, called oxymyoglobin, giving meat the fresh bright red color expected by the consumer. Fresh meat is usually packaged in 65-80% O₂ atmospheres in order to maintain the correct level of oxygenation (Kropf 2004; Phillips 1996). O₂ also affects the bacterial flora able to grow on the product. Generally it stimulates the growth of aerobic microorganisms and inhibits the growth of anaerobes. Low levels of O₂, <0.5%, result in a color change in meat and meat products to brown or brown/grey known as metmyoglobin (Church 1994). Conversely, a high O₂ concentration may cause rancidity due to oxidative mechanisms particularly in products with a high fat content, such as “streaky” unsmoked bacon. These products tend to be packaged in atmospheres without O₂, and a ratio of 35% CO₂: 65% N₂ would be used for bacon.

Although there have been suggestions that the atmosphere within the packaging of all the MAP products should contain a level of 5-10% O₂ (Hotchkiss 1988), O₂ will diffuse into the packaging during storage unless the packaging is impervious to O₂. This low level of O₂ may inhibit the surface growth of pathogenic anaerobic bacteria, particularly *C. botulinum*, although it would not prevent anaerobic conditions being present in the “body” of the product.

Nitrogen (N₂)

N₂ is an inert gas which has been used as a packaging filler for many years to prevent package collapse due to its low solubility in water and lipid compared to CO₂ (Phillips 1996). High solubility of CO₂ in fresh meat causes package collapse in high CO₂ MAP. It has no direct function in microbial control. It is used to replace O₂ in MAP products to prevent rancidity and inhibit growth of aerobic organisms (Farber and Perterski 1991).

Carbon dioxide (CO₂)

CO₂ is the major antimicrobial factor in MAP. Generally, the inhibitory effect is seen as increases in the lag phase and generation time during the logarithmic phase of growth of organisms. The effectiveness of CO₂ is influenced by the original and final concentrations of gas, the temperature storage and the original population of organisms. Microbial growth is reduced at high concentrations of CO₂ in a variety products and this effect increases as storage temperature decreases (Reddy and others 1992).

Genigeorgis (1985) suggested that the antimicrobial activity of CO₂ was a result of the gas being absorbed onto the surface of the food forming carbonic acid, subsequent ionization of the carbonic acid, and a reduction in pH. However, this minimal decrease in pH probably would not cause any any significant bacteriostatic activity.

The effectiveness of CO₂ as an antimicrobial agent is not universal and depends on the microbial flora present and the product characteristics. Yeasts which produce CO₂ are stimulated by high levels of CO₂ and so in some products where they are potentially a major cause of spoilage, this may not be an advisable option (Babic and others 1992). *Clostridium perfringens* and *C. botulinum* are not affected by the presence of CO₂ and their growth is encouraged by anaerobic conditions. In general, CO₂ is most effective in foods where normal spoilage organisms consist of aerobic, gram-negative psychrotrophic bacteria. For some fermented foods, such as Hungarian salami which develops a mold layer after manufacture, or for foods which develop a gram-positive flora during processing, such as cured bacon, CO₂ MAP may not be effective (Church 1994)

Effects of MAP on Lm

As a general rule, foods that are subjected to MAP should possess one or more of the following antibotulinal hurdles: $a_w < 0.93$, $pH \leq 4.6$, cured with NaNO₂, contain high levels of nonpathogenic competing organisms, maintained frozen, maintained at 4.0 °C or below, and have a definitive shelf-life (Jay 2005). Another factor in determining the microbiological safety of MAP food products is whether the food is sold as RTE, requires minimal further heating, or is raw. *Lm* is of great concern in RTE MAP products. This is due to its capability of growing in completely aerobic or anaerobic environments, as well as in the presence of low levels of CO₂. Bunic and others (1990) reported that *Lm* was able to grow in vacuum packaged frankfurters at

4°C. According to Daniels and others (1984), the effectiveness of CO₂ in inhibiting microorganisms generally increased as the concentration of the gas increased. Atmospheres containing >50% CO₂ were shown to decrease the growth rate of *Lm* as compared to *Lm* growth in aerobic or anaerobic atmospheres. A 100% CO₂ atmosphere for chicken breast meat stored at 6°C inhibited the growth of *Lm* (Hart and others 1991). This experiment showed that 100% CO₂ was more effective than an atmosphere containing 30% CO₂ plus air, or a 30% CO₂ and 70% N₂ environment. Hart and others (1991) also found no difference between 30% CO₂/70% air and the 30% CO₂/70% N₂ atmosphere. *Lm* in turkey roll slices was inhibited by >70% CO₂ at 4°C (Farber and Daly 1994).

CO₂ concentrations of 5-10% (with 5% O₂/95% N₂-10% O₂/90% N₂) had no inhibitory effect compared to air on the growth and survival of pure *Lm* cultures (Francis and O'Bierne 2002). An atmosphere of 100% N₂ allowed survival of pure *Lm* cultures, but populations did not change during storage, whereas a 3% O₂/95% N₂ atmosphere allowed significant growth of *Lm*. Combining MAP with some antimicrobials has been shown to have a synergistic effect on *Lm* inhibition. This was observed on poultry treated with a 10% lactate acid/sodium lactate buffer and packaged in 90% CO₂/10% O₂ (Zeitoun and Debevere 1991). This combination was more effective than packaging poultry in either treatment alone. Nilsson and others (1997) found that using a high CO₂ atmosphere with nisin at 5°C delayed growth of *Lm* in salmon compared to either treatment individually. A treatment of 2% lactic acid combined with a 74.8% CO₂/10.4% O₂/14.8% N₂ atmosphere resulted in an 8 d extended lag phase of *Lm* compared to a treatment of 2% lactic acid with vacuum or air packaging (Pothuri and others 1995). In addition, an environment of 44% CO₂/56% N₂ combined with sodium lactate extended the lag phase of *Lm* by 10 d at 3.5°C in cooked poultry cuts (Barakat and Harris 1999).

Microbiological Media for *Lm* Enumeration

Oxford Medium Base

Oxford Medium Base is a selective medium used for isolation of *Listeria* spp. It contains peptones and beef heart digest that provide nitrogen, carbon, amino acids and vitamins (Difco 2009). It also contains sodium chloride to maintain the osmotic balance of the media. Ferric ammonium citrate serves as the color indicator that aids in the differentiation of *Listeria* spp.

Listeria spp. reacts with ferric ions producing black colonies and a black zone of inhibition around the colony (Tanus 2007) indicating that *Listeria* spp. has hydrolyzed esculin. A blackening of the colony and surrounding medium in cultures containing esculin-hydrolyzing bacteria results from the formation of 6,7-dihydroxycoumarin which reacts with the ferric ions (Difco 2009). Selectivity is provided by the presence of lithium chloride in the formula.

Selectivity is increased by adding various antimicrobial agents to the medium base. Incorporating these agents into Oxford Medium Base completely inhibits gram-negative organisms and most gram-positive organisms after 24 h of incubation. The most widely recognized antimicrobial agent combinations are the Oxford Medium formulation (Curtis and others 1989) and the Modified Oxford Medium formulation (McClain and Lee 1989). The Oxford Medium formulation contains cycloheximide, colistin sulfate, acriflavine, cefotetan, and fosfomycin (available as Oxford Antimicrobial Supplement) (Difco 2009). The Modified Oxford Medium formulation contains moxalactam and colistin methane sulfonate or colistin sulfate (available as Modified Oxford Antimicrobial Supplement). Modified Oxford Medium is recommended for isolating and identifying *Lm* from processed meat and poultry products (McClain and Lee 1989). Oxford Medium is recommended for isolating *Listeria* from enrichment broth cultures (Horwitz 2000).

Table 2-1. Terminologies used in MAP

Terminologies	
Modified atmosphere packaging (MAP)	Replacement of air with a single gas or a mixture of gases. No further control over initial composition. ¹
Controlled atmosphere packaging (CAP)	Altered initial atmosphere with desired atmosphere gas composition and maintained gas composition over the whole period of storage. ²
Vacuum packaging	Air is evacuated from gas-impermeable package followed by sealing. Residual air pressure after packaging is 0.3-0.4 bar (1 bar=0.9869 atm). ³
Vacuum skin packaging	Softened film placed over product and vacuum applied. It is used for delicate products. ¹
Active packaging	Systems that "actively change the conditions of the packed food to extend shelf-life or to improve food safety or sensory properties while maintaining the quality of the food." This includes systems that absorb or release substances, and substances incorporated into the packaging itself or packed separately in the package. ⁴
Equilibrium modified atmosphere (EMA) packaging	It is a type of dynamic MAP where pack is flushed with gas or sealed without modification. Permeability of packaging and respiration of product results in an equilibrium modified atmosphere. It is used for fresh fruits and vegetables. ¹

¹Phillips 1996

²Jay and others 2005

³Kropf 2004

⁴Misco 2002

CHAPTER 3 - Preliminary Study: Evaluation of *Listeria monocytogenes* Inoculation Procedures in Whole Muscle Turkey Jerky and Pork and Beef Smoked Sausage sticks

Introduction

When studying *Lm* survival or reduction in RTE meat and poultry products, it is important to use an appropriate inoculation method that will result in at least 4 to 5 logs of attachment in order to observe significant *Lm* log reduction or survival. Several inoculation procedures have been used to study post-thermal processing survival of *Lm* in RTE meat and poultry products. To compare the efficiency and frequency of the USDA FSIS product composite method with the USDA Agricultural Research Service (ARS) package rinse method, Luchansky and others (2002) inoculated vacuum-packaged frankfurters with *Lm* by adding 4 mL of a diluted five-strain *Lm* culture onto the package. This inoculation was done to achieve an average final *Lm* concentration of 22 CFU or 20,133 CFU per package depending on treatment. Gounadaki and others (2007) inoculated sliced salami with *Lm* by spreading 0.25 mL of the *Lm* culture onto the surface of each side of a 6.0-7.0 g sample using a sterile bent glass rod. The salami was immediately packaged under air or vacuum, with a population density of 4.6 to 6.5 log CFU/g of *Lm*. A similar *Lm* inoculation procedure was done by Ingham and others (2004) to RTE meat products including summer sausage, elk sausage, buffalo sausage, pork rinds, pork cracklings, beef jerky, and snack sticks. An amount of 0.025 mL of *Lm* culture was added onto each product and distributed evenly using a sterile plastic spreader. The samples were dried for 15 min and then vacuum packaged. The resulting *Lm* inoculum level ranged from 3.1 to 4.4 log CFU/sample. Gande and Muriana (2003) used contact inoculation and a dipping method to inoculate turkey bologna, roast beef, corned beef, and ham with a four-strain *Lm* cocktail. This was done by dipping and rotating each sample in 160 ml (4 x 40 ml) of a four-strain mixture of *Lm* placed in a stainless steel bowl. Product pieces were then allowed to drain excess culture for 5 min in a sterile tray and 9 to 9.5 CFU per product was typically achieved. For the contact inoculation

method, sponge-foam padding material (5 to 6 cm thick) was cut to the shape of a petri plate, autoclaved in foil-covered beakers, and used to pick up the mixed strain inoculum lawn from inoculated petri plates after overnight incubation on agar plates with the use of a contact and twist motion. The inoculum was then contact inoculated onto the surface of the product with the same twist motion. The contact inoculation method also provided initial *Lm* levels of 9 to 9.5 CFU per product sample.

To evaluate *Lm* inoculation procedures for this research, four preliminary experiments were conducted for jerky and sausage sticks.

Materials and Methods

The following procedures for bacterial culture preparation, media preparation, and enumeration were used for all four preliminary experiments.

Bacterial culture preparation

Lyophilized reference stock cultures of five *Lm* strains (Table 3-1) were obtained (Kwik-Stik, Microbiologics, Inc., Grenoble Cedex 2, France). A pure subculture of each strain was prepared by transferring a loopful of stock culture to 10 mL of pre-sterilized tryptic soy broth (TSB; Difco BD and Company, Sparks, MD) and incubated at 37°C for 24 h. One liter of the five-strain *Lm* cocktail was prepared aseptically by first transferring 0.5 mL of each pure culture isolate to 200 mL pre-sterilized TSB, and then incubating at 37°C for 24 h. Next, each 200 mL inoculum was transferred into a sterile 1 L beaker to obtain a liter of the five-strain cocktail containing 8 to 9 log CFU/mL of *Lm*. A total of 2.0 L of *Lm* cocktail was prepared. One liter was used for smoked sausage stick inoculation and the other liter was used for turkey jerky inoculation.

Table 3-1. Strains of *Lm* used to inoculate turkey jerky and smoked sausage sticks

Strain	Original Source
ATCC 7644	Human
ATCC 19115	Human
ATCC 19118	Chicken (England)
ATCC 19112	Spinal fluid of man (Scotland)
SLR 2249 Cornell University	Human

Media preparation

TSB was prepared by adding 30 g of the media to 1 L of distilled water, mixed thoroughly, and then heated for 2 min. Then, it was transferred to a 1 L bottle and sterilized at 121 °C for 15 min.

Modified oxford agar (MOX; Difco BD and Company, Sparks, MD) was prepared by adding 57.5 g of the media to 1 L of distilled water, mixing, and then boiling for 1 min or until turbidity was lost. Next, it was transferred to 1 L bottle and sterilized at 121 °C for 15 min. Media was then cooled down at room temperature to about 45°C. Hydrolyzed Oxford antimicrobial supplement (10 mL vial) (Difco BD and Company, Sparks, MD) dissolved in 10 mL distilled water was immediately added into cooled media. This supplement makes the media selective for *Lm*. The supplemented media was then poured into disposable agar plates and allowed to set for at least 2 h at ambient temperature.

Peptone water (0.1%) was prepared by adding 1 g of Bacto™ Peptone (Difco BD and Company, Sparks, MD) to 1 L of distilled water, mixed thoroughly, and sterilized at 121 °C for 15 min. For preparing peptone blanks for serial dilution, 9 mL of 0.1% peptone was dispensed into 15.0 mL screw-capped tubes and sterilized at 121 °C for 15 min.

Enumeration of *Lm*

Inoculum level of the of the pre-prepared 1.0 L five-strain *Lm* cocktail was initially determined before inoculation by transferring 1.0 mL aliquot of the cocktail to 9.0 mL 0.1% peptone water blank. It was then serially diluted up to ninth dilution. Dilutions 5- 9 were spread plated in duplicate on MOX. Plates were then incubated at 35±2°C for 48±2 h and counts were reported as log CFU/cm².

Surviving *Lm* in inoculated samples was determined after inoculation procedure (described in Experiment 1-4). Each sample was aseptically transferred onto double-chamber stomacher bags (Fisherbrand, Ste-Julie, Quebec, Canada). Next, 50 mL of 0.1% peptone diluent was added, and contents were stomached for 1 min. Three and four serial dilutions were also prepared for smoked sausage stick and turkey jerky sample piece, respectively. This was done by transferring 1.0 mL aliquot of the stomached treatment to 9.0 mL 0.1% peptone water blank Dilutions 0-2 and dilutions 1-3 from smoked sausage stick and turkey jerky sample piece were

plated in duplicate on MOX. Plates were then incubated at $35\pm 2^{\circ}\text{C}$ for 48 ± 2 h and counts were reported as $\log \text{CFU}/\text{cm}^2$.

Experiment 1: Turkey Jerky Experiment

A preliminary study was conducted to evaluate the turkey jerky inoculation procedure. This preliminary experiment aimed to develop an inoculation procedure that would achieve at least 5.0 log CFU/cm² *Lm* attachment on turkey jerky.

Sample description

Whole muscle turkey jerky samples were obtained commercially and were packaged in a nitrogen flushed O₂ impermeable re-sealable 5 mil thick clear pouch, 7.5 cm x 14.0 cm with zipper and containing an oxygen absorber. The product contained turkey breast, soy sauce, salt, brown sugar, corn syrup, sugar, pineapple juice concentrate, vinegar, molasses, caramel color, high fructose corn syrup and citric acid, hydrolyzed soy and corn protein water, anchovies, onions, tamarind, garlic, cloves, chili peppers, natural flavorings and shallots, pineapple juice, and natural hickory smoke flavor.

Design of Experiment

A total of 12 pieces of turkey samples were used for the initial inoculation study and two surface areas were evaluated; 108 and 134 cm². The surface areas were chosen based on the size of jerky pieces that were available in each package. In addition, two samples were allotted to one of the three treatments, that include, initial attachment, 24 and 48 h. A total of eight turkey jerky pieces were used for a_w determination. Four samples were allotted to determine the initial a_w or a_w before inoculation and another four samples were allotted to determine the a_w after inoculation.

Turkey jerky sample preparation, inoculation, and packaging

Six turkey jerky pieces for each surface area of 108 and 134 cm² was cut aseptically using a sterile knife on a chopping board covered with sterile foil. Each sample was dipped aseptically using sterile binder clips in 1 L five-strain *Lm* cocktail for 1 min and hung to dry for 1 h at 25.5°C. Surviving *Lm* was then enumerated using the procedure described previously on two samples from each surface area to determine the initial *Lm* attachment to the sample. The remaining samples were then assigned to one of the two storage times and were vacuum

packaged using a vacuum packer (Multivac C100, Gepufte Sicherheit, Germany) under 600 mm Hg. Samples were stored for 24 or 48 h at 25.5°C.

Water activity measurement

The a_w of eight jerky pieces (surface area= 122 cm²) before and after inoculation was determined using an a_w meter (AQUALAB CX2 series 3TE; Decagon, Pullman WA). The a_w meter was calibrated using a 0.760 NaCl (6.0 molal in water; Decagon, Pullman WA) verification standard and distilled water at 25.2°C. To avoid contamination of the a_w meter with *Lm*, a simulation of the inoculation procedure described above was done by dipping turkey jerky pieces into 1 L sterile TSB media without the *Lm* inoculum. The a_w was measured by cutting each turkey jerky piece into a hexagonal shape with a diameter of approximately 3.2 cm and was placed in the sample container. Duplicate readings were taken for each sample at 25.5°C.

Statistical analysis

To compare a_w before and after dipping of turkey in TSB for 2 min, statistical analysis was performed using a t-Test for paired two sample means procedure in Microsoft Excel. Significance was set at $P < 0.05$.

Results and Discussion

Listeria monocytogenes survival in vacuum packaged turkey jerky after 24 and 48 h for preliminary Experiment 1 is shown in Table 3-2. Initial attachment of at least 5 log CFU/cm² was observed. After 24 h, *Lm* reduction was >1.0 log CFU/cm² on the 134 cm² surface area sample while <1.0 log CFU/cm² of *Lm* reduction was observed on the 108 cm² surface area sample. On the other hand, *Lm* reduction was >1.0 log CFU/cm² on both surface area samples after 48 h ambient temperature storage.

The mean a_w of turkey jerky increased ($P < 0.05$) from 0.76 to 0.78 as a result of the dipping procedure (Table 3-3). However, the 0.02 change in a_w would be too small to have an impact on the product properties.

Table 3-2. *Lm* survival in vacuum packaged turkey jerky after 24 and 48 h ambient temperature storage for preliminary Experiment 1

Sample number	Surface area (cm ²)	Initial attachment(a)	<i>Lm</i> population after storage (b)		<i>Lm</i> Log reduction after storage (a-b)	
			(Log CFU/cm ²)			
			24 h	48 h	24 h	48 h
1	134	5.87	4.76	4.34	0.50	1.42
2	108	5.42	4.92	4.00	1.11	1.53

Table 3-3. Water activity of turkey jerky before and after dipping in trypticase soy broth for preliminary Experiment 1

Sample number	a _w before inoculation	a _w after inoculation
1	0.75	0.78
2	0.76	0.78
3	0.76	0.77
4	0.76	0.77
Mean	0.76 ^a	0.78 ^b

^{ab}Means within rows with different superscript differ (p<0.05)

Preliminary Pork and Beef Smoked Sausage Sticks Experiment

Three preliminary experiments were conducted to evaluate smoked sausage sticks inoculation procedures based on the level of attachment ($\log \text{CFU}/\text{cm}^2$) that could be achieved. The objective of these preliminary studies was to develop an inoculation procedure that could achieve at least $4.0 \log \text{CFU}/\text{cm}^2$ attachment on for smoked sausage sticks.

Sample description

Pork and beef smoked sausage sticks with beef collagen casing were commercially obtained and were packaged in a nitrogen flushed O_2 impermeable packaging containing an oxygen absorber. O_2 concentration inside each package was $<0.1 \%$. Ingredients in the smoked sausage sticks were pork hearts, beef, pork fat, salt, dextrose, wheat flour, natural flavorings, paprika, potassium nitrate, lactic acid starter culture, sodium nitrite, sodium nitrate, BHA and treated with a solution of potassium sorbate to ensure freshness.

Design of experiment

A total of 9 commercial sausage sticks were used for each of the following experiments. Three commercial sausage sticks were used for microbiological analysis and 6 sausage sticks were used for a_w determination.

Water activity measurement

The a_w of samples before and after inoculation was determined using an a_w meter (Aqualab CX2 series 3TE; Decagon, Pullman WA). The a_w meter was calibrated using a 0.760 NaCl (6.0 molal in water; Decagon, Pullman WA) verification standard and distilled water at 25.5°C . Smoked sausage sticks were chopped into small pieces, and evenly placed in a sample container. Duplicate readings were taken for each sample at 25.5°C .

Experiment 2

Smoked sausage sticks inoculation

Three commercial sausage sticks were used for *Lm* inoculation. The inoculation was done by aseptically dipping each stick using a sterile binder clip into 1.0 L of the five-strain *Lm* cocktail for 1 min and then hanging to dry for 1 h at 25.5°C. *Lm* was enumerated by plating dilutions 0- 2 in duplicate on MOX.

Results and Discussion

Table 3-4 shows *Lm* attachment and a_w values of sausage sticks before and after dip inoculation and the hand massage procedures. The a_w remained constant before and after inoculation, *Lm* attachment ranged from 2.05-4.42 log CFU/cm² depending on inoculation method.

The dip inoculation procedure provided a mean attachment of 2.12 log CFU/cm² which is too low of an attachment level to be used for validation research (Table 3-4). This low *Lm* attachment was attributed to the cellulose casings on the smoked sausage sticks that made *Lm* attachment difficult. Due to low level of attachment that was achieved by the dip inoculation procedure, another procedure for inoculation was evaluated for smoked sausage sticks in the third experiment.

Experiment 3

Smoked sausage sticks inoculation

In this experiment, instead of dipping sticks into a *Lm* cocktail, three sausage sticks were placed into a stomacher bag and a proportional amount of *Lm* cocktail of 4 mL per stick was added (12 mL total *Lm* cocktail). With gloves on, the sausage sticks were then gently hand massaged inside the stomacher bag for 2 min followed by a 2 min holding period. The sausage sticks were hung to dry for 1 h, and *Lm* was enumerated using MOX. Dilutions 0- 2 were spread plated in duplicate.

Results and Discussion

The a_w remained constant before and after hand massage inoculation using proportion of 1:4.0 mL stick to *Lm* inoculum. The resulting average *Lm* attachment was 2.95 log CFU/cm² (Table 3-4). Due to the low level of attachment that was achieved by the hand massage and 1:4.0 mL stick to *Lm* inoculum inoculation procedure, another procedure was evaluated for smoked sausage sticks in the fourth experiment.

Experiment 4

Smoked sausage sticks inoculation

For this preliminary experiment, the procedure for inoculation described in Experiment 3 was modified by adding a proportional amount of 10 mL *Lm* cocktail per stick onto the three sausage sticks instead of 4 mL. The sausage sticks were hung to dry for 1 h, and *Lm* was enumerated using MOX and dilutions 0-2 were spread plate in duplicate.

Results and Discussion

When 1:10.0 mL stick to *Lm* inoculum was used inoculate sausage sticks in the fourth experiment, an average of 4.30 log CFU/cm² *Lm* attachment resulted without affecting a_w of sausage sticks (Table 3-5).

Table 3-4. *Lm* attachment and a_w of pork and beef smoked sausage sticks before and after dip inoculation and hand massage procedures for preliminary experiments 2, 3 and 4

Inoculation procedure	Sample #	<i>Lm</i> attachment (log CFU/cm²)	a_w before inoculation	a_w after inoculation
Experiment 2: Dip inoculation procedure	1	2.05	0.83	0.82
	2	2.20	0.84	0.83
	3	2.10	0.83	0.83
Experiment 3: Hand massage and 1:4 mL stick to inoculum level	1	3.00	0.83	0.81
	2	2.89	0.83	0.84
	3	2.97	0.82	0.82
Experiment 4: Hand massage and 1:10 mL stick to inoculum level	1	4.12	0.82	0.82
	2	4.37	0.83	0.83
	3	4.42	0.83	0.84

Summary and Conclusions

Lm attachment achieved a minimum of 5 log CFU/cm² on turkey jerky using dip inoculation. Although there was a significant change in a_w before and after dip inoculation, the change was too small to have an impact on the properties of the product. Since the a_w value was kept below 0.80, this method was selected to use for turkey jerky validation research described in Chapter 5 with a few modifications were to the sample surface area. Based on the three preliminary inoculation experiments conducted on pork and beef smoked sausage sticks, the inoculation procedure using a proportion of 1:10.0 mL stick to *Lm* inoculum in combination with 2 min hand massaging followed by a 2 min holding time was selected for smoked sausage sticks validation research described in Chapter 4. This method achieved >4.0 log CFU/cm² *Lm* attachment.

CHAPTER 4 - Smoked Sausage Sticks

The presence of *Listeria monocytogenes* (*Lm*) in ready-to-eat (RTE) cooked meat is a food safety concern and it is strictly regulated in the U.S. In 2003, the U.S. Department of Agriculture (USDA) released a final rule addressing control of *Lm* in RTE products due to the severity of listeriosis and the ability of *Lm* to grow at refrigeration temperatures. In 2002, the Centers for Disease Control and Prevention (CDC) reported that there are about 2,500 cases annually from listeriosis, including 500 deaths and nearly around \$200 million in monetary loss in the U.S. (CDC 2002). Between 1990 and 1999, Levine and others 2001 reported *Lm* contamination in nine different product categories including jerky and small-diameter cooked sausages. They found jerky and small diameter cooked sausage to account for 0.52% and 3.56% *Lm* prevalence. The CDC (1999) reported that a multistate outbreak of *Lm* in 1998 and 1999 was attributed to the consumption of frankfurters and deli meats, resulting in 101 cases and 21 deaths. In addition, the CDC also reported several other multistate outbreaks of *Lm* including a 2002 outbreak in the Northeastern U.S. where 12.5 million kg of fresh and frozen RTE poultry products that were recalled and was attributed to consumption of sliceable turkey deli meat. There were 46 confirmed cases, 7 deaths, and 3 stillbirths or miscarriages associated with this outbreak. Zhu and others (2005) noted that *Lm* contamination of cured and non-cured RTE meat is a major safety concern because: (1) RTE meats have a long shelf-life and are consumed without further heating; (2) *Lm* can grow to a threatening level during refrigerated storage because of its ability to grow in the presence of curing salt at refrigerated temperatures (Lou and Yousef 1999), and (3) the emergence of multiple resistance in *Listeria* spp. due to acquisition of a replicon from staphylococci (Lemaitre and others 1998).

Control of *Lm* in RTE meat and poultry products is addressed in the USDA FSIS (2003) interim final rule that specifies three alternatives to control *Lm* in RTE meat and poultry products. Under alternative 1, an establishment must employ both a post-lethality treatment and an antimicrobial agent or process to control *Lm* on RTE products. Under alternative 2, an establishment must employ either an antimicrobial agent or process or a post-lethality treatment. Lastly, under alternative 3, an establishment relies only on sanitation to control *Lm*. The

objective of this study was to determine the effects of four packaging systems and four storage time on survival of *Lm* inoculated smoked sausage sticks.

Materials and Methods

Bacterial culture preparation

Lyophilized reference stock cultures of five *Lm* strains (Table 3-1) were obtained (Kwik-Stik, Microbiologics, Inc, Grenoble Cedex 2, France). A pure subculture of each strain was prepared by transferring a loopful of stock culture to 10 mL of pre-sterilized tryptic soy broth (TSB; Difco BD and Company, Sparks, MD) and incubated at 37°C for 24 h. One liter of the five-strain *Lm* cocktail was prepared aseptically by first transferring 0.5 mL of each pure culture isolate to 200 mL pre-sterilized TSB, and then incubating at 37°C for 24 h. Next, each 200 mL inoculum was transferred into a sterile 1 L beaker to obtain a liter of the five-strain cocktail. A total of 2.0 L of *Lm* cocktail was prepared. One liter was used for smoked sausage stick inoculation and the other liter was used for turkey jerky inoculation (discussed in Chapter 5).

Media preparation

TSB was prepared by adding 30 g of the media to 1 L of distilled water, mixed thoroughly, and then heated for 2 min. Then, TSB was transferred to a 1 L bottle and sterilized at 121 °C for 15 min.

Modified oxford agar (MOX; Difco BD and Company, Sparks, MD) was prepared by adding 57.5 g of the media to 1 L of distilled water, mixing, and then boiling for 1 min or until turbidity was lost. Next, MOX was transferred to 1 L bottle and sterilized at 121 °C for 15 min. Media was then cooled down at room temperature to about 45°C. Hydrolyzed Oxford antimicrobial supplement (10 mL vial) (Difco BD and Company, Sparks, MD) dissolved in 10 mL distilled water was immediately added into cooled media. This supplement makes the media selective for *Lm*. The supplemented media was then poured into disposable agar plates and allowed to set for at least 2 h at ambient temperature.

Peptone water (0.1%) was prepared by adding 1 g of Bacto™ Peptone (Difco BD and Company, Sparks, MD) to 1 L of distilled water, mixed thoroughly, and sterilized at 121 °C

for 15 min. For preparing peptone blanks for serial dilution, 9 mL of 0.1% peptone was dispensed into 15.0 mL screw-capped tubes and sterilized at 121 °C for 15 min.

Treatments, experimental design, and statistical analysis

For microbiological data, four packaging treatments were used including heat seal (HS), heat seal with oxygen scavenger (HSOS), nitrogen flush with oxygen scavenger (NFOS), and vacuum (VAC). All treatments were subjected to four different storage times that included 24, 48, and 72 h, and 30 d. The experimental design used was a completely randomized design (CRD) using a 4 x 4 factorial treatment with smoked sausage stick as the experimental unit. The model included the main effects of packaging treatments and storage times and the interaction of packaging treatments × storage times as fixed effects. Analysis of variance (ANOVA) was performed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Least square means were calculated for each independent variable. Statistical significance and tendencies were set at $P < 0.05$ level of significance. The experiment was done in triplicate. Two samples were assigned in each treatment and a total of 96 samples were used in all three replications.

To determine the effect of storage times (0, 24, 48, and 72 h, and 30 d) on a_w , pH, and moisture protein ratio (MPR) of sausage sticks packaged in HSOS, and on O_2 concentration of inoculated sausage sticks packaged in HS, HSOS, and NFOS, a one-way classification model using a one-way ANOVA was used. Analysis was performed using the PROC GLM procedure of SAS. Least square means were calculated for each independent variable. Statistical significance and tendencies were set at $P < 0.05$ level of significance.

Sample description and packaging material

Pork and beef smoked sausage sticks with beef collagen casing were commercially obtained and had a MPR of 0.89, pH of 5.1, and an a_w of 0.82. Samples were packaged in a nitrogen flushed O_2 impermeable packaging containing an oxygen absorber. O_2 concentration inside each package was < 0.1 %. Ingredients in the smoked sausage sticks were pork hearts, beef, pork fat, salt, dextrose, wheat flour, natural flavorings, paprika, potassium nitrate, lactic acid starter culture, sodium nitrite, sodium nitrate, BHA and treated with a solution of potassium sorbate to ensure freshness.

The packaging material used in this study was an O_2 impermeable re-sealable clear pouch 7.5 cm x 14.0 cm (5 mils thick) with zipper (TPG Co., LTD, Gyeonggi-do, Korea). This was cut

to a final dimension of 7.5 cm x 7 cm and O₂ absorbers (O₂-Zero BJ100; TPG Co., LTD, Gyeonggi-do, Korea) were used for HSOS and NFOS treatments. Reduction of the size of the packaging was done in order to at least simulate the actual ratio of the size of the packaging to the amount of the product that was received originally.

Sample inoculation and packaging

Prior to sample inoculation, a 1 mL portion of the *Lm* cocktail was transferred to a 9 mL 0.1 % peptone blank to determine the initial inoculum level. A total of 34 commercial smoked sausage sticks (Length=14.0 cm; diameter= 1.0 cm) for each replicate were inoculated with *Lm*. Smoked sausage sticks were aseptically transferred four at a time to a non-filtered stomacher bag (Fisherbrand, Ste-Julie, Quebec, Canada) with 40 mL of *Lm* cocktail. By using both hands, samples inside the bag were gently massaged for two min and then held for another two min. Next, samples were hung using sterile binder clips (Figure 4-1) to dry for 1 h. Surviving *Lm* was immediately enumerated on two samples to determine the *Lm* initial attachment to the sample. Samples were then assigned to one of the four packaging treatments. Samples were prepared in duplicate for each packaging treatment and holding time for a total of 32 samples. Samples assigned to NFOS were flushed for 10 sec using food grade 100% N₂ and a vacuum packer (Multivac C100, Gepufte Sicherheit, Germany). VAC samples were packaged using the vacuum packer with 600 mm Hg. HS and HSOS samples were packaged using an impulse sealer (Model H-1029; ULINE, Chicago, IL). An oxygen absorber was added to each sample packaged in NFOS or HSOS prior to heat sealing or N₂ flushing.

Water activity, package oxygen content, and pH measurement

A total of 36 smoked sausage sticks were used for a_w, pH, and moisture and protein content so MPR could be determined. This was done to check whether there were significant changes in chemical properties of the smoked sausage sticks throughout storage as a result of the dipping procedure. Since it was not possible to measure the chemical characteristics of inoculated samples, a simulation of the inoculation procedure was done by adding four smoked sausage sticks to a stomacher bag with 40.0 mL sterile TSB media. The a_w, pH, and moisture and protein content were measured initially before dipping, immediately after drying, and after 24, 48, and

72 h and 30 d of ambient temperature storage. After drying, non-inoculated TSB samples were packaged in HSOS.

The a_w of treatments was determined using an a_w meter (Aqualab CX2 series 3TE; Decagon, Pullman WA). The a_w meter was calibrated using a 0.760 NaCl (6.0 molal in water; Decagon, Pullman WA) verification standard and distilled water at 25.5°C. Smoked sausage sticks were chopped into small pieces, and evenly placed in a sample container. Duplicate readings were taken for each sample at 25.5°C.

Determination of pH was done using a pH meter (Acumet 925, Fischer Scientific, Kent City, Michigan). This was calibrated using a pH 4.0 and a pH 7.0 buffer solution (Fisher Scientific, Fair Lawn, NJ). The pH determination was done by preparing a slurry containing 10 g of smoked sausage sticks with 90 mL distilled water in a double-chamber filter bag (Fisherbrand, Ste-Julie, Quebec, Canada). The mixture was stomached for 1 min and then filtered using a Whatman grade no.1 filter paper (The Lab Depot, Inc., Dawsonville, GA). The filtrate pH was measured using the pH meter fitted with a flat-surface combination electrode (476550; Corning Incorporated, Corning, NY).

The moisture and protein content of each sample packaged in HSOS was determined following the SMART system 5 (CEM Corp., NC) procedure for moisture analysis (AOAC Official Methods 985.14, 977.11, 985.26 and 2008.6) and LECO FP-2000 Protein/Nitrogen Analyzer (model 602-600; LECO Corp., MI) procedure for nitrogen/protein content (AOAC Official Method 990.03). These values were used to calculate MPR for smoked sausage sticks.

The O₂ content of the packaged samples was measured after 0, 24, 48, 72 h and 30 d ambient temperature storage using an O₂ analyzer (Checkpoint-O₂; PBI Dansensor, DK-400 Kingsted, Denmark). Oxygen concentration in each package type except VAC was measured prior to *Lm* enumeration. This was done by piercing the package, 1.0 cm away from the zipper, with the needle O₂ detector of the equipment inserted at a 45° angle.

Enumeration of *Lm*

Surviving *Lm* populations in packaged samples were enumerated using spread plating on MOX. This was done by aseptically removing inoculated smoked sausage sticks from packages and transferring them to double-chamber stomacher bags. Next, 50 mL of 0.1% peptone diluent was added, and contents were stomached for 1 min (0 dilution). Three serial dilutions were

prepared by transferring a 1.0 mL aliquot of the stomached treatment to 9.0 mL of 0.1% peptone blanks. To determine the initial inoculum level, dilutions 0-3 were plated in duplicate on MOX. For treatments that were stored for 24, 48, and 72 h, dilutions 0-2 were plated, while dilutions 0 and 1 were plated in duplicate on MOX for treatments that were stored for 30 d.

Another set of plating was done to test for true zero. This was done in order to determine the actual level of *Lm* that was present on the first set of plates with 0 count. Counts observed on these plates were reported. Instead of plating 0.1 mL of sample on duplicate plates, an aliquot of 0.25 mL was plated on quadruplet plates. MOX plates were incubated at 35 ± 2 °C for 48 ± 2 h and counts were reported as log CFU/cm².

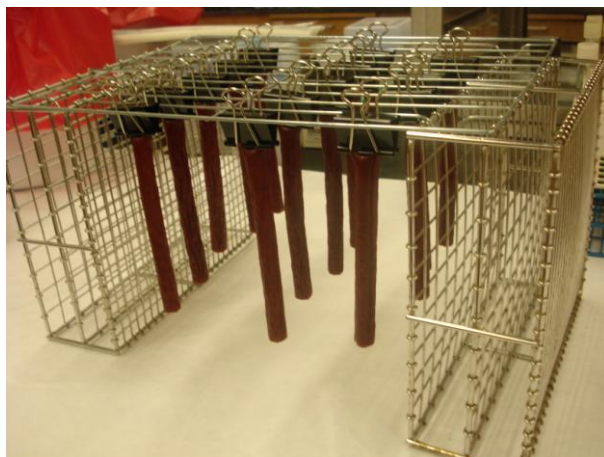


Figure 4-1. Drying arrangement for smoked sausage sticks after *Lm* inoculation.

Results and Discussion

The chemical analysis and package O₂ concentration for non-inoculated smoked sausage sticks dipped in sterile TSB media are shown in Table 4-1. The a_w remained constant (P>0.05) at 0.82 throughout storage, while the MPR and pH ranged from 0.86-0.94 and 4.99-5.10, respectively. There was a fluctuation that was observed in pH however, the change was too small to have an impact on the product. MPR decreased due to inoculation (P>0.05) after 72 h to 30 d ambient temperature storage. Immediately after packaging, HSOS, NFOS, and HS contained 19.0, 0.1, and 18.9% O₂, respectively. The O₂ concentration in NFOS and HS remained constant (P>0.05) throughout storage. On the other hand, the O₂ concentration in HSOS decreased

($P < 0.05$) dramatically from 19.0% to 0.1% O_2 after 24 h of ambient temperature storage and then remained constant ($P > 0.05$) up to 30 d. The addition of O_2 scavenger in HSOS was effective in reducing the O_2 concentration after 24 h of ambient temperature storage. The MPR of smoked sausage sticks remained constant from 0 to 24 h, and then decreased ($P < 0.05$) after 48 h to 30 d ambient temperature storage. Smoked sausage stick pH remained constant from 0 to 24 h ambient temperature storage, then increased ($P < 0.05$) at 48 to 72 h ambient temperature storage, followed by a decrease ($P < 0.05$) to its initial pH after 30 d of ambient temperature storage.

There was no interaction ($P > 0.05$) between packaging treatments and storage times indicating that packaging and storage times acted independently on *Lm* reduction on smoked sausage sticks. *Lm* log reductions were similar ($P > 0.05$) on smoked sausage sticks packaged in HS, VAC, and HSOS (Figure 4-2). NFOS had the lowest log reduction with less than 2 log CFU/cm² while HS, VAC, and HSOS had more than a 2 log CFU/cm² reduction. As a facultative anaerobic organism, *Lm* can grow in the presence and absence of O_2 . It utilizes O_2 for ATP production via aerobic respiration but can easily shift to fermentation when O_2 is absent. The high concentration of O_2 in HS did not enhance the survival of *Lm*. Even VAC or HSOS which provided anaerobic or low oxygen conditions, did not enhance *Lm* survival. These results do not support the findings of Thomas and others (2007) who found air and vacuum packaging allowed *Lm* proliferation in RTE shrimp. Buchanan and Klawitter (1990) found *Lm* grew at intermediate temperatures (19 and 28°C) under aerobic conditions at pH 4.5 in tryptose phosphate broth. On the other hand, *Lm* recovered and survived for extended periods at 37°C under anaerobic conditions. They found that O_2 restriction also enhanced *Lm* growth at 19°C. NFOS was less effective ($P < 0.05$) in reducing *Lm* on smoked sausage sticks. Francis and O'Beirne (2002) found that an atmosphere of 100% N_2 allowed survival of pure cultures of *Lm*, but populations did not significantly change ($P > 0.05$) during storage.

The mean *Lm* log reduction (CFU/cm²) on smoked sausage sticks following ambient temperature storage up to 30 d is shown in Figure 4-3. Increasing storage time from 24 to 72 h or 30 d significantly increased *Lm* reduction in smoked sausage sticks. A > 2.0 log CFU/cm² *Lm* log reduction was achieved after 24 h. After 30 d of ambient temperature storage, *Lm* had been reduced by 3.25 log CFU/cm², regardless of packaging system. Ingham and others (2004) reported a mean *Lm* log reduction of 1.4 log CFU/sample on vacuum packaged small beef snack

sticks that were stored for 5 wks at 5°C. They used a much cooler storage temperature than the ambient temperature used in this study.

The increasing mean *Lm* log reduction observed in smoked sausage sticks might be possibly attributed to metabolic exhaustion of the organism, and eventually autosterilization (Leistner 2000). Microbiologically stable foods that are produced by hurdle technology become safer during ambient storage (Leistner 2000) due to autosterilization. In this phenomenon, vegetative microorganisms that will not grow will die, and will die faster if the stability is close to threshold for growth, or if there are hurdles such as antimicrobials or elevated storage temperatures, causing microorganisms to become sublethally injured (Leistner 1995 a). In a shelf-stable meat product that is processed by hurdle technology, microorganisms use up their energy and die because of metabolic exhaustion resulting from exertion of every possible repair mechanism for their homeostasis to overcome an unfavorable environment (Leistner 1995 b).

Table 4-1. Chemical analyses of pork and beef smoked sausage sticks packaged in HSOS and package O₂ concentration of smoked sausage sticks packaged in four packaging environments and stored at ambient temperature

Time	a _w ¹	pH	MPR ²	Package O ₂ concentration (%)			
				HSOS ³	NFOS ⁴	HS ⁵	VAC ^{6,7}
0 h ^{**}	0.82 ^a	4.99 ^a	0.94 ^d	19.0 ^b	0.1 ^a	18.9 ^a	-
24 h	0.82 ^a	5.01 ^a	0.93 ^d	0.1 ^a	0.1 ^a	18.9 ^a	-
48 h	0.82 ^a	5.10 ^b	0.88 ^{bc}	0.1 ^a	0.1 ^a	19.0 ^a	-
72 h	0.82 ^a	5.10 ^b	0.86 ^a	0.1 ^a	0.1 ^a	18.8 ^a	-
30 d	0.82 ^a	5.00 ^a	0.87 ^{ab}	0.1 ^a	0.1 ^a	18.7 ^a	-

¹ Water activity

² Moisture protein ratio

³ Heat sealed with oxygen absorber

⁴ Nitrogen flushed with oxygen absorber

⁵ Heat sealed

⁶ Vacuum packaged

⁷ O₂ not measured

^{**} Initial a_w prior to packaging

^{a-d} Means within columns having different superscripts differ (P<0.05)

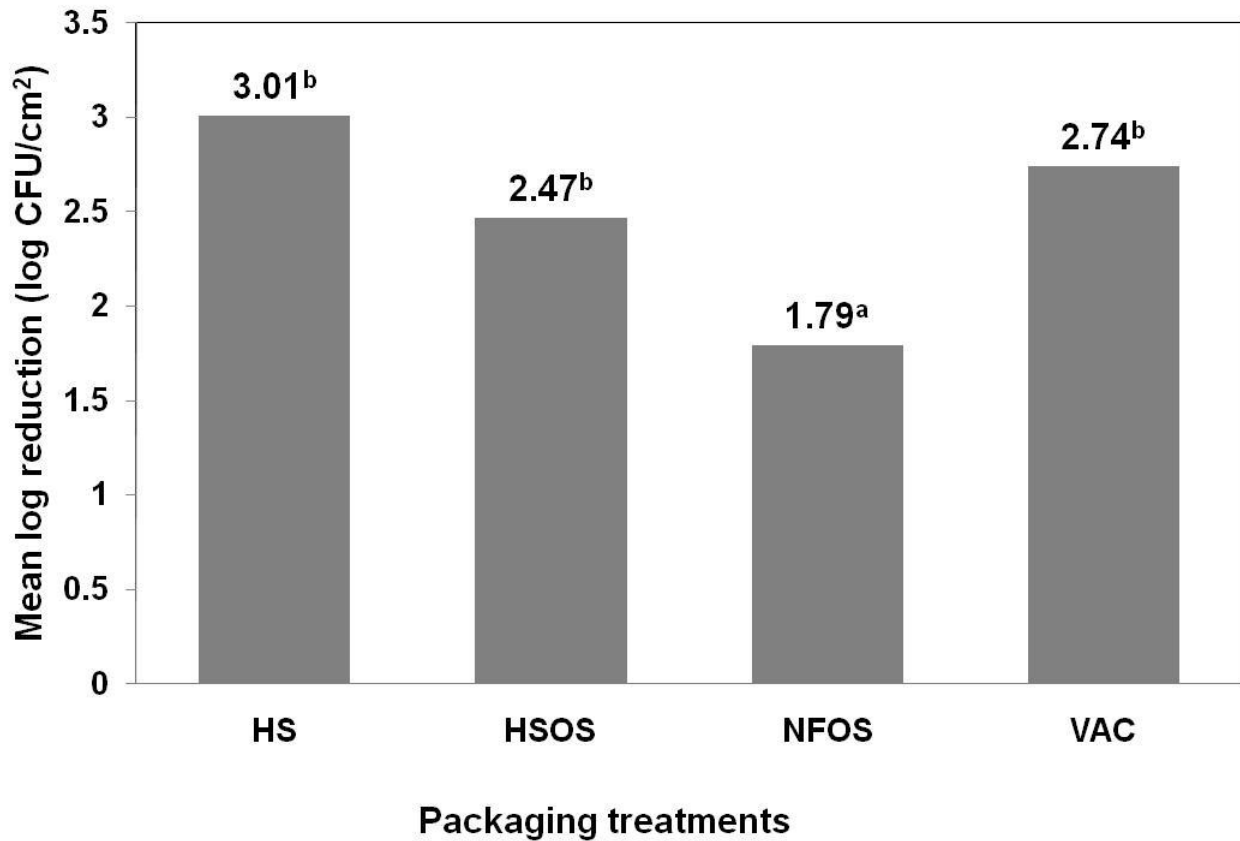


Figure 4-2. Mean *Lm* log reduction (log CFU/cm²) on smoked sausage sticks packaged in four packaging environments. HS: heat sealed; HSOS: heat sealed with oxygen scavenger; NFOS: nitrogen flushed with oxygen scavenger; VAC: vacuum packaged. ^{ab} Means having different superscripts differ (p<0.05).

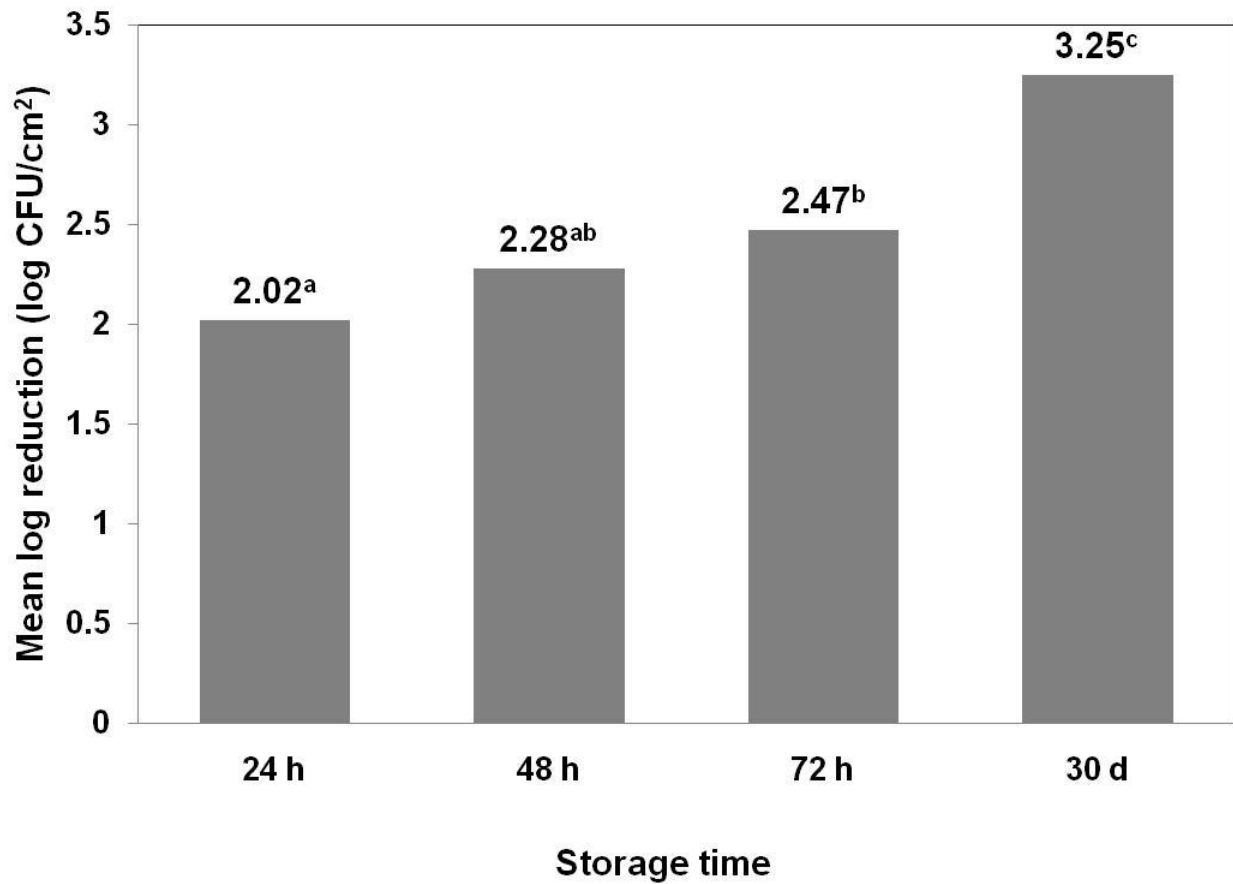


Figure 4-3. Mean *Lm* log reduction (log CFU/cm²) on smoked sausage sticks during ambient temperature storage up to 30 d. HS: heat sealed; HSOS: heat sealed with oxygen scavenger; NFOS: nitrogen flushed with oxygen scavenger; VAC: vacuum packaged. ^{ab} Means having different superscripts differ (p<0.05).

Summary and Conclusion

There was no interaction on packaging systems and storage time on *Lm* reduction on smoked sausage sticks. HS, HSOS, and VAC reduced *Lm* by >2.0 log CFU/cm², and >2.0 log CFU/cm² reduction in *Lm* was achieved after 24 h of ambient temperature storage, regardless of package type. NFOS was less effective by more than 0.5 log CFU/cm² than HS, HSOS or VAC in generating a *Lm* reduction. After 30 d of ambient storage, *Lm* had been reduced by 3.3 log CFU/cm² for all packaging environments.

Results of this study indicate that packaging smoked sausage sticks with HS, HSOS or VAC or by storing product at least 24 h at ambient temperature prior to shipping reduces potential *Lm* populations by at least 2 log CFU/cm² and could serve as post lethality treatment. USDA FSIS defines post-lethality treatment as a process that reduces *Lm* by at least 1.0 log. NFOS could serve as a post-lethality treatment, however, it would be subjected to more sampling from USDA FSIS.

CHAPTER 5 - Turkey Jerky

The 12 to 20% prevalence and up to 10^3 /g of *Listeria monocytogenes* (*Lm*) on turkey became a growing concern of the potential poultry related listeriosis outbreaks (Ryser 1991). *Lm* is a ubiquitous organism that can contaminate any type of foods, including ready-to-eat (RTE) products such as pate, deli meats, and frankfurters (Farber 1991; Mitchell 2000). Due to the severity of listeriosis in humans and the ability of *Lm* to grow at refrigeration temperatures, presence of *Lm* in RTE meat is a major safety concern. In response, in 2003, the U.S. Department of Agriculture (USDA) released a final rule in 2003 addressing control of *Lm* in RTE products. In 2002, the Centers for Disease Control and Prevention (CDC) reported that there were about 2500 cases annually from listeriosis, including 500 deaths and nearly around \$200 million in monetary loss in the U.S. (CDC 2002). Between 1990 and 1999, Levine and others (2001) reported the incidence of *Lm* contamination in nine different product categories including jerky and small-diameter cooked sausages. They found jerky and small diameter cooked sausage to account for 0.52% and 3.56% *Lm* prevalence. CDC (1999) reported that a multistate outbreak of *Lm* in 1998 and 1999 was attributed to the consumption of frankfurters and deli meats, resulting in 101 cases and 21 deaths. In addition, the CDC also reported several other multistate outbreaks of *Lm* including a 2002 outbreak in Northeastern U.S. where 12.5 million kg of fresh and frozen RTE poultry products were recalled and was attributed to consumption of sliceable turkey deli meat. There were 46 confirmed cases, 7 deaths, and 3 stillbirths or miscarriages associated with this outbreak. Zhu and others (2005) noted that *Lm* contamination of cured and non-cured RTE meat is a major safety concern because: (1) RTE meats have a long shelf-life and are consumed without further heating; (2) *Lm* can grow to a threatening level during refrigerated storage because of its ability to grow in the presence of curing salt at refrigerated temperatures (Lou and Yousef 1999), and (3) the emergence of multiple resistance in *Listeria* spp. due to acquisition of a replicon from staphylococci (Lemaitre and others 1998).

Control of *Lm* in RTE meat and poultry products is addressed in USDA FSIS (2003) interim final rule that specifies three alternatives to control *Lm* in RTE meat and poultry products. Published in June 6, 2003, the alternatives involve varying levels of control and

microbiological testing. Under alternative 1, an establishment must employ both a post-lethality treatment and an antimicrobial agent or process to control *Lm* on RTE products. Under alternative 2, an establishment must employ either an antimicrobial agent or process or a post-lethality treatment. Lastly, under alternative 3, an establishment relies only on sanitation to control *Lm*. The objective of this study was to determine the effects of four packaging systems and four storage times on survival of *Lm* inoculated whole muscle turkey jerky.

Materials and Methods

Bacterial culture preparation

Lyophilized reference stock cultures of five *Lm* strains (Table 3-1) were obtained (Kwik-Stik, Microbiologics, Inc., Grenoble Cedex, France). A pure subculture of each strain was prepared by transferring a loopful of stock culture to 10 mL of pre-sterilized tryptic soy broth (TSB; Difco BD and Company, Sparks, MD) and incubated at 37°C for 24 h. One liter of the five-strain *Lm* cocktail was prepared aseptically by first transferring 0.5 mL of each pure culture isolate to 200 mL pre-sterilized TSB, and then incubating at 37°C for 24 h. Next, each 200 mL inoculum was transferred into a sterile 1 L beaker to obtain a liter of the five-strain cocktail. A total of 2.0 L of *Lm* cocktail was prepared. One liter was used for smoked sausage stick inoculation (discussed in Chapter 4) and the other one liter was used for turkey jerky inoculation.

Media preparation

TSB was prepared by adding 30 g of the media to 1 L of distilled water, mixed thoroughly, and then heated for 2 min. Then, TSB was transferred to a 1 L bottle and sterilized at 121 °C for 15 min.

Modified oxford agar (MOX; Difco BD and Company, Sparks, MD) was prepared by adding 57.5 g of the media to 1 L of distilled water, mixing, and then boiling for 1 min or until turbidity was lost. Next, MOX was transferred to 1 L bottle and sterilized at 121 °C for 15 min. Media was then cooled down at room temperature to about 45°C. Hydrolyzed Oxford antimicrobial supplement (10 mL) (Difco BD and Company, Sparks, MD) dissolved in 10 mL distilled water was immediately added into cooled media. This supplement makes the media

selective for *Lm*. The supplemented media was then poured into disposable agar plates and allowed to set for at least 2 h at ambient temperature.

Peptone water (0.1%) was prepared by adding 1 g of Bacto™ Peptone (Difco BD and Company, Sparks, MD) to 1 L of distilled water, mixed thoroughly, and sterilized at 121 °C for 15 min. For preparing peptone blanks for serial dilution, 9 mL of 0.1% peptone was dispensed into 15.0 mL screw-capped tubes and sterilized at 121 °C for 15 min.

Treatments, experimental design, and statistical analysis

Four packaging treatments were used including heat seal (HS), heat seal with oxygen scavenger (HSOS), nitrogen flush with oxygen scavenger (NFOS), and vacuum (VAC). All treatments were subjected to four different storage times that included 24, 48, and 72 h, and 30 d. The experimental design used was a completely randomized design (CRD) using a 4 x 4 factorial treatment with turkey jerky as the experimental unit. The model included the main effects of packaging treatments and storage times and the interaction of packaging treatments × storage times as fixed effects. Analysis of variance (ANOVA) was performed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Least square means were calculated for each independent variable. Statistical significance and tendencies were set at $P < 0.05$ level of significance. The experiment was done in triplicate.

To determine the effect of storage times (0, 24, 48, and 72 h, and 30 d) on a_w , pH, and moisture protein ratio (MPR) of non-inoculated turkey jerky pieces packaged in HSOS, and on O_2 concentration of inoculated turkey jerky pieces packaged in HS, HSOS, and NFOS, a one-way classification model using a one-way ANOVA was used. Analysis was performed using the PROC GLM procedure of SAS. Least square means were calculated for each independent variable. Statistical significance and tendencies were set at $P < 0.05$ level of significance.

Sample description

Turkey jerky samples were commercially obtained and had an MPR of 0.66, pH of 5.5, and an a_w of 0.701. The product contained turkey breast, soy sauce, salt, brown sugar, corn syrup, sugar, pineapple juice concentrate, vinegar, molasses, caramel color, high fructose corn syrup and citric acid, hydrolyzed soy and corn protein water, anchovies, onions, tamarind, garlic, cloves, chili peppers, natural flavorings and shallots, pineapple juice, and natural hickory smoke flavor. The turkey jerky was packaged in a nitrogen flushed O_2 impermeable re-sealable

5 mil thick clear pouch, 7.5 cm x 14.0 cm in size with zipper and containing an oxygen absorber. The O₂ concentration inside each package was 0.1 %.

The packaging material used in this study was an O₂ impermeable re-sealable clear pouch 7.5 cm x 14.0 cm x 5 mil thick with zipper (TPG Co., LTD, Gyeonggi-do, Korea). This was cut to a final dimension of 7.5 cm x 7 cm and O₂ absorbers (O₂-Zero BJ100; TPG Co., LTD, Gyeonggi-do, Korea) were used for HSOS and NFOS treatments. Reduction of the size of the packaging was done in order to at least simulate the actual ratio of the size of the packaging to the amount of the product that was received originally.

Sample inoculation and packaging

Prior to sample inoculation, 1 mL portion of the *Lm* cocktail was transferred to 9 mL 0.1 % peptone blank to determine the initial inoculum level. A total of 34 commercial turkey pieces for each replicate, measuring 4.0 cm X 4.0 cm, were obtained aseptically and were dipped two at a time in 1 L *Lm* cocktail for 1 min. Samples were immediately hung using sterile binder clips (Figure 5-1) and dried for 1-2 h or until the a_w was below 0.80 . Surviving *Lm* was immediately enumerated on two samples to determine the *Lm* initial attachment to the sample. Samples were then assigned to one of the four packaging treatments. Samples were prepared in duplicate for each packaging treatment and holding time for a total of 32 samples. Samples assigned to NFOS were flushed for 10 sec using food grade 100% N₂ and a vacuum packer (Multivac C100, Gepufte Sicherheit, Germany). VAC samples were packaged using the vacuum packer with 600 mm Hg. HS and HSOS samples were packaged using an impulse sealer (Model H-1029; ULINE, Chicago, IL). An oxygen absorber was added to each sample packaged in NFOS or HSOS prior to heat sealing or N₂ flushing.

Water activity, package oxygen content, and pH measurement

A total of 36 turkey jerky pieces for each replicate, measuring 4.0 cm x 4.0 cm, were used for determination of a_w, pH and moisture and protein content. This was done to check whether there were significant changes in chemical properties of the jerky pieces throughout storage as a result of the dipping procedure. Since it was not possible to measure the chemical characteristics of inoculated samples, a simulation of the inoculation procedure was done by dipping turkey jerky pieces into sterile TSB media without the *Lm* inoculum. The a_w, pH and

moisture and protein content were measured initially before dipping and immediately after drying, and after 24, 48, and 72 h and 30 d of ambient temperature storage. After drying, the non-inoculated TSB dipped jerky pieces were packaged in HSOS.

The a_w of treatments was determined using an a_w meter (AQUALAB CX2 series 3TE; Decagon, Pullman WA). The a_w meter was calibrated using a 0.760 NaCl (6.0 molal in water; Decagon, Pullman WA) verification standard and distilled water at 25.5°C. Turkey jerky pieces were cut into hexagonal shape with a diameter of approximately 3.2 cm and were placed in sample container. Duplicate readings were taken for each sample at 25.5°C.

Determination of pH was done using a pH meter (Acumet 925, Fischer Scientific, Kent City, MI). This was calibrated using a pH 4.0 and a pH 7.0 buffer solution (Fisher Scientific, Fair Lawn, NJ). The pH determination was done by preparing a slurry containing 10 g of turkey jerky with 90 mL distilled water in a double-chamber filter bag (Fisherbrand, Ste-Julie, Quebec, Canada). The mixture was stomached for 1 min and then filtered using a Whatman grade no.1 filter paper (The Lab Depot, Inc., Dawsonville, GA). The filtrate pH was measured using the pH meter fitted with a flat-surface combination electrode (476550; Corning Incorporated, Corning, NY).

The moisture and protein content of each sample packaged in HSOS was determined following the SMART system 5 (CEM Corp., NC) procedure for moisture analysis (AOAC Official Methods 985.14, 977.11, 985.26 and 2008.6) and LECO FP-2000 Protein/Nitrogen Analyzer (model 602-600; LECO Corp., MI) procedure for nitrogen/protein content (AOAC Official Method 990.03). These values were used to calculate MPR for turkey jerky.

The O₂ content of the packaged samples was measured after 0, 24, 48, 72 h and 30 d ambient temperature storage using an O₂ analyzer (Checkpoint-O₂; PBI Dansensor, DK-400 Kingsted, Denmark). O₂ concentration in each package type except VAC was measured prior to *Lm* enumeration. This was done by piercing the package, 1.0 cm away from the zipper, with the needle O₂ detector of the equipment inserted at a 45° angle.

Enumeration of *Lm*

Surviving *Lm* populations in packaged samples were enumerated using spread plating on MOX plates. This was done by aseptically removing inoculated turkey jerky samples from packages and transferring them to double-chamber stomacher bags. Next, 50 mL of 0.1%

peptone diluent was added, and contents were stomached for 1 min (0 dilution). Three serial dilutions were prepared by transferring a 1.0 mL aliquot of the stomached treatment to 9.0 mL of 0.1% peptone blanks. To determine the initial inoculum level, dilutions 0-3 were plated in duplicate on MOX. For treatments that were stored for 24, 48, and 72 h, dilutions 0-2 were plated, while dilutions 0 and 1 were plated in duplicate on MOX for treatments that were stored for 30 d. MOX plates were incubated at 35 ± 2 °C for 48 ± 2 h and counts were reported as log CFU/cm².

Another set of plating was done to test for true zero. This was done in order to determine the actual level of *Lm* that is present on the first set of plates with 0 counts. Counts observed on these plates were reported. Instead of plating 0.1 mL of sample on duplicate plates, an aliquot of 0.25 mL was plated on quadruplet plates. MOX plates were incubated at 35 ± 2 °C for 48 ± 2 h and counts were reported as log CFU/cm².



Figure 5-1. Drying arrangement for turkey jerky samples after *Lm* inoculation.

Results and Discussion

The chemical analysis and package O₂ concentration for non-inoculated turkey jerky dipped in sterile TSB media are shown in Table 5-1. The a_w remained constant (P>0.05) at 0.73-0.74 throughout storage, while the MPR and pH ranged from 0.66-0.76 and 5.55-5.58, respectively. Immediately after packaging, HSOS, NFOS, and HS contained 18.9, 0.1, and 18.8% O₂, respectively. The O₂ concentration in NFOS and HS remained constant (P>0.05) throughout storage. On the other hand, the O₂ concentration in HSOS decreased (P<0.05) dramatically from 18.9% to 0.1% O₂ after 24 h of ambient temperature storage and then

remained constant ($P>0.05$) up to 30 d. The addition of oxygen scavenger in HSOS was effective in decreasing the O_2 concentration. The MPR of turkey jerky decreased ($P<0.05$) from 0 to 24 h, and then remained constant ($P>0.05$) throughout ambient temperature storage. Turkey jerky pH fluctuated slightly during storage.

Lm reduction in turkey jerky was affected by the interaction ($P<0.05$) of packaging treatment and storage time. Figure 5-2 shows the mean log reduction ($\log CFU/cm^2$) of *Lm* on turkey jerky packaged in the four packaging environments and held at ambient temperature up to 30 d. Regardless of packaging treatment, the mean *Lm* log reduction was $<1.0 \log CFU/cm^2$ in turkey jerky after 24, 48, or 72 h of storage. However, after 30 d, the mean *Lm* log reduction ranged from 1.38 to 3.64 $\log CFU/cm^2$ in turkey jerky. The most effective combination ($P<0.05$) in reducing *Lm* was packaging turkey jerky in HS and storing it for 30 d at ambient temperature. It has been reported that vacuum packaging resulted in slower destruction of *Lm* inoculated on sliced salami than air packaging (Gounadaki and others 2007). Williams and Golden (2001) reported that aerobic conditions resulted in higher destruction of *Lm* in a simulated salami matrix than under vacuum at 20°C. The mean *Lm* log reduction achieved by VAC and 30 d storage was similar to research reported by Ingham and others (2004), where a mean *Lm* log reduction of 2.7 $\log CFU/sample$ on beef jerky packaged in vacuum and stored for 5 weeks at 21°C was observed. NFOS was the least effective ($P<0.05$) packaging method to reduce *Lm* after 30 d. This supports the findings of Francis and O'Beirne (2002), where an atmosphere of 100% N_2 allowed survival of pure cultures of *Lm*, and populations did not significantly change during storage.

Bacterial cells maintain intracellular osmotic pressure which is greater than that of the suspending medium in order to generate cell turgor pressure (Collin and others 2002). Turgor pressure is considered to be the driving force for cell extension, growth and division (Brown 1976; Taiz 1984; Csonka 1989). In addition, it is essential for growth and survival of bacterial cells to adapt to changes in the osmolarity of the external environment, such as generating a number of osmoadaptive mechanisms to overcome fluctuations (Collin and others 2002). Galinski and others (1995) noted that osmoadaptation describes both the physiological and genetic manifestations to a low a_w environment. The a_w of turkey jerky may have contributed to the slow reduction in *Lm* in this study.

Very little data has been published discussing how different packaging atmospheres affect survival of *Lm* in RTE meat. The mechanism for *Lm* reduction under these conditions is not fully understood and additional research is needed.

Table 5-1. Chemical analyses of turkey jerky packaged in HSOS and package O₂ concentration of turkey jerky packaged in four packaging environments and stored at ambient temperature

Time	Package O ₂ concentration (%)						
	a _w ¹	pH	MPR ²	HSOS ³	NFOS ⁴	HS ⁵	VAC ^{6,7}
0 h ^{**}	0.73 ^a	5.58 ^b	0.76 ^b	18.9 ^b	0.1 ^a	18.8 ^a	-
24 h	0.73 ^a	5.55 ^a	0.66 ^a	0.1 ^a	0.1 ^a	18.9 ^a	-
48 h	0.73 ^a	5.57 ^{ab}	0.69 ^a	0.1 ^a	0.1 ^a	18.8 ^a	-
72 h	0.74 ^a	5.58 ^b	0.68 ^a	0.1 ^a	0.1 ^a	18.9 ^a	-
30 d	0.74 ^a	5.57 ^{ab}	0.70 ^a	0.1 ^a	0.1 ^a	18.8 ^a	-

¹ Water activity

² Moisture protein ratio

³ Heat sealed with oxygen absorber

⁴ Nitrogen flushed with oxygen absorber

⁵ Heat sealed

⁶ Vacuum packaged

⁷ O₂ not measured

^{**} Initial a_w prior to packaging

^{a-b} Means within columns having different superscripts differ (P<0.05)

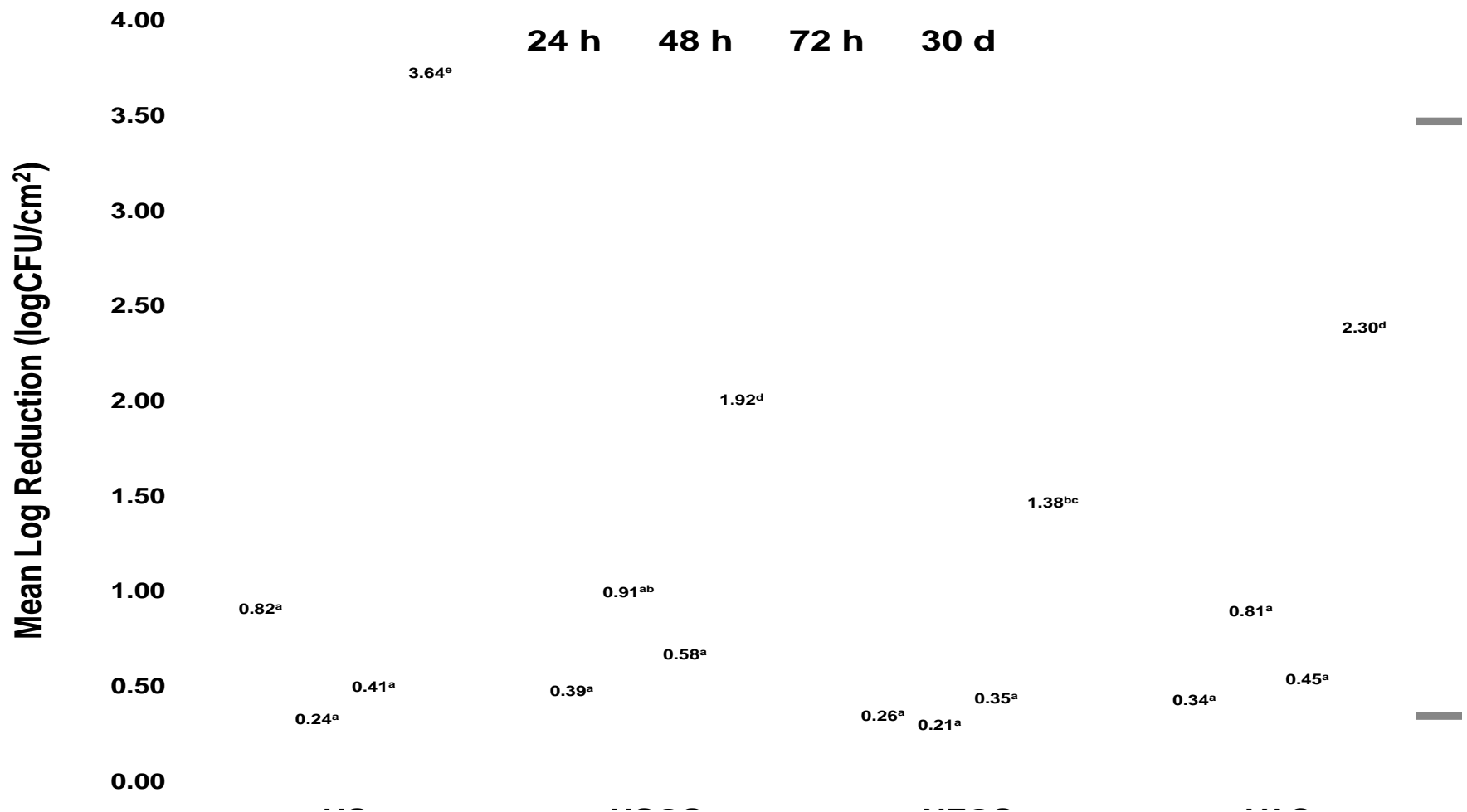


Figure 5-2. Mean *Lm* log reduction (log CFU/cm²) on turkey jerky packaged in four packaging environments and stored at ambient temperature for up to 30 d. HS: heat sealed; HSOS: heat sealed with oxygen scavenger; NFOS: nitrogen flushed with oxygen scavenger; VAC: vacuum packaged. ^{abcde} Means having different superscripts differ within and across packaging types (p<0.05).

Summary and Conclusion

Lm reduction in turkey jerky was affected by the interaction of packaging treatments and storage times. Results of this study indicate that HS, HSOS, NFOS, or VAC in combination with 24, 48, or 72 h ambient temperature storage could not be used as post-lethality treatment for turkey jerky. Processors could package turkey jerky in HS, HSOS, NFOS or VAC and store for 30 d at ambient temperature as a post-lethality treatment since all these packaging strategies reduced *Lm* by >1.0 mean log CFU/cm². Alternatively, processors could package turkey jerky in HS, HSOS, NFOS, or VAC in combination with 24 to 72 ambient storage period to be an antimicrobial process. USDA FSIS defines an antimicrobial process as a process that allows no more than 2 log increase in *Lm* population.

There is little published research describing how different packaging atmospheres affect survival of *Lm* in RTE meat. The mechanism of packaging environment as additional hurdle for *Lm* is not fully understood. Metabolic characteristics of *Lm* under aerobic and anaerobic or low O₂ environments must be investigated further in order to understand how *Lm* survives so effective intervention strategies can be developed.

Overall Summary and Conclusion

The USDA FSIS interim final rule addressing the control of *Lm* on RTE meat and poultry products mandated processors implement one of the three alternatives to control *Lm* in their products. Under alternative 1, an establishment can use both a post-lethality treatment and an antimicrobial agent or process to control *Lm* on RTE products. Under alternative 2, an establishment can use either an antimicrobial agent or process or a post-lethality treatment. Lastly, under alternative 3, an establishment can use sanitation measures to control *Lm*. A potential intervention strategy evaluating packaging and storage times to control *Lm* on whole muscle turkey jerky and pork and beef smoked sausage sticks was investigated. In order for a process can be considered a post-lethality treatment, it be validated and show that it can reduce *Lm* by at least 1 log.

The effect of packaging and ambient temperature storage time on *Lm* reduction in pork and beef smoked sausage sticks were independent of each other. Packaging smoked sausage sticks in HS, HSOS or VAC or by storing product at least 24 h at ambient temperature prior to shipping reduced potential *Lm* populations by at least 2 log CFU/cm² and could serve as post lethality treatment. NFOS achieved >1 log CFU/cm² *Lm* reduction and could also serve as a post-lethality treatment, however, it would be subject to more sampling from USDA FSIS.

Packaging turkey jerky with HS, HSOS, NFOS, or VAC for 24 to 72 h did not reduce *Lm* by at least 1 log CFU/cm², hence could not serve as post-lethality treatment. It was only after 30 d of ambient temperature storage that an *Lm* log reduction of >1.0 log CFU/cm² was achieved for all packaging systems. Thus, processors can use all of these packaging systems in combination with 30 d ambient temperature storage as a post-lethality treatment. Alternatively, processors could package turkey jerky in HS, HSOS, NFOS, or VAC in combination with 24 to 72 h ambient storage period to be an antimicrobial process. USDA FSIS defines antimicrobial process as a process that allows no more than 2 log increase in *Lm* population.

There is little published research describing how packaging atmosphere affects *Lm* survival in RTE products. Metabolic characteristics of *Lm* in the presence of aerobic and anaerobic or low O₂ environments must be investigated further in order to understand how *Lm* survives so effective intervention strategies can be developed. In addition, physiological

characteristics of *Lm* in the presence of different ingredients and physical properties of meat and poultry products must be investigated.

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