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Use of Silver Zeolite As An Antimicrobial Agent
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USE OF SILVER ZEOLITE AS AN ANTIMICROBIAL AGENT IN PACKAGING FILM

By

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ABSTRACT

USE OF SILVER ZEOLITE AS AN ANTIMICROBIAL AGENT IN PACKAGING FILM

By

Renae F. McKinley

Antimicrobial properties of a commercially processed polyethylene film coated with AgION™ silver zeolite were tested for its ability to inhibit growth of four strains of *Listeria monocytogenes* (CWD 95 and 246 from silage, CWD 201 from raw milk, and CWD 1503 from ground turkey). The surface of the films was inoculated with *Listeria monocytogenes*, and was inhibited 1.34 logs, respectively, on trypticase soy agar (TSA) containing 0.6%yeast extract (TSA-YE) after 24 hours of storage at 37°C.

The antimicrobial properties of the film were also tested over 48 hours of storage at 4°C and 12°C, on beef bologna packaged in film inoculated with *Listeria monocytogenes* at a level of 10^6 CFU/cm^2. The bologna was examined at specified intervals for numbers of *Listeria monocytogenes*, mesophilic aerobic bacteria (MAB), and lactic acid bacteria (LAB). During 48 hours of storage, *Listeria* populations decreased 0.39 and 0.05 logs, at 4°C and 12°C, respectively. At both temperatures, MAB populations decreased 0.07 logs at 4°C and 0.08 logs at 12°C using antimicrobial film compared to antimicrobial-free control film. At 4°C LAB populations were unchanged at 6.91 logs, while at 12°C the antimicrobial film decreased LAB populations 0.25 logs compared to antimicrobial-free film.

To my family, B.C., and Keisha

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ABBREVIATIONS

ANOVA	Analysis of variance
ASTM	American Society for Testing and Materials
CDC	Center for Disease Control
CFR	Code of Federal Regulations
CFU	Colony forming units
FDA	Food and Drug Administration
FSIS	Food Safety Inspection Service
GRAS	Generally Recognized As Safe
LAB	Lactic acid bacteria
MAB	Mesophilic aerobic bacteria
MOX	Modified Oxford Agar
PBS	Butterfield's phosphate buffer solution
PVDC	Polyvinylidene chloride
SAS	Statistical Analysis System
RPM	Rotations per minute
TSA	Triptycase soy agar
TSA-YE	Triptycase soy agar + yeast extract
TSB	Triptycase soy broth
TSB-YE	Triptycase soy broth + yeast extract
USDA	United States Department of Agriculture
UVM	University of Vermont Broth

INTRODUCTION

With the growing demand for convenience food products, today's consumers are demanding food products with fewer or no preservatives. While meeting the demands of food consumers is important, an even greater concern in the food industry is post-processing food contamination of ready-to-eat food products from food-borne microorganisms. The processed meat industry is constantly threatened by *Listeria monocytogenes*. Outbreaks of listeriosis (which is caused by *Listeria monocytogenes*) are major contributors to Class I microbial related food recalls. There have been several cases of listeriosis from consumption of processed meat that have resulted in fatalities. The Food and Drug Administration (FDA) estimates that *Listeria monocytogenes* causes estimated 2,500 cases of listeriosis and 500 deaths each year in the United States (FDA, 2001). In December 2000, an outbreak involving 29 cases in 10 states prompted the recall of approximately 14.5 million pounds of turkey and chicken deli meat due to probable *Listeria monocytogenes* contamination, which resulted in 7 fatalities (CDC, 2000).

In an effort to address this growing concern, there has been enhanced interest during the past few years in the development of films containing antimicrobial additives. Siragusa, Cutter, and Willett incorporated bacteriocin in polyethylene to inhibit bacterial growth on beef carcass surface tissue sections (Siragusa et al, 1999). Devlieghere et al (2000) used hexamethylenetetramine incorporated in low-density polyethylene to extend the shelf life of cooked ham. Labuza and Breene (1989) define antimicrobial film as a type of active packaging in which undesirable microorganisms in food are eliminated due to incorporation of antimicrobial additives into or coating of them onto food packaging materials. Because of the ability of such films to inhibit undesirable microorganisms,

their availability for direct food contact use is increasing. These films also have the ability to potentially increase shelf life of food products.

Colorcon's No-Tox Products Division produces specialty silver zeolite antimicrobial inks and coatings for pharmaceutical, medical, and food packaging industries. The silver zeolite coatings, called AgION™, are compliant with Food and Drug Administration (FDA) regulations for direct food contact applications for silver. AgION™ has been found to inhibit growth and migration of bacteria, yeast, fungus and mold, while maintaining package performance (Podhajny, 2002). While the use of AgION™ has proven beneficial in inhibiting surface contamination, there has been no published information regarding its effectiveness when in direct contact with food products and at differing storage temperatures.

The hypothesis of this study was that polyethylene film coated with AgION™ will inhibit the growth of *Listeria monocytogenes* both on the surface of the film and when in direct contact with sliced beef bologna stored at 4°C and 12°C. This study builds upon American Society for Testing and Materials (ASTM) E2180-01 and observations made by Colorcon. The objectives of this work are (1) to examine the effectiveness of polyethylene film coated with AgION™ to inhibit the growth of four strains of *Listeria monocytogenes* (CWD 95 and 246 from silage, CWD 201 from raw milk, and CWD 1503 from ground turkey), and (2) to assess the coated film's ability to retain its antimicrobial properties while in direct contact with beef bologna slices stored at 4°C and 12°C.

CHAPTER 1

LITERATURE REVIEW

1.1. Antimicrobial Film

Antimicrobial film is a new and effective type of active packaging that inhibits undesired growth of microorganisms in food products. These films also have the ability to potentially increase product shelf life and enhance food safety. The use of antimicrobial additives is necessary in order for an antimicrobial film to be effective. Antimicrobial additives are materials used in various forms of packaging to reduce or prevent microbial growth (Han, 2000).

There are a variety of compounds that have been proposed as potential antimicrobial additives. Some of the common antimicrobial additives used to preserve food products include bacteriocins, organic acids and organic acid salts, parabens, natural antimicrobials, and curing agents. Whichever of these antimicrobial additives is used for food packaging, safety must be ensured. Typically, all antimicrobial agents used in film are approved by the Food and Drug Administration (FDA) in accordance with the Code of Federal Regulations (CFR) (Brody et al, 2001).

1.1.1. Antimicrobial Packaging Films

Antimicrobial films may be categorized as antimicrobial packaging films or antimicrobial edible films. The antimicrobial additives used for both types of antimicrobial films can be developed in two ways. The first is directly incorporating the antimicrobial additive(s) into the film through extrusion or extrusion coating. The second is to the coat the surface of the film with the antimicrobial additive(s). While each of these methods have advantages and disadvantages, both categories have proven effective

in killing target organisms (Ming et al, 1997;Siragusa et al, 1999; Devlieghere et al, 2000).

Antimicrobial packaging films are non-edible films that serve as protection for a food product. Through the development of antimicrobial polyvinylidene chloride (PVDC) coploymer packaging films containing nisin, potassium sorbate, and sorbic acid, Limjaroen (2002) was able to inhibit the growth of four strains of *Listeria monocytogenes* using a diffusion assay. Limjaroen also determined that PVDC copolymer film containing 1.5% and 3.0% (w/v) sorbic acid decreased populations of *L. monocytogenes* 4.0-7.0 logs on beef bologna slices after 28 days of refrigerated storage at 4°C.

1.1.2. Antimicrobial Edible Films

Antimicrobial edible films can be prepared from proteins, polysaccharides, and/or proteins and lipids. They can be used in a variety of food packaging applications such as casings for hot dogs and sausages. Cagri et al (2002) determined that whey protein isolate edible films containing sorbic acid or p-aminobenzoic acid inhibited growth of *Listeria monocytogenes, Escherichia coli* 0157:H7, and *Salmonella typhimurium* DT104 on trypticase soy agar (TSA). Cagri also determined that 0.5% to 1.0% sorbic acid or p-aminobenzoic acid was effective in decreasing populations of *Listeria monocytogenes, Escherichia coli* 0157:H7, and *Salmonella typhimurium* DT104 on beef bologna slices after 21 days storage at 4°C by 3.4-4.1 logs, 3.1-3.6 logs, and 3.1-4.1 logs, respectively.

1.2. Antimicrobial Additives

In order for an antimicrobial film to be effective, an antimicrobial additive can be incorporated into or coated onto a packaging material. A variety of antimicrobial additives can be utilized to extend product shelf life. Some food components are naturally

present or added during food formulation and processing and contain antimicrobial activity, thus contributing to food preservation. Of these antimicrobial additives, some are applied mainly to control microflora, while others have dual or multiple functions (Lou and Yousef, 1999). Some of the common antimicrobial additives used to preserve food products include organic acids such as benzoic, sorbic, and propionic acid; bacteriocins such as nisin; parabens; natural antimicrobials such as lactoferrin; curing agents such as sodium chloride; and metals such as silver ions. Each will be discussed individually in terms of their possible use as antimicrobial additives in antimicrobial packaging films.

1.2.1. Organic Acids

Most organic acids permitted in food are typically applied as acidulants (e.g. acetic and lactic acids), while their salt forms are used as preservatives (e.g. potassium sorbate and sodium benzoate). The effectiveness of these organic acids as antimicrobial agents is related to the amount of the undissociated form of the organic acid, which is related to pH of the medium and pKa of the acid (Lou and Yousef, 1999). Undissociated organic acids can pass through the cell membrane, dissociate inside the cytoplasm and interfere with the metabolic processes in the microbial cell. The antimicrobial activity of these acids can be attributed to cytoplasm acidification, and the antimicrobial effect of the particular anionic species (Lou and Yousef, 1999).

1.2.1.1. Benzoic acid or sodium benzoate

In 1875, H. Fleck was the first to describe the preservative action of benzoic acid (Cagri, 2002). Sodium benzoate is the first chemical preservative permitted in food by the FDA. Both benzoic acid and sodium benzoate are Generally Recognized As Safe (GRAS) preservatives. Benzoic acid acts essentially as a mold and yeast inhibitor, but

studies have shown that it can also inhibit *Listeria monocytogenes* using tryptose broth after 60 days storage (El-Shenawy and Marth, 1988).

The antimicrobial activity of benzoate is related to pH. It is most effective in its undissociated form, with 60% of the compound undissociated at pH 4.0. Benzoic acid reduces the intracellular pH and also induces change in the morphology and appearance of microbial cells. Benzoic acid can also alter cell membrane function by producing pores that interfere with substrate uptake and electron transport.

Sodium benzoate in combination with organic acids has proven effective in decreasing the microbial population of raw chicken. Hwang and Bechaut (1995) showed that raw chicken inoculated with *Listeria monocytogenes, Salmonella, Campylobacter jejuni, Staphylococcus aureus*, and *Escherichia coli* O157:H7 decreased in population by 1.0 log when immersed in a solution of 0.05% sodium benzoate/0.5% lactic acid (pH 2.64) for 30 minutes.

1.2.1.2. Sorbic acid and sorbates

Sorbic acid is a straight chain α, β-unsaturated monocarboxylic acid (Sofos, 1989), and is classified as a GRAS preservative. The typical usage level is 0.02% to 0.3% in commercial foods. These concentrations are added to bakery, fruit, dairy, and meat products to inhibit yeasts, bacteria, and molds (Lueck 1980; Sofos and Busta, 1993; Kasrazadeh and Genigeorgis, 1995). Potassium sorbate is a commonly used salt of sorbic acid, and is highly soluble in water.

Sorbic acid induces changes in the morphology and appearance of microbial cells. Its antimicrobial activity is generally enhanced at low temperatures. El-Shenawy and Marth (1988) showed that *Y. enterocolitica, Pseudomonas putida, L. monocytogenes*, and

A. hydrophila were more sensitive to potassium sorbate at refrigerated temperatures than at higher temperatures.

1.2.2. Bacteriocins

Bacteriocins are antimicrobial substances that encompass a peptide or protein component that is essential for their activity. With the exception of nisin, most bacteriocins have a narrow spectrum of inhibition, and only inhibit closely related species. Nisin exhibits a broad spectrum of inhibition against gram-negative bacteria.

1.2.2.1. Nisin

Nisin, a polypeptide that is synthesized by *Lactococcus lactis subspecies lactis*, is one of the most widely studied bacteriocins. In 1998, the FDA approved nisin for pasteurized processed cheese with no more than 250 parts per million (ppm) nisin in the finished product (Lou and Yousef, 1999). Being a natural additive and having the status of GRAS contributes to its wide use (Bower et al, 1995). Composed of 34 amino acids (Jung, 1991), nisin possess amphiphilic characteristics, with clusters of hydrophobic and hydrophilic residues at the N- and C-terminus. At the N- and C-terminus Cross and Morell (1971) identified didehydroalanyllysine and isoleucine. The highly reactive double bonds and thioether cross-linkages are suggested to be responsible for important properties of the nisin molecule, such as thermostability, acid tolerance, and bactericidal.

The most significant application of nisin may be in the inhibition of gram-positive bacteria, such as *Listeria monocytogenes* and *Clostridium spp.* (Ming et al, 1997, Bower et al, 1995, Padgett et al, 1998). However, when combined with chelating agents, it has been suggested that nisin may be effective against gram-negative bacteria (Wells et al, 1998; Boziaris and Adams, 1999).

Liu and Hansen (1990) determined that the biological activity of nisin decreased at high pH values. The stability, solubility, and biological stability are pH dependent, and nisin becomes insoluble at alkaline and neutral conditions.

Nisin has been effective in cheese products such as cheese spreads and cold-pack cheese (Delves-Broughton, 1990). It is effective against bacteria in meat and meat products. Bell and Dlacy (1986) reported that nisin inhibited growth of *Bacillus licheniformis* in fermented and cured meat products.

1.2.3. Parabens

Parabens are esters of p-hydroxybenzoic acids, which are made by esterification of the carboxyl group of benzoic acid. Due to their ability to remain undissociated at pH values of up to 8.5, most parabens are active at pH values of 3.0 to 8.0. Of these esters, methyl, propyl, and heptyl parabens are approved in some countries for direct food use (Lou and Yousef, 1999). While parabens can be effective in a wide range of foods, they are mostly used in butter, margarine, meat products, and maple syrup (Limjaroen, 2002).

Parabens are more effective in the inhibition of gram-positive bacteria than of gram-negative bacteria, due to their decreased polarity. Parabens are generally more effective against mold and yeasts than against bacteria. Concentrations of 32 to 1000 μg/ml are normally needed to inhibit bacteria and fungi using esters of *p*-hydroxybenzoic acid in laboratory media after 24 and 48 hours storage (Kato and Shibasaki, 1975; Jermini and Schmidt-Lorenz, 1987; Thompson, 1991; Juneja and Davidson, 1993).

Since parabens are phenolic derivatives, their antimicrobial mechanisms are similar to those of phenols and phenol-related compounds. Judis (1963) proposed that phenol physically damages the cytoplasmic membrane of microorganisms, resulting in

the release of cytoplasmic compounds. Eklund (1980) showed that parabens inhibit nutrient uptake through the cytoplasmic membrane. This results in inhibition of membrane transport and the electron transport system.

1.2.4. Natural Antimicrobials

1.2.4.1. Lactoferrin

A glycoprotein found in mammalian milk, lactoferrin exerts its antimicrobial activity through the binding of iron, and can bind two iron atoms per molecule (Lou and Yousef, 1999). This protein has proven to be effective against *Micrococcus spp.*, *Bacillus subtilis*, *Listeria monocytogenes*, *Bacillus sterothermophilus*, and *Escherichia coli* (Oram and Reiter, 1968; Reiter, 1978; Payne et al, 1990).

It was reported that lactoferrin causes cell death by chelation of iron, calcium, and magnesium ions (Ashton and Busta, 1968). *Listeria monocytogenes* was inhibited by lactoferrin, which was directly related to iron availability in the medium, with *Listeria monocytogenes* surviving best in an iron-rich media (Payne et al, 1989). However, it was reported that lactoferrin inhibited many bacteria in an iron-rich environment. For *Escherichia coli,* lactoferrin chelated cations that stabilized lipopolysaccharides, which led to increased permeability of the outer membrane to hydrophobic compounds (Arnold et al, 1982).

1.2.5. Curing Agents

1.2.5.1. Sodium Chloride

Sodium chloride is a food preservative used since ancient times. It can be used alone or in combination with other food preservation techniques (e.g. fermentation or pasteurization). Most bacterial food-borne pathogens are susceptible to sodium chloride,

with the exception of Staphylococcus aureus, which grows at a low water activity (0.83 to 0.86) (McLean et al, 1968). *Listeria monocytogenes* can grow at concentrations of up to 10% sodium chloride.

Sodium chloride exerts its antimicrobial activity through its ability to reduce water activity in food. Microbial cells lose water when the water activity of the external environment is reduced, which results in growth inhibition or possible death (Sperber, 1983). Sodium chloride also alters pH, limits oxygen solubility, and is toxic for microbial cells (Banward, 1979).

1.2.6. Metal

1.2.6.1. Silver Ions

Silver ions have the strongest antimicrobial activity among other metallic ions (Brody et al, 2001). When compared to other metallic ions metallic silver does not release the ion easily, thus making its antimicrobial activity not as strong in its metallic state. Silver ions are naturally occurring and effective microbe inhibitors. The use of silver zeolite as an antimicrobial additive is a new approach to prevent microbial growth in food products. Silver is safe and relatively inert, making it safe for direct human contact. FDA clearance allows for a maximum of 2% silver content by weight of the polymer (FDA, 2003). Human exposure to silver and silver compounds can occur orally, dermally, and by inhalation. Ingestion, dermal absorption, or inhalation of silver nitrates my cause argyria. Argyria is a disease caused by long term exposure to silver nitrate resulting in a gray or blue-gray permanent discoloration of the skin.

Silver salts possess the greatest potential for antimicrobial activity (e.g. silver nitrate and Ag-zeolite) (Brody et al, 2001). Silver nitrate that forms silver ions in water has strong antimicrobial activity. The antimicrobial activity of silver is achieved through an interference with metabolic functions of the respiratory and electron-transport systems of microorganisms. Silver ion is absorbed by the surface of the microbial cell through active transport, thus inhibiting a range of metabolic processes necessary for sustaining life (Brody et al, 2001). To facilitate antimicrobial activity, Ag-zeolite retains Ag^+ ions in stable and effective conditions (Brody et al, 2001). In order for Ag- zeolite to be formed, sodium ions in a zeolite are substituted with silver ions (Figure 1.1). Ag-zeolite is effective against bacteria, fungi, and yeast, but does not demonstrate antimicrobial activity against spores of heat-resistant bacteria (Brody et al, 2001).

Because of its expense, Ag-zeolite for packaging is usually laminated as a thin coextruded layer (3-6 μm) (Brody et al, 2001). To order to provide adequate heat-seal and other physical film properties, the normal incorporation level of Ag-zeolite is 1 to 3% (Brody et al, 2001). Silver ions are activated and released by moisture in the air, which allows for a steady rate to maintain the antimicrobial surface, thus ensuring product longevity and integrity. Slow release of Ag-zeolite is critical for optimal antimicrobial activity. Slow diffusion of an antimicrobial agent is important because it allows for longer and more effective antimicrobial action, which provides longer product shelf life (Brody et al, 2001; Brody 2001).

The No-Tox Products Divisions of Colorcon produces specialty antimicrobial silver zeolite coatings called AgION™ for pharmaceutical, medical, and food packaging industries. AgION™ coatings that are compliant with FDA regulations for direct food

contact applications use less than 0.001% of silver by weight of dry coating (AgION, 2001). According to Colorcon, zeolite, ion exchange, and silver must be present in order for the AgION™ to be effective (Cooksey, 2001) (Figure 1.1).

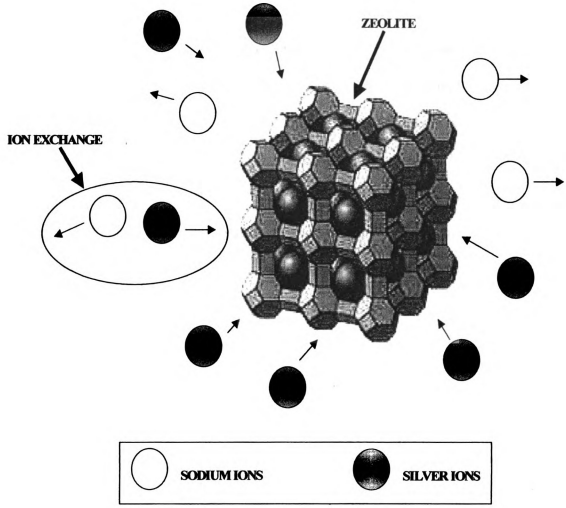

Figure 1.1. Illustration of ion exchange in the formation of silver zeolite.

AgION™ is dispersed as a fine solid in a proprietary water or solvent based coating. Solvent-based coatings were found to provide better release of the additive when tested against *Listeria monocytogenes* (Podhajny, 2002). The inorganic silver in zeolite carriers is antimicrobial on contact and technically feasible to fabricate, but the required migration from polymers is minimal (Brody, 2001). The coating itself is so effective that its shelf life is typically the shelf life of the product (Podhajny, 2002). Using such antimicrobial packaging materials is not a substitute for good sanitation and handling practices. These antimicrobial packaging materials should be used as an additional protective measure to help ensure the safety and high quality of food products (Cooksey, 2001).

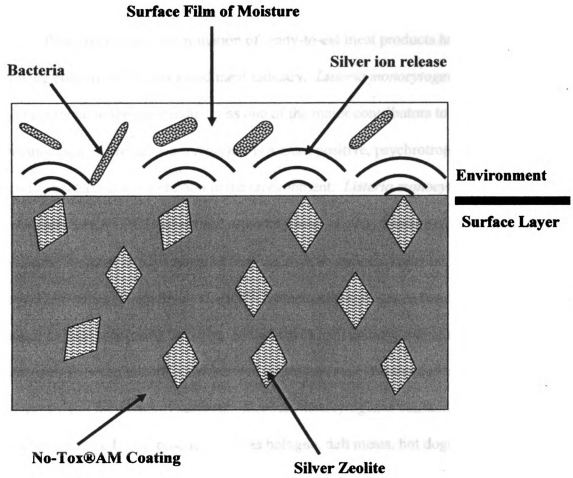

Figure 1.2. Illustration of silver ion release from film.

1.3. *Listeria monocytogenes* and Listeriosis

Post-processing contamination of ready-to-eat meat products has become a growing concern in the processed meat industry. *Listeria monocytogenes* is continuously posing a threat to the meat industry as one of the major contributors to post-processing contamination. *Listeria monocytogenes* is a gram positive, psychrotropic, non-spore forming bacteria that is common in the environment. *Listeria monocytogenes* typically grows between 3-45°C, with an optimum temperature growth range of 30-37°C. This pathogen can grow at a pH range of 5.6-9.6, with an optimum range of slightly alkaline to neutral pH values (Donnelly et al, 1992). *Listeria monocytogenes* has proven difficult to control in food processing facilities. Mafu et al (1990) demonstrated that *Listeria monocytogenes* can attach to and survive on rubber, stainless steel, glass, and polypropylene food contact surfaces. *Listeria monocytogenes* contamination can often occur in processed meat products such as bologna, deli meats, hot dogs, poultry, and meat spreads.

Consumption of a food product contaminated with *Listeria monocytogenes* can cause listeriosis. Listeriosis is a food-borne illness that is a major contributor to Class I microbial related food recalls. It is estimated that 5,000 Americans die each year from food-borne illnesses (CDC, 2002). While anyone can be affected by listeriosis, it can be fatal for the elderly, newborns, the fetus of pregnant women, and the immunocompromised (USDA-FSIS, 2002). Those with listeriosis often develop meningitis, sepsis, or meningoencephalitis (Slutsker and Schuchat, 1999). Pregnant women with listeriosis typically develop mild flu-like symptoms, fever, headaches, or gastrointestinal symptoms (Slutsker and Schuchat, 1999). Some infections in pregnant

women may result in preterm labor, amnionitis, spontaneous abortion, or stillbirth (Slutsker and Schuchat, 1999). During the second half of 2000, an outbreak involving 29 cases in 10 states (including 7 fatalities) prompted the recall of approximately 14.5 million pounds of turkey and chicken deli meat due to probable *Listeria monocytogenes* contamination (CDC, 2000).

The United States Department of Agriculture (USDA) and the Food Safety Inspection Service (FSIS) have been working diligently to develop methods to reduce the number of listeriosis outbreaks. On June 4, 2003, an interim final rule that requires federal establishments that produce ready-to-eat meat and poultry products to reduce incidences of *Listeria monocytogenes*. This rule requires those establishments that produce ready-to-eat products that are exposed to the environment after cooking to devise written programs such as Hazard Analysis and Critical Control Point (HACCP) systems, Sanitation Standard Operating Procedures (Sanitation SOPs), or other preliminary programs to control *Listeria monocytogenes*. Verification of the program's effectiveness will be achieved through testing, and results will be shared with the FSIS. The establishments will also provide the FSIS with information on production volumes as well as any other information on the product affected by regulation (USDA-FSIS, 2003). While the use of antimicrobial films does not replace this ruling, their implementation can assist with this effort.

CHAPTER 2

ANTIMICROBIAL PROPERTIES OF SILVER ZEOLITE-COATED POLYETHYLENE PACKAGING FILM

2.1. MATERIALS AND METHODS

2.1.1. Film Preparation

Commercially processed antimicrobial and control films were aseptically cut (5.08 x 5.08 cm) and sterilized by UV light for 20 minutes using a Class II A/B3 Biological Safety Cabinet (Forma Scientific Inc., Marietta, OH). The film samples were then aseptically placed in 15-mm disposable polystyrene petri dishes.

2.1.2. Bacterial Strains

Four strains of *Listeria monocytogenes* (CWD 95 and CWD 246 from silage CWD 201 from raw milk, and CWD 1503 from ground turkey) were obtained from C.W. Donnelly (Department of Nutrition and Food Sciences, University of Vermont, Burlington, VT). All strains were maintained at -70°C in trypticase soy broth (TSB) (Difco Laboratories, Detroit, MI) containing 10% (v/v) glycerol and subcultured twice in TSB containing 0.6% (w/v) yeast extract (TSB-YE) (Difco) at 35°C 18 to 24 hours before use. Following incubation, 2.5 ml of each culture was combined and centrifuged using a Sorvall Super T21at 10,000 rpm for 10 minutes at 4°C (Kendro Laboratory Products, Newtown, CT), and was resuspended in 10 ml sterile Butterfield's phosphate buffer (PBS) (Sigma Chemical Company, St. Louis, MO) (neutralized to pH 7.2 with 1 N sodium hydroxide). One ml of the inoculum was added to 99 ml of sterile PBS (Sigma) (neutralized to pH 7.2 with 1 N sodium hydroxide).

2.1.3. Film Inoculation and Storage

Antimicrobial and control films were aseptically placed in 15-mm disposable polystyrene petri dishes and inoculated with 0.5 ml of the diluted culture. Immediately after the films were inoculated, stomacher bags (5.08 x 5.08 cm) were aseptically placed

on each film sample to maintain inoculum on the film. These petri dishes were covered and stored in plastic containers with 200 ml of boiled deionized water. The plastic containers were stored at 37°C. All experiments were replicated three times.

2.1.4. Antimicrobial Properties

Samples were examined for numbers of *Listeria* immediately after inoculation and again following 24 hours of storage at 37°C. For analysis, the films were aseptically added to 20 ml of sterile PBS (Sigma) and homogenized using a Stomacher 400 (Tekmar Co., Cincinnati, OH) for 2 minutes, after which 0.1 ml of the dilution was surface plated on trypticase soy agar (TSA) containing 0.6% yeast extract (TSA-YE). Following 24 hours of incubation at 37°C, the number of colonies was counted. All experiments were replicated three times.

2.1.5. Statistical Analysis

All experiments were performed in triplicate. Film type and storage time data was analyzed by two-way analysis of variance (ANOVA) using SAS Statistical Analysis System (SAS Institute Inc., 2001). Means were compared using Tukey-Kramer adjustment at $p=0.05$.

2.2. RESULTS AND DISCUSSION

Control films without silver zeolite showed no antimicrobial activity against the *L. monocytogenes* during storage. However, the antimicrobial film showed antimicrobial activity against *L. monocytogenes* (Table 2.1 and Figure 2.1.). At the initial incubation of the film samples, there was no significant difference between the control and antimicrobial film ($p < 0.05$). There was a significant difference between the control and

Table 2.1. Population of *L. monocytogenes* after 24 hours storage at 37°C.

	Storage Time (Hours)	
Film Types	0	24
Silver Zeolite	$5.23^{a} \pm 0.21$	$<3.89^{c} \pm 0.00$
Control	$5.33^{a} \pm 0.5$	$6.26^{b} \pm 0.03$

Unit=CFU/cm^2 ($\log_{10}$) Geometric mean ± standard deviation (n=3). Means with different superscripts are significantly different ($p<0.05$).

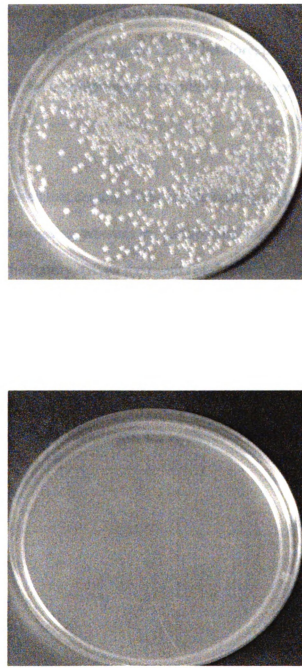

Figure 2.1. TSA-YE plates from control film (top) and antimicrobial film (bottom) after 24 hours storage at
37°C.

antimicrobial film after 24 hours ($p<0.05$) (Table 2.1). There was no growth observed in TSA-YE for the antimicrobial film (Figure 2.2). Therefore, the antimicrobial film used in this study proved effective in inhibiting the growth of *L. monocytogenes* in direct contact after 24 hours.

2.3. CONCLUSION

This study showed that coating silver zeolite coating on polyethylene led to the inhibition of *L. monocytogenes*; whereas the control film without antimicrobial coating was found to be non-inhibitory. Given the results of this work, it is likely that this film may prove useful for reducing post-processing contamination of to ready-to-eat meat products.

CHAPTER 3

INHIBITION OF *LISTERIA MONOCYTOGENES* ON BEEF BOLOGNA USING SILVER ZEOLITE-COATED POLYETHEYLENE PACKAGING FILM

3.1. MATERIALS AND METHODS

3.1.1. Product

Pre-sliced (~ 3 mm), commercially processed beef bologna (diameter 9.6 cm) was obtained from a local supermarket. As reported by the manufacturer, the all-beef bologna contained beef, salt, sodium lactate, flavor, dextrose, hydrolyzed beef stock, autolyzed yeast, sodium phosphate, sodium diacetate, sodium erythorbate, sodium nitrite, and extractives of paprika. At the time of purchase, the beef bologna had a pH value of 6.0. The product used for all trials was from the same production lot. Enrichment analysis was conducted to confirm the absence of *L. monocytogenes* in the bologna prior to testing as outlined in the Compendium of Methods for the Microbiological Examination of Foods (Donnelly et al, 2001).

3.1.2. Film Preparation

Commercially processed film samples were then aseptically cut (6.35 x 3.81 cm) and sterilized by UV light using a Class II A/B3 Biological Safety Cabinet (Forma Scientific Inc., Marietta, OH) for 20 minutes. The film was then aseptically placed in 15-mm disposable polystyrene petri dishes.

3.1.3. Bacterial Strains

Four strains of *Listeria monocytogenes* (CWD 95 and CWD 246 from silage CWD 201 from raw milk, and CWD1503 from ground turkey) were acquired from C.W. Donnelly (Department of Nutrition and Food Sciences, University of Vermont, Burlington, VT). Each strain was maintained at -70°C in trypticase soy broth (TSB) (Difco Laboratories, Detroit, MI) containing 10% (v/v) glycerol and subcultured twice in TSB containing 0.6% (w/v) yeast extract (TSB-YE) (Difco) at 35°C 18 to 24 hours prior

to use. The four strains (2.5 ml each) of *L. monocytogenes* were combined and centrifuged (Sorvall Super T21, Kendro Laboratory Products, Newtown, CT) for 10 minutes at 4 °C at 10,000 rpm, and resuspended in 10 ml of sterile 0.1% (w/v) peptone water (Difco).

3.1.4. Product Inoculation and Storage

Antimicrobial and control films were inoculated with 0.1 ml of a diluted culture to obtain an inoculum level of log 6.0 (± 0.1) CFU/cm^2. The inoculum was evenly spread on the film surface that was to be in contact with the bologna samples using a sterile glass rod. Bologna slices were individually wrapped in the inoculated film samples and stacked 3 slices high in sterile 150-mm diameter petri dishes. The bologna was then covered and stored aerobically at 4°C and 12°C.

3.1.5. Microbiological Analysis

The slices of bologna were examined for level of inoculum, lactic acid bacteria, and mesophilic aerobic bacteria immediately after inoculation, and again following 2, 4, 6, 8, 24, and 48 hours of storage at 4°C and 12°C. 10-g bologna slices were each added to 40 ml of sterile 0.1% (w/v) peptone water (Difco) and homogenized in a stomacher (Tekmar Co., Cincinnati, OH) for 3 minutes. Appropriate dilutions of peptone water (Difco) were plated on Modified Oxford Agar (MOX) (Difco), Plate Count Agar (Difco), and MRS Lactobacillus Agar (Difco) to quantify *Listeria monocytogenes*, mesophilic aerobic bacteria (MAB), and lactic acid bacteria (LAB).

3.1.6. Statistical Analysis

All experiments were conducted in triplicate. Film type, storage time, and temperature data was analyzed by three-way analysis of variance (ANOVA) using SAS

Statistical Analysis System (SAS Institute Inc., 2001). Analysis of means was compared at a 95% confidence level (p=0.05) using Tukey-Kramer adjustment.

3.2. RESULTS AND DISCUSSION

3.2.1. *Listeria monocytogenes*

Antimicrobial and control films were initially inoculated to contain 10^6 CFU/cm^2 and examined after 48 hours storage at 4°C and 12°C. The population distributions of *L. monocytogenes* during 48 hours at the two temperatures are shown in Tables 3.1 and 3.2. The antimicrobial film decreased populations of *L. monocytogenes* by 0.39 logs, from 6.79 to 6.40 logs, for the bologna slices after 48 hours of storage at 4°C (Figure 3.1). A similar effect was observed for bologna slices after 48 hours of storage at 12°C, with *L. monocytogenes* decreasing by 0.05 logs, from 6.78 to 6.73 logs. After 48 hours storage at 4°C, *Listeria* counts increased 0.1 logs using film without silver zeolite antimicrobial coating, and 0.09 logs at 12°C.

3.2.2. Mesophilic Aerobic Bacteria

Numbers of mesophilic aerobic bacteria (MAB) remained unchanged at about 6.91 logs for bologna slices stored 48 hours at 4°C with antimicrobial film (Figure 3.2). MAB populations increased by 0.07 logs, from 6.73 to 6.80 logs, with antimicrobial-free film samples. The MAB population, after 48 hours storage at 12°C, decreased by 0.25 log, from 6.90 to 7.15 logs with antimicrobial film, while MAB with antimicrobial-free film increased by 0.16 logs (Figure 3.2).

3.2.3. Lactic Acid Bacteria

Using the antimicrobial film, LAB increased from 6.73 to 6.80 logs on the bologna slices after 48 hours storage at 4°C; a similar effect was observed with

antimicrobial-free film (Figure 3.3). After 48 hours storage at 12°C, LAB populations increased by 0.08 logs with the antimicrobial film, and antimicrobial-free film increased by 0.16 logs.

3.3. CONCLUSION

Polyethylene coated with silver zeolite reduced the growth of *L. monocytogenes* when in contact with bologna slices at 4°C and 12°C for 48 hours. The film shows little promise for extending the shelf life of temperature-abused sliced beef bologna due to low population decreases. A more extensive shelf-life study of the antimicrobial film would provide the processed meat industry with an alternative means of protecting meat products from post-processing contamination.

Table 3.1. Inhibition of *Listeria monocytogenes*, lactic acid bacteria, and mesophilic bacteria on bologna slices using control and antimicrobial films at 4°C.

Film Type	Temperature	Time	*L. monocytogenes*	Mesophilic bacteria	Lactic acid bacteria
Control	4	0	6.80 ± 0.01^{a}	6.91 ± 0.00^{a}	6.73 ± 0.01^{a}
		2	6.80 ± 0.01^{ab}	6.90 ± 0.01^{a}	6.75 ± 0.01^{a}
		4	6.81 ± 0.00^{ac}	6.92 ± 0.00^{a}	6.76 ± 0.01^{a}
		6	6.80 ± 0.01^{ad}	6.91 ± 0.01^{a}	6.74 ± 0.01^{a}
		8	6.82 ± 0.02^{ae}	6.91 ± 0.01^{a}	6.75 ± 0.02^{a}
		24	6.85 ± 0.00^{abf}	6.94 ± 0.02^{ab}	6.78 ± 0.01^{b}
		48	6.90 ± 0.00^{acg}	6.99 ± 0.01^{cd}	6.80 ± 0.02^{c}
Antimicrobial	4	0	6.79 ± 0.01^{a}	6.91 ± 0.01^{a}	6.73 ± 0.01^{a}
		2	6.74 ± 0.01^{b}	6.92 ± 0.01^{a}	6.74 ± 0.01^{a}
		4	6.66 ± 0.01^{c}	6.92 ± 0.01^{a}	6.74 ± 0.02^{ab}
		6	6.60 ± 0.02^{d}	6.91 ± 0.01^{a}	6.76 ± 0.01^{c}
		8	6.50 ± 0.00^{e}	6.91 ± 0.01^{a}	6.73 ± 0.01^{a}
		24	6.42 ± 0.07^{ef}	6.91 ± 0.01^{a}	6.79 ± 0.02^{ad}
		48	6.40 ± 0.01^{f}	6.91 ± 0.01^{a}	6.80 ± 0.00^{ae}

Unit=CFU/cm^2 ($\log_{10}$) Geometric mean ± standard deviation (n=3). Means with different superscripts are significantly different ($p<0.05$).

Table 3.2. Inhibition of *Listeria monocytogenes*, lactic acid bacteria, and mesophilic bacteria on bologna slices using control and antimicrobial films at 12°C.

Film Type	Temperature	Time	*L. monocytogenes*	Mesophilic bacteria	Lactic acid bacteria
Control	12	0	6.79 ± 0.01^{a}	6.90 ± 0.01^{a}	6.74 ± 0.01^{a}
		2	6.86 ± 0.01^{a}	6.90 ± 0.00^{a}	6.74 ± 0.01^{a}
		4	6.87 ± 0.00^{a}	6.91 ± 0.01^{a}	6.74 ± 0.01^{a}
		6	6.89 ± 0.00^{a}	6.92 ± 0.01^{ad}	6.74 ± 0.01^{a}
		8	6.91 ± 0.02^{ad}	6.91 ± 0.01^{ae}	6.76 ± 0.01^{ab}
		24	7.12 ± 0.01^{b}	7.02 ± 0.01^{bf}	6.90 ± 0.00^{ce}
		48	7.18 ± 0.00^{c}	7.15 ± 0.01^{c}	6.90 ± 0.01^{de}
Antimicrobial	12	0	6.78 ± 0.00^{a}	6.91 ± 0.01^{a}	6.73 ± 0.00^{a}
		2	6.76 ± 0.01^{ab}	6.87 ± 0.01^{ab}	6.72 ± 0.01^{a}
		4	6.73 ± 0.01^{ach}	6.81 ± 0.01^{ac}	6.74 ± 0.00^{a}
		6	6.73 ± 0.01^{adh}	6.79 ± 0.01^{acd}	6.79 ± 0.01^{bf}
		8	6.72 ± 0.00^{aeh}	6.81 ± 0.01^{ace}	6.79 ± 0.01^{cfg}
		24	6.73 ± 0.00^{fh}	6.81 ± 0.01^{acf}	6.81 ± 0.01^{dfgh}
		48	6.73 ± 0.01^{gh}	6.82 ± 0.01^{acg}	6.81 ± 0.01^{efgh}

Unit=CFU/cm^2 ($\log_{10}$) Geometric mean ± standard deviation (n=3). Means with different superscripts are significantly different ($p<0.05$).

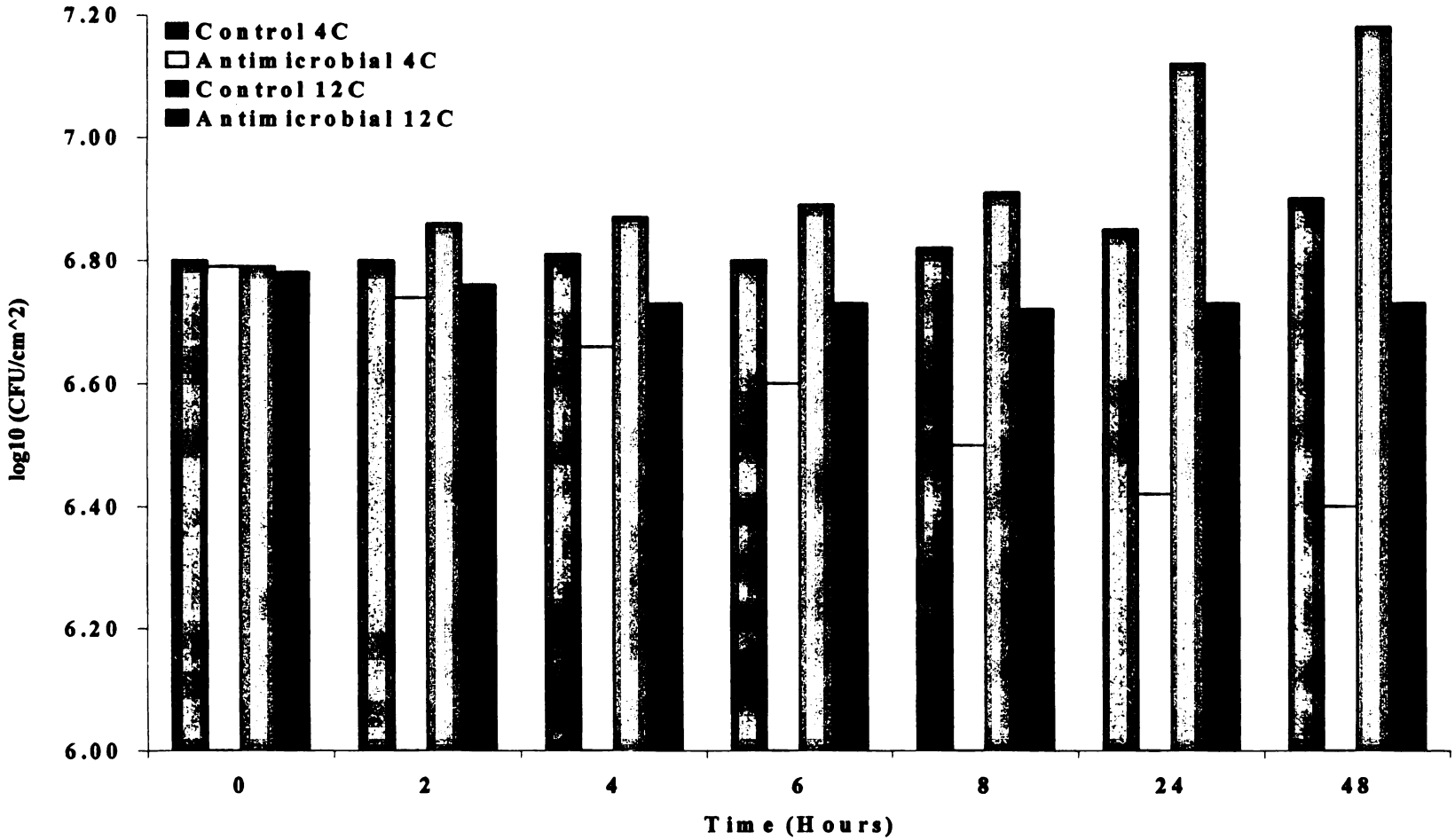

Figure 3.1. Inhibition of *L. monocytogenes* on bologna samples wrapped in control and antimicrobial film during storage at 4°C and 12°C.

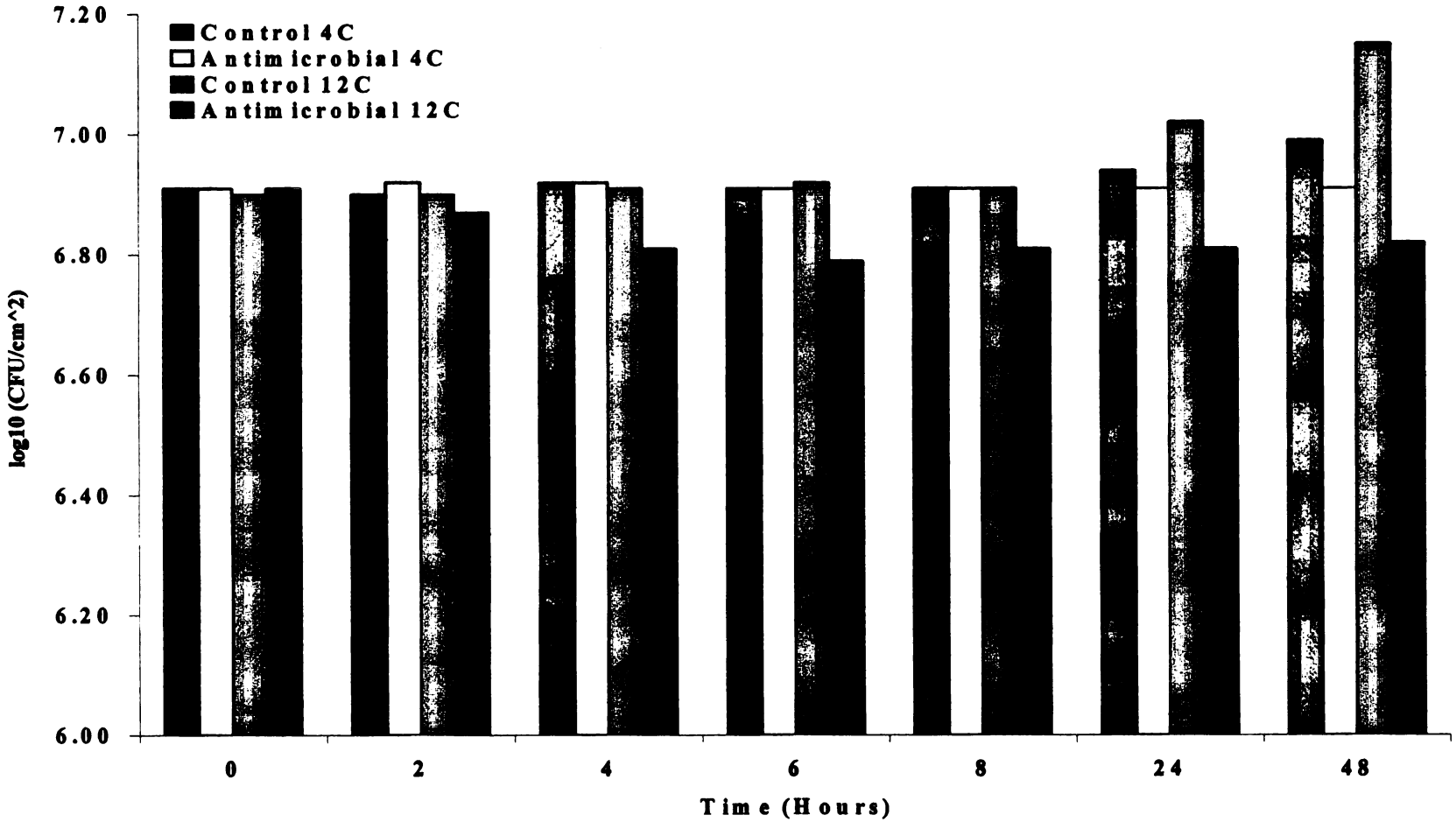

Figure 3.2. Inhibition of mesophilic aerobic bacteria on bologna samples wrapped in control and antimicrobial film during storage at 4°C and 12°C.

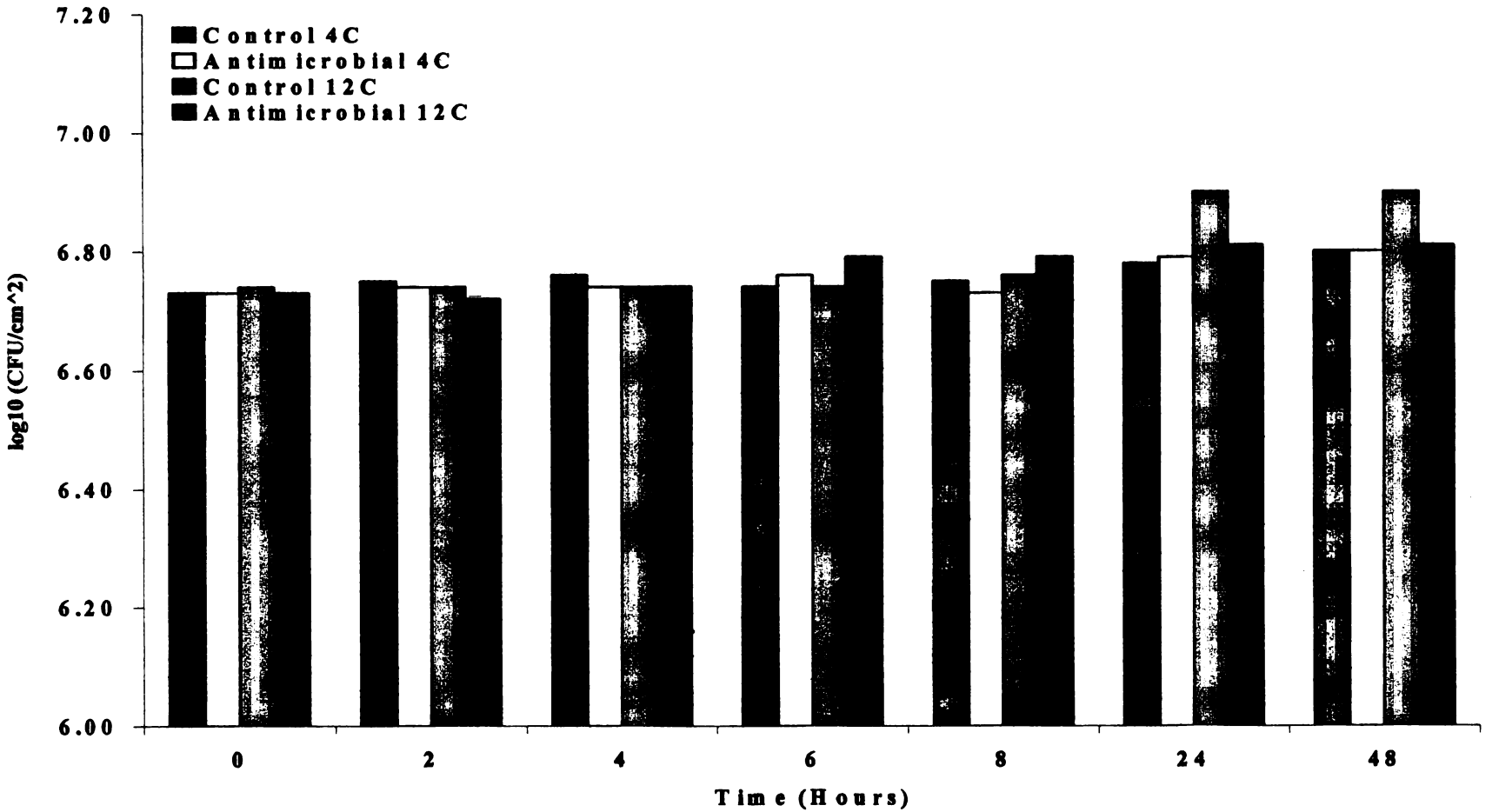

Figure 3.3. Inhibition of lactic acid bacteria on bologna samples wrapped in control and antimicrobial film during storage at 4°C and 12°C.

CONCLUSIONS

Antimicrobial properties of a commercially processed polyethylene film coated with AgION™ silver zeolite were tested for its ability to inhibit growth of four strains of *Listeria monocytogenes* (CWD 95 and 246 from silage, CWD 201 from raw milk, and CWD 1503 from ground turkey). The antimicrobial activity of the film inhibited the growth of *Listeria monocytogenes* by 1.34 logs, on trypticase soy agar (TSA) containing 0.6% yeast extract (TSA-YE) after 24 hours of storage at 37°C.

Subsequently, beef bologna slices were packaged in the silver zeolite coated film inoculated with 10^6 CFU/cm^2 *Listeria monocytogenes* to observe the effectiveness of the antimicrobial properties of the film while in direct contact with the bologna slices. Populations of *Listeria monocytogenes* decreased on bologna slices while in contact during 48 hours storage at 4°C and 12°C. MAB populations remained unchanged during 48 hours at 4°C; whereas during 48 hours at 12°C populations decreased. At both temperatures, MAB populations were decreased using antimicrobial film compared to antimicrobial-free control film. The antimicrobial film increased in populations of LAB during 48 hours at 4°C and 12°C. At 4°C, LAB populations were unchanged using antimicrobial film compared to antimicrobial-free film; while at 12°C the antimicrobial film decreased LAB populations compared to the antimicrobial-free film.

From this work, it can be concluded that silver zeolite coated polyethylene was effective in inhibiting the growth of *Listeria monocytogenes* (CWD 95, CWD 246, CWD 201, and CWD 1503). While the silver zeolite coating was not as effective in inhibiting the growth of *Listeria monocytogenes* at 10^6 CFU/cm^2 in contact with beef bologna, the populations were reduced over 48 hours. Temperature did not seem to hinder the

antimicrobial properties of the coating, which shows that it may be possible to use the film as meat packaging to reduce the risk of *Listeria* contamination film for temperature abused meat products.

Overall, this research represents a first step in the development of silver zeolite as an antimicrobial packaging film for the inhibition of *Listeria monocytogenes*. Directions for future research should include observing the antimicrobial properties of silver zeolite over the entire shelf life of a processed meat product. According to the research, there should be inhibition of *Listeria* over time. Also, a detailed study of the effects of extreme temperature abuse on the antimicrobial properties of the film is recommended. While the research showed temperature did not affect the antimicrobial properties of the film, it would be interesting to see how the film would behave if it underwent temperature shock, i.e. storage at 4°C for 2 hours, followed by storage at 37°C for one hour. This is relevant and worthy of consideration since products may be temperature abused during their distribution. Lastly, a detailed study of the silver zeolite coating's ability to inhibit the growth of other food-borne microorganisms is recommended. This would provide information regarding the versatility of the film's ability to prevent post–processing contamination.

APPENDIX I

OBJECTIVE 1 DATA

***L. monocytogenes* Log_{10} (CFU/cm^2) population of film samples during storage at 37°C.**

Colony Count	Average Colonies	Dilution Factor	*L. monocytogenes*
14 12	13.00	2.02E+05	5.30
9 24	16.50	2.56E+05	5.41
13 15	14.00	2.17E+05	5.34
10 8	9.00	1.40E+05	5.14
6 9	7.50	1.16E+05	5.07
24 13	18.50	2.87E+05	5.46
114 112	113.00	1.75E+06	6.24
130 125	127.50	1.98E+06	6.30
123 120	121.50	1.88E+06	6.28
1 0	0.50	7.75E+03	3.89
0 0	0.00	0.0	0.00
0 0	0.00	0.0	0.00

APPENDIX II

OBJECTIVE 2 DATA

A. Population counts of *L. monocytogenes* (*Listeria*), mesophilic aerobic bacteria (MAB), and lactic acid bacteria (LAB) Log_{10} (CFU/cm^2) for bologna slices wrapped in control film over 48 hours at 4°C.

Time	Colony Count	Average Colonies	Dilution Factor	*Listeria*	Colony Count	Average Colonies	Dilution Factor	MAB	Colony Count	Average Colonies	Dilution Factor	LAB
0	75	73.00	6.28E+06	6.80	95	94.50	8.13E+06	6.91	63	62.00	5.33E+06	6.73
	71				94				61			
	72	71.50	6.15E+06	6.79	96	93.50	8.04E+06	6.91	65	63.50	5.46E+06	6.74
	71				91				62			
	73	74.00	6.37E+06	6.80	96	95.50	8.22E+06	6.91	60	63.00	5.42E+06	6.73
	75				95				66			
2	75	74.50	6.41E+06	6.81	92	93.00	8.00E+06	6.90	63	64.00	5.51E+06	6.74
	74				94				65			
	70	71.50	6.15E+06	6.79	90	91.50	7.87E+06	6.90	62	64.50	5.55E+06	6.74
	73				93				67			
	70	73.00	6.28E+06	6.80	96	95.50	8.22E+06	6.91	69	67.50	5.81E+06	6.76
	76				95				66			
4	74	75.00	6.45E+06	6.81	98	96.50	8.30E+06	6.92	68	67.00	5.76E+06	6.76
	76				95				66			
	75	75.00	6.45E+06	6.81	95	95.50	8.22E+06	6.91	65	64.50	5.55E+06	6.74
	75				96				64			
	74	74.50	6.41E+06	6.81	95	96.00	8.26E+06	6.92	68	69.00	5.94E+06	6.77
	75				97				70			

Table A (Con't)

Time	Colony Count	Average Colonies	Dilution Factor	*Listeria*	Colony Count	Average Colonies	Dilution Factor	MAB	Colony Count	Average Colonies	Dilution Factor	LAB
6	71 75	73.00	6.28E+06	6.80	95 99	97.00	8.34E+06	6.92	61 62	61.50	5.29E+06	6.72
	73 76	74.50	6.41E+06	6.81	96 97	96.50	8.30E+06	6.92	70 61	65.50	5.63E+06	6.75
	76 73	74.50	6.41E+06	6.81	93 92	92.50	7.96E+06	6.90	62 65	63.50	5.46E+06	6.74
8	79 80	79.50	6.84E+06	6.83	97 95	96.00	8.26E+06	6.92	63 67	65.00	5.59E+06	6.75
	78 77	77.50	6.67E+06	6.82	93 90	91.50	7.87E+06	6.90	60 66	63.00	5.42E+06	6.73
	74 72	73.00	6.28E+06	6.80	92 94	93.00	8.00E+06	6.90	67 68	67.50	5.81E+06	6.76
24	83 82	82.50	7.10E+06	6.85	100 97	98.50	8.47E+06	6.93	69 67	68.00	5.85E+06	6.77
	84 81	82.50	7.10E+06	6.85	98 97	97.50	8.39E+06	6.92	70 72	71.00	6.11E+06	6.79
	80 83	81.50	7.01E+06	6.85	107 105	106.00	9.12E+06	6.96	71 70	70.50	6.06E+06	6.78
48	91 93	92.00	7.91E+06	6.90	111 113	112.00	9.63E+06	6.98	69 70	69.50	5.98E+06	6.78
	92 90	91.00	7.83E+06	6.89	112 114	113.00	9.72E+06	6.99	71 79	75.00	6.45E+06	6.81
	94 90	92.00	7.91E+06	6.90	117 118	117.50	1.01E+07	7.00	74 78	76.00	6.54E+06	6.82

B. Population counts of *L. monocytogenes* (*Listeria*), mesophilic aerobic bacteria (MAB), and lactic acid bacteria (LAB) Log_{10} (CFU/cm^2) for bologna slices wrapped in antimicrobial film over 48 hours at 4°C.

Time	Colony Count	Average Colonies	Dilution Factor	*Listeria*	Colony Count	Average Colonies	Dilution Factor	MAB	Colony Count	Average Colonies	Dilution Factor	LAB
0	73	71.50	6.15E+06	6.79	90	92.50	7.96E+06	6.90	62	63.00	5.42E+06	6.73
	70				95				64			
	71	71.50	6.15E+06	6.79	93	93.50	8.04E+06	6.91	60	61.50	5.29E+06	6.72
	72				94				63			
	75	74.50	6.41E+06	6.81	96	96.00	8.26E+06	6.92	62	61.50	5.29E+06	6.72
	74				96				61			
2	68	64.50	5.55E+06	6.74	96	97.50	8.39E+06	6.92	65	65.50	5.63E+06	6.75
	61				99				66			
	62	62.50	5.38E+06	6.73	96	95.50	8.22E+06	6.91	63	62.50	5.38E+06	6.73
	63				95				62			
	60	63.00	5.42E+06	6.73	94	94.50	8.13E+06	6.91	63	62.00	5.33E+06	6.73
	66				95				61			
4	53	52.50	4.52E+06	6.65	96	96.50	8.30E+06	6.92	63	64.00	5.51E+06	6.74
	52				97				65			
	51	52.50	4.52E+06	6.65	95	93.50	8.04E+06	6.91	67	68.00	5.85E+06	6.77
	54				92				69			
	50	54.50	4.69E+06	6.67	99	97.50	8.39E+06	6.92	61	62.00	5.33E+06	6.73
	59				96				63			

Table B (Con't)

Time	Colony Count	Average Colonies	Dilution Factor	*Listeria*	Colony Count	Average Colonies	Dilution Factor	MAB	Colony Count	Average Colonies	Dilution Factor	LAB
6	49 48	48.50	4.17E+06	6.62	95 92	93.50	8.04E+06	6.91	66 62	64.00	5.51E+06	6.74
	49 47	48.00	4.13E+06	6.62	94 96	95.00	8.17E+06	6.91	68 67	67.50	5.81E+06	6.76
	43 45	44.00	3.78E+06	6.58	93 92	92.50	7.96E+06	6.90	67 67	67.00	5.76E+06	6.76
8	38 36	37.00	3.18E+06	6.50	97 95	96.00	8.26E+06	6.92	62 62	62.00	5.33E+06	6.73
	39 35	37.00	3.18E+06	6.50	91 95	93.00	8.00E+06	6.90	60 64	62.00	5.33E+06	6.73
	38 37	37.50	3.23E+06	6.51	95 92	93.50	8.04E+06	6.91	62 69	65.50	5.63E+06	6.75
24	36 37	36.50	3.14E+06	6.50	96 96	96.00	8.26E+06	6.92	70 71	70.50	6.06E+06	6.78
	30 26	28.00	2.41E+06	6.38	98 97	97.50	8.39E+06	6.92	78 72	75.00	6.45E+06	6.81
	28 27	27.50	2.37E+06	6.37	91 95	93.00	8.00E+06	6.90	71 70	70.50	6.06E+06	6.78
48	36 32	34.00	2.92E+06	6.47	98 93	95.50	8.22E+06	6.91	78 74	76.00	6.54E+06	6.82
	35 34	34.50	2.97E+06	6.47	94 93	93.50	8.04E+06	6.91	75 74	74.50	6.41E+06	6.81
	34 31	32.50	2.80E+06	6.45	95 96	95.50	8.22E+06	6.91	76 74	75.00	6.45E+06	6.81

C. Population counts of *L. monocytogenes* (*Listeria*), mesophilic aerobic bacteria (MAB), and lactic acid bacteria (LAB) Log_{10} (CFU/cm^2) for bologna slices wrapped in control film over 48 hours at 12°C.

Time	Colony Count	Average Colonies	Dilution Factor	*Listeria*	Colony Count	Average Colonies	Dilution Factor	MAB	Colony Count	Average Colonies	Dilution Factor	LAB
0	72	71.00	6.11E+06	6.79	90	90.50	7.78E+06	6.89	62	63.00	5.42E+06	6.73
	70				91				64			
	73	72.50	6.24E+06	6.79	92	93.00	8.00E+06	6.90	63	64.50	5.55E+06	6.74
	72				94				66			
	72	71.00	6.11E+06	6.79	96	95.50	8.22E+06	6.91	64	63.00	5.42E+06	6.73
	70				95				62			
2	84	83.50	7.18E+06	6.86	91	91.50	7.87E+06	6.90	63	63.50	5.46E+06	6.74
	83				92				64			
	89	87.50	7.53E+06	6.88	94	92.50	7.96E+06	6.90	65	64.00	5.51E+06	6.74
	86				91				63			
	80	83.00	7.14E+06	6.85	92	91.00	7.83E+06	6.89	65	65.00	5.59E+06	6.75
	86				90				65			
4	87	86.00	7.40E+06	6.87	97	96.00	8.26E+06	6.92	62	62.50	5.38E+06	6.73
	85				95				63			
	88	86.50	7.44E+06	6.87	92	92.50	7.96E+06	6.90	63	64.50	5.55E+06	6.74
	85				93				66			
	88	86.50	7.44E+06	6.87	95	94.50	8.13E+06	6.91	63	64.50	5.55E+06	6.74
	85				94				66			

Table C (Con't)

Time	Colony Count	Average Colonies	Dilution Factor	*Listeria*	Colony Count	Average Colonies	Dilution Factor	MAB	Colony Count	Average Colonies	Dilution Factor	LAB
6	89 90	89.50	7.70E+06	6.89	98 99	98.50	8.47E+06	6.93	62 63	62.50	5.38E+06	6.73
	90 91	90.50	7.78E+06	6.89	100 97	98.50	8.47E+06	6.93	64 65	64.50	5.55E+06	6.74
	90 88	89.00	7.66E+06	6.88	95 96	95.50	8.22E+06	6.91	65 65	65.00	5.59E+06	6.75
8	90 90	90.00	7.74E+06	6.89	95 98	96.50	8.30E+06	6.92	68 68	68.00	5.85E+06	6.77
	93 98	95.50	8.22E+06	6.91	99 95	97.00	8.34E+06	6.92	67 69	68.00	5.85E+06	6.77
	97 95	96.00	8.26E+06	6.92	94 92	93.00	8.00E+06	6.90	65 66	65.50	5.63E+06	6.75
24	153 154	153.50	1.32E+07	7.12	122 126	124.00	1.07E+07	7.03	92 90	91.00	7.83E+06	6.89
	157 152	154.50	1.33E+07	7.12	121 120	120.50	1.04E+07	7.02	90 91	90.50	7.78E+06	6.89
	158 142	150.00	1.29E+07	7.11	119 118	118.50	1.02E+07	7.01	93 92	92.50	7.96E+06	6.90
48	177 170	173.50	1.49E+07	7.17	165 166	165.50	1.42E+07	7.15	90 91	90.50	7.78E+06	6.89
	176 178	177.00	1.52E+07	7.18	164 163	163.50	1.41E+07	7.15	92 93	92.50	7.96E+06	6.90
	179 174	176.50	1.52E+07	7.18	162 160	161.00	1.38E+07	7.14	94 95	94.50	8.13E+06	6.91

D. Population counts of *L. monocytogenes* (*Listeria*), mesophilic aerobic bacteria (MAB), and lactic acid bacteria (LAB) Log_{10} (CFU/cm^2) for bologna slices wrapped in antimicrobial film over 48 hours at 12°C.

Time	Colony Count	Average Colonies	Dilution Factor	*Listeria*	Colony Count	Average Colonies	Dilution Factor	MAB	Colony Count	Average Colonies	Dilution Factor	LAB
0	71	70.00	6.02E+06	6.78	92	93.50	8.04E+06	6.91	62	61.50	5.29E+06	6.72
	69				95				61			
	67	68.50	5.89E+06	6.77	93	94.00	8.09E+06	6.91	60	61.50	5.29E+06	6.72
	70				95				63			
	68	69.50	5.98E+06	6.78	96	97.00	8.34E+06	6.92	65	62.50	5.38E+06	6.73
	71				98				60			
2	73	72.00	6.19E+06	6.79	87	87.50	7.53E+06	6.88	60	59.50	5.12E+06	6.71
	71				88				59			
	70	71.00	6.11E+06	6.79	87	88.00	7.57E+06	6.88	61	61.00	5.25E+06	6.72
	72				89				61			
	74	75.00	6.45E+06	6.81	86	85.50	7.35E+06	6.87	62	62.50	5.38E+06	6.73
	76				85				63			
4	61	61.50	5.29E+06	6.72	73	73.50	6.32E+06	6.80	65	63.50	5.46E+06	6.74
	62				74				62			
	64	63.50	5.46E+06	6.74	76	74.00	6.37E+06	6.80	63	63.50	5.46E+06	6.74
	63				72				64			
	60	62.00	5.33E+06	6.73	79	76.00	6.54E+06	6.82	65	64.50	5.55E+06	6.74
	64				73				64			

Table D (Con't)

Time	Colony Count	Average Colonies	Dilution Factor	*Listeria*	Colony Count	Average Colonies	Dilution Factor	MAB	Colony Count	Average Colonies	Dilution Factor	LAB
6	60 63	61.50	5.29E+06	6.72	71 72	71.50	6.15E+06	6.79	71 73	72.00	6.19E+06	6.79
	60 62	61.00	5.25E+06	6.72	74 71	72.50	6.24E+06	6.79	72 74	73.00	6.28E+06	6.80
	67 61	64.00	5.51E+06	6.74	70 71	70.50	6.06E+06	6.78	71 71	71.00	6.11E+06	6.79
8	60 61	60.50	5.20E+06	6.72	74 75	74.50	6.41E+06	6.81	70 72	71.00	6.11E+06	6.79
	63 60	61.50	5.29E+06	6.72	77 76	76.50	6.58E+06	6.82	73 74	73.50	6.32E+06	6.80
	61 60	60.50	5.20E+06	6.72	74 77	75.50	6.49E+06	6.81	70 71	70.50	6.06E+06	6.78
24	65 60	62.50	5.38E+06	6.73	76 71	73.50	6.32E+06	6.80	75 74	74.50	6.41E+06	6.81
	63 62	62.50	5.38E+06	6.73	70 76	73.00	6.28E+06	6.80	72 73	72.50	6.24E+06	6.79
	61 64	62.50	5.38E+06	6.73	79 76	77.50	6.67E+06	6.82	77 75	76.00	6.54E+06	6.82
48	65 63	64.00	5.51E+06	6.74	77 78	77.50	6.67E+06	6.82	80 71	75.50	6.49E+06	6.81
	62 63	62.50	5.38E+06	6.73	75 77	76.00	6.54E+06	6.82	78 75	76.50	6.58E+06	6.82
	65 64	64.50	5.55E+06	6.74	74 75	74.50	6.41E+06	6.81	75 74	74.50	6.41E+06	6.81

APPENDIX III

FILM PREPARATION

Corona-treated polyethylene was aseptically cut (17.78 x 22.86 cm) and taped onto manila folders. Two ml of FGN-5415 silver zeolite coating was then coated onto the film with using a #3 Meyer rod (Colorcon, West Point, Pennsylvania). The coating was dried using a hot gun (Colorcon).

APPENDIX IV

ENRICHMENT PROCEDURE FOR ISOLATION OF *L. MONOCYTOGENES*

Sample Preparation (from section 38.513 USDA's Enrichment Procedure for Isolation of *L. monocytogenes* From Meat Products, Compendium of Methods for the Microbiological Examination of Foods, 1992)

Surfaces of beef-bologna packages were swabbed with 3% hydrogen peroxide before opening. Bologna slices were cut into 25-g pieces on a sterilized aluminum baking pan using a sterilized knife and forceps. The meat samples were placed in 225 ml of sterile University of Vermont (UVM) broth (Difco Laboratories, Detroit, MI) and homogenized in a stomacher (Tekmar Co., Cincinnati, OH) for 2 minutes, and then incubated at 30°C for 24 hours. After storage, 0.1 ml of the UVM culture was placed in 10 ml of sterile Fraser's broth (Difco), and incubated at 35°C for 24 and 40 hours. After incubation, a sterile cotton swab was dipped into the Fraser's broth (Difco) and then swabbed and streaked onto Modified Oxford Agar (MOX) (Difco). The MOX was incubated at 35°C for 48 hours and examined for *Listeria* colonies.

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