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DEVELOPMENT OF AN ANTIMICROBIAL FILM
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DEVELOPMENT OF AN ANTIMICROBIAL FILM FOR FOOD PACKAGING

By

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ABSTRACT

DEVELOPMENT OF AN ANTIMICROBIAL FILM FOR FOOD PACKAGING

By

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Polyvinylidene chloride (PVDC) copolymer films containing nisin, potassium sorbate, lactoferrin, sodium diacetate or sorbic acid were developed to have antimicrobial activity against four strains of *Listeria monocytogenes* (CWD 95, CWD 246, CWD 201 and CWD 1503). Only films containing nisin, potassium sorbate and sorbic acid had antimicrobial activity. The minimum concentrations of nisin, sorbic acid and potassium sorbate that had antimicrobial activity using a disc diffusion assay were 1.0%, 1.5% and 2.0% (w/v), respectively. Films containing sorbic acid had the most antimicrobial activity, best barrier and mechanical properties, and greatest distribution of sorbic acid in the polymer structure. The polyvinylidene chloride copolymer coating containing 3.0% (w/v) sorbic acid in a 0.75 mil coating thickness on polyethylene terephthalate (PET) film had antimicrobial activity against *L. monocytogenes*.

PVDC films containing 1.5% and 3.0% (w/v) sorbic acid were selected to verify their antimicrobial activity on Cheddar cheese and bologna, which were previously surface inoculated with *L. monocytogenes* (CWD 95) at inoculum levels of 10^5 or 10^3 CFU/g. Both products were examined at selected intervals for numbers of *L. monocytogenes*, mesophilic aerobic bacteria, lactic acid bacteria, and yeast/mold. Films containing 1.5 and 3.0% (w/v) sorbic acid decreased *L. monocytogenes* populations 0.1-1 log and 4.0-7.0 logs on cheese and bologna after 35 and 28 days refrigerated storage, respectively. Potential reduction in the number of mesophilic and lactic acid bacteria was

also found on both cheese and bologna using film containing sorbic acid. Mold growth was found only on cheese wrapped with sorbic acid-free film.

The migration of sorbic acid from PVDC antimicrobial films into Cheddar cheese and beef bologna was determined using high performance liquid chromatography (HPLC). At the end of refrigerated storage, 40 and 93% of the sorbic acid migrated from the film into Cheddar cheese and bologna, respectively. The rate constant for Cheddar cheese was 0.007 per day, and for bologna was 0.040 per day.

To my mother

Panadda Amornjarusiri

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INTRODUCTION

Contamination of food by foodborne pathogens and spoilage microorganisms is of great concern to the food industry. Microbial contamination of food may occur in post-processing during packaging and distribution. *Listeria monocytogenes* continues to pose a major threat to the food industry as a post-processing contaminant. Commonly found in home refrigerators (Ziney and Debevere, 1998), this psychotropic pathogen can readily contaminate refrigerated foods and grow to potentially hazardous levels. *L. monocytogenes* has remained the leading cause of Class I microbiologically related recalls for more than 15 years. Class I is a health hazard situation where there is a reasonable probability that the use of the product will cause serious, adverse health consequence or death. A multistate outbreak involving at least 100 cases of listeriosis in 22 states from consumption of hot dogs during August 1998 to February 1999 (which caused 21 fatalities) heightened concerns regarding foodborne listeriosis (CDC 1999). From April 1998 to October 2001 there were more than 75 million pounds of cooked ready-to-eat meats from over 75 Class I recalls (USDA-FSIS, 2001).

L. monocytogenes can cause mastitis leading to excretion of the organism in milk from infected animals (Gitter et al, 1980). Ryser and Marth (1987) reported that *L. monocytogenes* can survive more than 1 year in Cheddar cheese. In cottage cheese *L. monocytogenes* survived during fermentation and manufacture (Hicks and Lund 1991, Ryser et al., 1985) with viable cells recovered from refrigerated cheese (Piccin and Shelef, 1995). The outbreaks originated in both domestic and imported cheese (Ryser and Marth, 1988). In 2000, there were 2,298 cases of listeriosis reported in the US,

which cost approximately $1 million per case, thus it is the costliest foodborne disease on a per-case basis (USDA-FISS, 2001).

Antimicrobial films may be an effective approach to inhibit foodborne pathogens or spoilage microorganisms, to enhance food safety and decrease product spoilage. Incorporation of chemical preservatives or antimicrobial agents into a film may provide a way to enhance microbial safety. An antimicrobial agent in films can diffuse into the food to inactivate target microorganisms. Weng and Hotchkiss (1992) developed a polyethylene-based antimycotic film using the antimycotic substance imazalil to inhibit mold growth on the surface of cheese. Han and Floros (1997) incorporated potassium sorbate into low-density polyethylene film to inhibit the growth of yeast (*Saccharomyces cerevisiae*). Nisin and lysozyme in combination with EDTA can be used in corn zein and soy protein films to inhibit *Escherichia coli* O157: H7 (Padgett et al., 1998).

The research hypothesis of this study is that an antimicrobial food packaging polyvinylidene chloride (PVDC) film can be made by incorporating some well-known antimicrobial agents in the PVDC. In order to prove the hypothesis the objectives of this work were:

(1) To develop antimicrobial packaging films using nisin, lactoferrin, sorbic acid, potassium sorbate or sodium diacetate.

(2) To determine the antimicrobial activity of the films against the *Listeria monocytogenes* in laboratory media.

(3) To verify the film's antimicrobial activity against *L. monocytogenes* on two food products, Cheddar cheese and beef bologna.

(4) To assess the barrier, water and oxygen permeability, and mechanical properties, and surface energy of the antimicrobial film. Electron microscopy is used to assess the three-dimensional structure of the film.

(5) To verify the antimicrobial activity of the films coated on base film, polyethylene terephthalate (PET), against the foodborne pathogen *Listeria monocytogenes* in laboratory media.

(6) To determine the migration rates of the antimicrobial agent (sorbic acid) from the film into the food, Cheddar cheese and beef bologna.

CHAPTER 1

LITERATURE REVIEW

1.1. ANTIMICROBIAL FILM

Antimicrobial films have been in continuing development since the 1990s (Rice, 1995). The potential application of antimicrobial films is mostly in food packaging to reduce surface contamination of solid or semisolid food. When antimicrobial agents are incorporated into a film, the film may then have the ability to prevent or inhibit microbial growth (Han, 2000). Antimicrobial films can be classified into two types. The first type is film containing antimicrobial agents that migrate to the surface of the packaging material, and then migrate to the food inhibiting food spoilage organism or foodborne pathogen. The second type is film containing antimicrobial agents that move to the surface of the film, and inhibit microbial growth on food surfaces without migration of the active agent into the food. Several synthetic and naturally occurring compounds have been proposed as antimicrobial agents in packaging film (Table 1.1). Brody et al. (2001) listed the citeria that should be considered when developing antimicrobial films:

- Consideration of the spectrum of microorganisms against which the package system might be effective. Films may inhibit food spoilage organisms without affecting the growth of pathogenic microorganisms, which will raise safety questions similar to those arising from technologies such as modified atmosphere packaging.

Table 1.1. Antimicrobial agents used in food packaging (Hotchkiss, 1995)

Class	Examples
Organic acids	Propionic, benzoic, sorbic acid
Bacteriocins	Nisin
Spice extracts	Thymol, *p*-cymene, wasabi
Thiosulfinates	Allicin
Enzymes	Peroxidase, lysozyme
Proteins	Conalbumin
Isothiocyanates	Allylisothiocyanate
Antibiotics	Imazalil
Fungicides	Benomyl
Chelating agents	EDTA
Metals	Silver
Parabens	Heptylparaben

- Consideration of the effects the antimicrobial additive may have on the mechanical and physical properties of the plastic packaging material structure.
- Does the antimicrobial activity cause a reduction in growth rate, while there is still an increase in cell numbers, or does it cause cell death, with a decline in cell number?
- Consideration of the migration of the antimicrobial agent into the food, and any toxicological and regulatory concern that may exist.
- Consideration of the effect of food characteristics such as pH and water activity (Aw). Some antimicrobial agents are effective only at a specific pH.

For food packaging applications or direct contact with humans, safety must be ensured. The antimicrobial agents used in films have to be approved by the Food and Drug Administration (FDA). Ben-Yehoshua et al. (1987) incorporated Imazalil (a commercial antimycotic) into low density polyethylene (LDPE) film for wrapping fruits and vegetables. This film also effectively prevented mold growth on cheese surfaces. Imazalil is not, however, approved for use with cheese in the United States. Metallic ions of silver and copper are regarded in Japan (Brody et al., 2001) and in the US as safe antimicrobial agents.

Antimicrobial films may include antimicrobial packaging films and antimicrobial edible films. An example of an antimicrobial packaging film is LDPE impregnated with benzoic anhydride. This film exhibited antimycotic activity on laboratory media and cheese (Hotchkiss, 1995). Nisin and lysozyme in combination with EDTA in corn zein

and soy protein films inhibited *Escherichia coli* O157: H7 (Padgett et al., 1998), and is an example of an antimicrobial edible film.

1.2. ANTIMICROBIAL SUBSTANCES

A chemical preservative can be incorporated in a packaging material to add antimicrobial activity. Common antimicrobial chemicals for food products include bacteriocins (e.g. nisin), organic acids (such as sorbic acid and its salts, benzoic acid and sorbate), parabens, curing agents (e.g. sodium chloride), natural preservatives (e.g. wasabi used in Japan), lactoferrin, and metals (e.g. silver ions). Antimicrobial agents which may have possible use in antimicrobial packaging films will be addressed individually.

1.2.1. Bacteriocins

Nisin

Nisin, an antimicrobial agent, belongs to a unique group called bacteriocins. Bacteriocins are defined as a group of microbially synthesized proteinaceous antimicrobial substances, which have a narrow spectrum of inhibition and only inhibit bacteria closely related to the producer organism. Nisin is a small peptide bacteriocin produced by several *Lactococcus lactis* strains (Sahl et al., 1995). It is produced during the exponential phase of bacterial growth (Buchanan et al., 1988). The complete structure was elucidated by Gross and Morell (1971) and confirmed by chemical synthesis (Fukase et al., 1988), DNA sequencing and NMR resonance (Chan et al., 1989). Nisin is composed of 34 amino acids (AA) (Jung, 1991) and possesses amphiphilic characteristics with clusters of hydrophilic and hydrophobic residues at the C and N-terminus,

respectively. Gross and Morell (1967; 1971) identified didehydroalanyllysine at the C-terminal and isoleucine as the N-terminal AA. Nisin contains a number of uncommon amino acids, which are formed in a post-translational modification process including ∞ and β unsaturated amino acids, dehydroalanine (Dha), β-methyldidehydroalanine or didehydrobutyrine (Dhb) and thioether amino acids lanthionine and β-methyllanthionine (Rollema et al., 1996). It is suggested that the unusual amino acids might be responsible for important properties of the nisin molecule such as acid tolerance, thermostability, and its bactericidal mode of action.

Nisin was the first bacteriocin to attain GRAS (General Recognized As Safe) status by FDA. Nisin is active against a broad range of gram-positive microorganisms (e.g. *L. monocytogenes* and *Clostridium botulinum*). It has been used as a preservative in cold-pack cheeses (Delves-Broughton, 1990).

The exact molecular mechanism by which nisin inhibits microbial growth is still not fully understood, although several studies have found that nisin interacts with the bacterial cell membrane forming pores ultimately leading to cell lysis (Hurst, 1981; Gao et al., 1991; Driessen et al., 1995; Sahl et al., 1995). When combined with chelating agents such as EDTA, nisin effectively inhibits Gram-negative bacteria such as *Salmonella* and *Escherichia coli* (Shelef et al., 1995; Wells et al., 1998). The chelating agent reacts with the outer membrane (lipopolysaccharide) of gram-negative bacteria to allow nisin to penetrate the cell membrane, forming pores which induces cell lysis (Stevens et al., 1991).

1.2.2. Organic acids

Sorbic acid and sorbate

Sorbic acid is a well-known food preservative and is classified as GRAS. It is used in bakery, meat products, fruit and dairy products to inhibit a wide range of yeasts (Lueck, 1980), molds such as *Fusarium*, *Aspergillus*, *Mucor* (Sofas and Busta, 1981), and bacteria including *E. coli* 0157:H7, *Staphylococcus aureus*, *C. botulinum* and coliform (Kasrazadeh and Genigeorgis, 1995; Gandhi et al., 1973; Briozzo et al., 1985). It can also inhibit aflatoxin and enterotoxin production (Luck, 1980). The typical usage level in food is from 0.02% to 0.3%. Sorbic acid ($CH_3CH=CHCH=CHCOOH$) is a straight chain α, β-unsaturated monocarboxylic acid (Windholdz et al., 1976).

Potassium sorbate is the well-known salt of sorbic acid, which is highly soluble in water. Sorbic acid is most effective in its undissociated form (pKa = 4.8). Sorbic acid exists in its undissociated (86%) form at pH 4.75 (Sofos et al., 1980) which is able to penetrate the bacterial cytoplasmic membrane (Chichester and Tanner, 1972). Sorbic acid is most effective in acidic foods (Cowles, 1941).

Sorbic acid induces changes in the morphology and appearance of microbial cells. Sorbic acid can inhibit specific biosynthesis pathways (Sofos et al., 1986). For example, sorbate prevents the amino acids, L-serine and L-histidine from being taken up by *Salmonella Typhimerium* at low pH (Tuncan and Martin, 1985). Sorbic acid also produced pores on cell membrane (Freese and Levin, 1978). Induction of an energy-expensive protective membrane has been reported as one mode of action. Proton pumping increases (H^+, ATPase) to ensure that the pH will not decline or compensate for

any disruption of intracellular pH to maintain homeostasis, which results in less available energy for normal growth.

Benzoic acid and benzoate

Benzoic acid and its sodium salt (sodium benzoate) are also GRAS preservatives. Benzoic acid is well known as a mold and yeast inhibitor, and it can also inhibit pathogenic bacteria. The antimicrobial activity of benzoic acid against foodborne pathogen increases when combined with organic acids. Huwang et al. (1995) showed that the population of *L. monocytogenes*, *Escherichia coli* O157:H7, *Salmonella*, *Campylobacter jejuni* and *Staphylococcus aureus* decreased significantly on raw chicken wings treated with 0.05% sodium benzoate/0.5% lactic acid solution (pH 2.6) for 30 minutes during storage at 4°C. The typical use level is up to 0.1% in commercial foods. The antimicrobial activity of benzoic acid is related to pH. The pKa of benzoic acid is 4.2. It is most effective in its undissociated form with 60% undissociated at pH 4.0. Benzoic acid induces change in the morphology and appearance of microbial cells. Benzoic acid also reduces the intracellular pH. Salmond et al. (1984) reported on the reduction in intracellular pH in *E. coli*. Benzoic acid also alters cell membrane function by producing pores that interfere with the uptake of substrate, electron transport and proton-motive forces.

1.2.3. Parabens

Parabens are made by esterification of the carboxyl group of benzoic acid (phenolic derivatives). Parabens in the undissociated form (active form) exist at pH 3.0-8.0. Parabens are used as food preservatives in the methyl, propyl and heptyl forms. Parabens with longer alkyl chains possess more antimicrobial activity than those with

shorter alkyl chain (Shibasaki, 1969). Parabens are mostly used in butter, margarine, maple syrup and meat products. Parabens are generally more active against molds and yeasts than against bacteria. Parabens are more effective against gram-positive bacteria than gram-negative bacteria.

Parabens damage the cytoplasmic membrane of microorganisms leading to the release of cell cytoplasmic compounds (Judis, 1963). Furr and Russel (1972) reported that parabens caused leakage of RNA in *Serratia marcescens*, with the extent of leakage proportional to their alkyl chain length. Parabens were also reported to inhibit membrane transport, electron transport, and nutrient uptake through the cytoplasmic membrane (Freese et al., 1973; Eklund, 1980).

1.2.4. Curing agent

Sodium chloride

Sodium chloride (NaCl) is a well-known curing agent used as a food preservative since ancient times. The antimicrobial activity of sodium chloride is related to its ability to reduce water activity in food (plasmolytic effect). Microbial cells lose water when the water activity of the external environment is reduced, which results in growth inhibition or cell death (Sperber, 1983). Sodium chloride also limits oxygen solubility (osmotic effect) and alters pH (Banward, 1979). While most foodborne pathogens are susceptible to sodium chloride, *Staphylococcus aureus*, can grow at low water activity, and *Listeria monocytogenes*, is a salt-tolerant pathogen that can grow at concentrations of up to 10% NaCl.

1.2.5. Natural preservative

Wasabi

Wasabi is a compound extracted from Japanese horseradish. Japan's Sekisui Jushi has developed an antimicrobial food packaging material by incorporation of a wasabi derivative, allylisothiocyanate (AIT), into polyethylene film (Brody et al., 2001). This packaging material is claimed to inhibit the proliferation of bacteria and fungi on food surfaces, thus extending product shelf life. This antimicrobial film is intended to be placed between food layers such as in a lunch box or ready-to-eat foods. It is now available to consumers in Japan.

An allylisothiocyanate (AIT) substance has received approval to be used as a food additive to deliver wasabi flavor in food (Brody et al., 2001). It is known to have antimicrobial activity against bacteria and fungi. However, it has a strong odor and pungent taste. Therefore, when used as an antimicrobial, this substance is generally used indirectly in the packaging material.

1.2.6. Lactoferrin

Lactoferrin is a newly isolated antimicrobial peptide derived from bovine lactoferrin present in cow's milk (Wakabayashi et al, 1992). It is an iron binding glycoprotein, which can bind two iron atoms per molecule. Lactoferrin has antimicrobial activity against many bacteria including *Listeria monocytogenes*, *Bacillus subtilis*, *B. stearothermophilus*, *Micrococcus* spp., and *E. coli* (Hutchens et al, 1994; Payne et al., 1990; Reiter, 1978; Oram and Reiter, 1968). Lactoferricin B is the active component of lactoferrin, containing 25 amino acid residues, and can be isolated by acid-pepsin

hydrolysis from the N-terminal region of the molecule (Bellamy et al., 1992; Wakabayashi et al, 1992).

Lactoferrin chelates iron, calcium and magnesium ions, causing cell dealth. Payne et al. (1989) reported that the inhibition of *L. monocytogenes* using lactoferrin was directly related to iron availability in the medium because *L. monocytogenes* survives best in iron-rich media. Arnold et al. (1982) reported that lactoferrin inhibited several bacteria in an iron-rich environment. For gram-negative bacteria such as *E. coli*, lactoferrin caused the chelation of cations that stabilize lipopolysaccharides, which increased permeability of the outer membrane to hydrophobic compounds.

1.2.7 Silver ion

Among metallic ions, silver ion has the strongest antimicrobial activity (Brody et al., 2001). Metallic silver does not release the ion easily, compared with other metallic ions. Therefore, its antimicrobial activity is not as strong in its metallic state, and its greatest potential appears to be as releasable silver salts such as silver nitrate and Ag-zeolite. Silver nitrate, which forms silver ions in a water solution, has strong antimicrobial activity. Microbial cells absorb the silver ion by active transportation, inhibiting a range of metabolic enzymes which inhibit metabolic processes necessary for sustaining life (Brody et al., 2001). Ag-zeolite maintains Ag^+ ions in a stable and effective condition. It has antimicrobial activity against bacteria (no effect against spores of heat-resistant bacteria), yeast and fungi (Brody et al., 2001). For application of Ag-zeolite to packaging, it is usually laminated as a thin coextruded layer (3-6 μm) because of its expense.

1.3. *LISTERIA MONOCYTOGENES* AND FOODBORNE DISEASE

Listeria monocytogenes is a foodborne pathogen, and is of major concern to the food industry. *L. monocytogenes* is a gram-positive, non-spore-forming, rod-shaped bacterium (Figure 1.1). *L. monocytogenes* grows at temperatures of 1-45 °C, with the optimum growth at 30-37 °C. *L. monocytogenes* can cause the human disease called listeriosis. *Listeria monocytogenes* is ubiquitous in nature, being commonly found in soil, water, silage and plants. Consequently, this pathogen is frequently found on raw materials used in food processing. Cox et al. (1989) demonstrated that *Listeria* spp. could be found in all types of food production environments. *L. monocytogenes* also has the ability to attach to various food contact surfaces such as stainless steel, polypropylene, glass and rubber (Herald and Zottola, 1988; Mafu et al., 1990; Blackman and Frank, 1996). Once present in the processing environment, control of *L. monocytogenes* has proven to be difficult. *L. monocytogenes* is present in various foods. Dairy foods have received the most scrutiny as vehicles for listeriosis. Dairy products can be particularly susceptible to contamination by *Listeria* because cows can shed the organism in the milk. Thus, contaminated raw milk could serve to introduce the bacterium into dairy plants or foods made from raw milk. Contamination of *L. monocytogenes* can be found in poultry and meat products including processed meat products such as fermented sausage. During August 1998 to February 1999, one listeriosis outbreak from hot dogs resulted in 101 cases of listeriosis (including 21 fatalities) in 22 states and greatly heightened concerns regarding the presence of *L. monocytogenes* in ready-to-eat foods (CDC 1999). *L. monocytogenes* can be found in

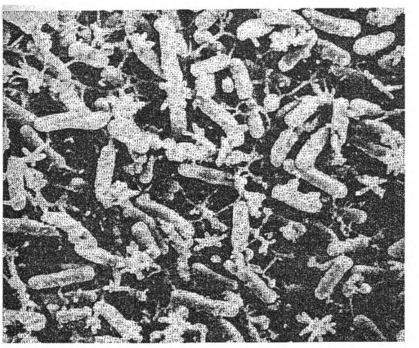

Figure 1.1. Electron micrograph of Listeria monocytogenes (From *Listeria*, Listeriosis and Food Safety; Ryser and Marth, 1991)

slaughterhouse and meat packaging areas. Fruits and vegetables can be contaminated with *L. monocytogenes*. In 1981, there was an outbreak involving cabbage (Schlech et al., 1983). It was concluded that the sheep manure used to fertilize the cabbage was the source of the contamination. Anyone can become infected with *L. monocytogenes*. However, some groups of people are more susceptible than others, including pregnant women, newborns, infants, and adults with a compromised immune system such as cancer patients. For listeriosis in pregnant women, infection commonly is manifested by fever, chills, headache, backache, and discolored urine. The flu-like symptoms are the expression of *Listeria* bacteremia, and *Listeria monocytogenes* can be isolated from blood. Infection in pregnant women leads to infection of the fetus either via the transplacental route or during delivery (Ryser and Marth, 1991). Furthermore, in some cases abortion or stillbirth occurred immediately after the mother experienced flu-like symptom (Ryser and Marth, 1991). Neonatal listeriosis is now among the most dangerous forms of listeriosis, and is a major cause of fetal damage and infant death. The symptoms of neonatal listeriosis include vomiting, refusal to drink, respiratory distress, heart failure and forced respiratory (Ryser and Marth, 1991). *L. monocytogenes* can also cause meningitis in healthy adults.

CHAPTER 2.

DEVELOPMENT OF A FOOD PACKAGING FILM WITH ANTIMICROBIAL ACTIVITY

2.1. ABSTRACT

Polyvinylidene chloride copolymer films containing nisin or potassium sorbate or sorbic acid were developed to have antimicrobial properties against four strains of *Listeria monocytogenes* (CWD 95, CWD 246, CWD 201 and CWD 1503). The minimum inhibitory concentrations of nisin, sorbic acid and potassium sorbate were 1%, 1.5% and 2%, respectively. Films containing 1%-2.5% nisin yielded average inhibition zones of 18.7 to 26.7 mm. Films containing 2% and 3% potassium sorbate had average inhibition zones of 18.3 and 22.0 mm, respectively, whereas films containing 1.5%-3%sorbic acid yielded average inhibition zones of 20.5-32.7 mm. Incorporating nisin, potassium sorbate or sorbic acid increased moisture and oxygen permeability (except for films containing sorbic acid), decreased tensile strength, and toughness of the films, and significantly altered elongation at break. Surface energy of the films remained unchanged after incorporating these antimicrobial agents. The three-dimensional structure of the films was also observed using an environmental scanning electron microscope (ESEM). Films containing sorbic acid were partly homogenous, while films containing nisin had nisin particles distributed throughout the film. Adding potassium sorbate caused pits/minuscule holes throughout the film structure.

2.2. INTRODUCTION

Contamination of food by foodborne pathogens and spoilage microorganisms is of great concern in the food industry. Microbial contamination of food may occur during post-process handling, packaging and distribution. *Listeria monocytogenes*, which causes listeriosis, is the major cause of class I microbiologically related recalls. Microbial

growth on food surfaces is the key determinant of spoilage and safety for many food products including refrigerated meats and intermediate moisture foods (IMF) (Torres et al, 1985; Vojdani and Torres, 1989a, b; Rico-Pena and Torres, 1991; Roth and Loncin, 1985). Several approaches have been developed to reduce the risk of surface contamination.

Antimicrobial films are a new and effective approach to inhibit foodborne pathogens or spoilage microorganisms, to enhance food safety and decrease product spoilage. Incorporating of chemical preservatives or antimicrobial agents into the film may provide a way to enhance microbial safety. Antimicrobial agents in films can diffuse into the food to inactivate target microorganisms. Weng and Hotchkiss (1992) developed a polyethylene-based antimycotic film using imazalil to inhibit mold growth on the surface of cheese. Han and Floros (1997) incorporated potassium sorbate into low-density polyethylene film to inhibit the growth of yeast (*Saccharomyces cerevisiae*). Nisin and lysozyme in combination with EDTA were similarly used in corn zein and soy protein films to inhibit *Escherichia coli* O157: H7 (Padgett et al., 1998).

Common antimicrobial agents include organic acids such as sorbic, lactic, acetic and benzoic acids and bacteriocins such as nisin. Nisin, the best-known antimicrobial peptide and most widely studied bacteriocin, is a secondary metabolite from *Lactococcus lactis* subsp. *lactis*. This natural compound has been well characterized as a food preservative and has attained GRAS status. Nisin inhibits a broad spectrum of gram-positive pathogenic bacteria such as *L. monocytogenes* and *Clostridium botulinum* (Shefet et al, 1995), as well as bacterial spores (Stevens et al, 1991). Hansen (1994) stated that nisin was a model food preservative.

Lactoferrin is an antimicrobial peptide derived from bovine lactoferrin present in cow's milk (Wakabayashi et al, 1992). The susceptibility of *L. monocytogenes* to bovine lactoferrin has been studied on laboratory media (Hutchens et al, 1994). Sodium diacetate has been studied for its antimicrobial activity against foodborne pathogens and spoilage microorganisms. Sodium diacetate had antimicrobial activity against *L. monocytogenes* in brain heart infusion broth (Shelef and Addala, 1994) and turkey breast meat (Schlyter et al., 1993). Sorbic acid and potassium salts (potassium sorbate) are well-known food preservatives that have attained GRAS status. They are effective inhibitors of most molds, yeasts and some bacteria. Sorbic acid in combination with lactic acid or acetic acid can be successfully used to inhibit *L. monocytogenes* in cold-pack cheese and many low acid foods (Ryser and Marth, 1988). Potassium sorbate has been used to preserve meat products including refrigerated packaged beef (Zamora and Zaritzky, 1987a, b).

The objectives of this work were to (1) develop an antimicrobial film wrap for foods by incorporating nisin, lactoferrin, sodium diacetate, potassium sorbate or sorbic acid into a polyvinylidene chloride copolymer film, with targeted inhibition of *L. monocytogenes*, (2) assess the effect of film casting technique on mechanical and barrier properties including water vapor and oxygen permeability, tensile strength, and surface energy of the test films, and (3) examine the morphological characteristics of the films using scanning electron microscopy, and (4) verify the antimicrobial activity of film coated on base film (PET) against *L. monocytogenes*.

2.3. MATERIALS AND METHODS

2.3.1. Film preparation

To make film, polyvinylidene chloride (PVDC) copolymer resin (Saran^R F-310, Dow Chemical, Midland, MI) (18% w/v) was added to methyl ethyl ketone (J.T Baker, Phillipsburg, NJ) at room temperature and continuously agitated until completely dissolved. Then, 0.5%, 1%, 1.5%, 2% or 3% (w/v) of either nisin, lactoferrin, sodium diacetate, potassium sorbate, or sorbic acid (Sigma Chemical Co., St. Louis, MO) was added. For film with pH 5.2, film solution was adjusted to pH 5.2 using lactic acid (1 N) and 2 M sodium hydroxide (Sigma). The original pH of film was 3.5. PVDC film solutions (14 ml) were cast in a glass petri dish (150 mm diameter× 15 mm height) or 40 ml solutions were cast to a glass plate (9 × 13 inches). Films containing sorbic acid, potassium sorbate or sodium diacetate were dried in a hot air oven at 86 ± 0.5 °C for 5 minutes and peeled off from the plates. Films containing nisin or lactoferrin were dried at room temperature (23°C) in a chemical hood with airflow of 45 ft/min, for 2-3 hours or until dried, and peeled from the plates. The thickness of the film was determined using a micrometer (model 549, Testing Machines Inc., Amityville, NY). The average thickness was obtained from 10 measurements from different locations on each sample.

2.3.2. Coating film preparation

To coat films, Saran^R F-310 (Dow Chemical, Midland, MI) at a concentration of 18% w/v was dissolved gradually in methyl ethyl ketone (J.T Baker, Phillipsburg, NJ) at room temperature with continuous agitation until completely dissolved. Sorbic acid concentration 1.5%, 2.0% or 3.0% (w/v) (Sigma Chemical Co., St. Louis, MO) was incorporated into the coating solution. This solution was coated on the base film, 0.5 mil

polyethylene terephthalate (PET) film (Dow Chemical, Midland, MI), using a wire-rod and dried at 86 °C. The wire-rods (Dow Chemical, Midland, MI) were fabricated by the Saran Barrier Division at Dow Chemical (Midland, MI). The coating thicknesses were 0.2, 0.5 and 0.75 mil ± 0.03 mil.

2.3.3. Culture preparation

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 MSU culture collection (Dept. of Food Sciences and Nutrition, Michigan State University, East Lansing, MI). The cultures were maintained at –70°C in trypticase soy broth (TSB) (Difco Laboratories, Detroit, MI) containing 10% (v/v) glycerol (J.T. Baker, Phillipsburg, NJ) and subcultured twice in TSB containing 0.6% (w/v) yeast extract (Difco) at 35 °C/ 18-24 hours before use (Figure 2.1).

2.3.4. Disc diffusion-type assay

Antimicrobial films were cut aseptically into 16-mm diameter discs using a sterile cork borer and sterilized using UV light for 20 minutes. These discs were then aseptically placed on 20 ml melted trypticase soy agar (TSA) containing 0.6% yeast extract (TSA-YE)(Difco) (acidified to pH 5.2 using 1 N hydrochloric acid) which was previously inoculated with 0.2 ml of an 18-24 h *L. monocytogenes* culture. Following incubation at 35 °C for 48 hours, the diameter of inhibition zone around each antimicrobial film disc was measured to the nearest millimeter (Figure 2.2-2.3). An average of two measurements was used as the result. All experiments were replicated three times.

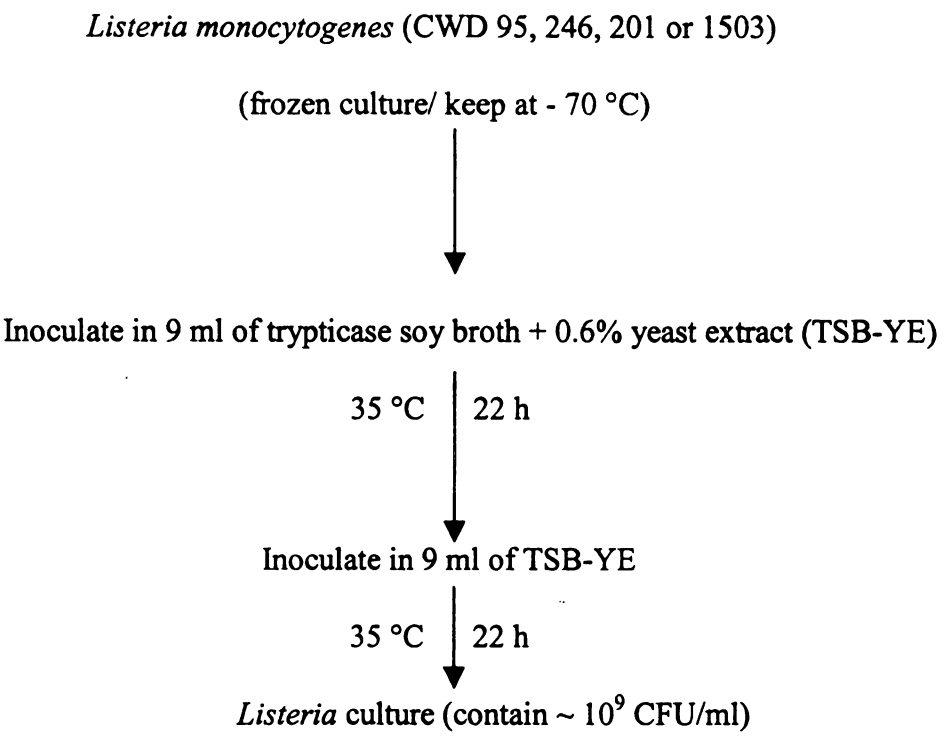

Figure 2.1. *Listeria monocytogenes* culture preparation for using as *Listeria* stock culture for disc diffusion-type assay.

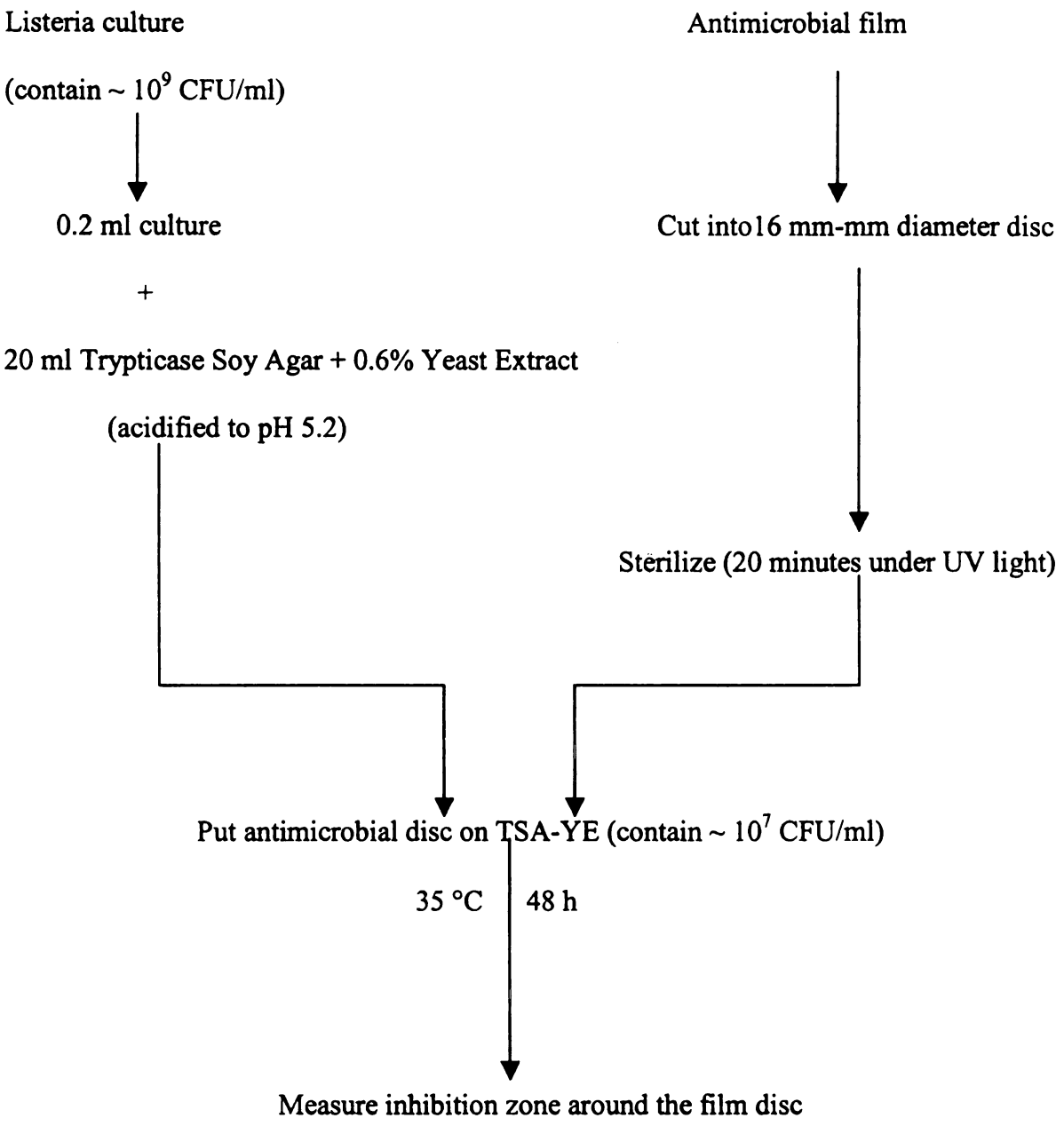

Figure 2.2. Disc diffusion-type assay for measuring antimicrobial activity of inhibition zone of the films against *Listeria monocytogenes*.

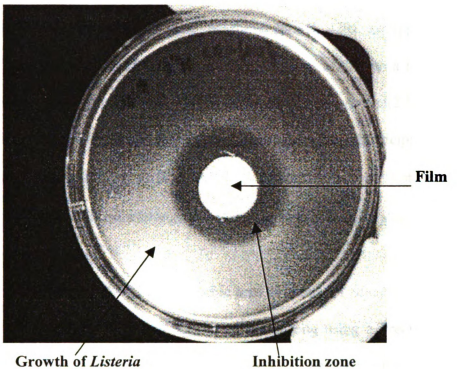

Figure 2.3. Inhibition zone of the foodborne pathogen *Listeria monocytogenes* around the disc of polyvinylidene copolymer film containing sorbic acid after 48 hours at 35 °C

2.3.5. Mechanical properties

Tensile properties of the films were determined according to ASTM D 882-91 using the INSTRON Universal Testing Machine Model 4201 equipped with an INSTRON Chart Recorder (INSTRON Corporation, Canton, MA) with a load cell of 1 kN and crosshead speed of 50.8 cm/min. Films were cut into strips (2.54 cm width) using a Precision Sample Cutter (Thawing Albert Instrument Co., Philadelphia, PA), and conditioned according to ASTM D 618 at 23 ± 2 °C/ 50% ± 5% RH. All tests were run at 23 ± 2°C/50% ± 5% RH. Tensile strength, % elongation and toughness were determined.

2.3.6. Seal strength testing

Films containing 1.5%, 2% or 3% sorbic acid (without pH adjust to 5.2) were cut into strips 2.54 centimeters wide and 25.4 centimeters long using a Precision Sample Cutter (Thawing Albert Instrument Co., Philadelphia, PA). Each strip was folded in half (lengthwise) and sealed approximately 0.5 cm from the fold with a heat sealer (Sencorp Systems Inc., Hyannis, MA). The sealing condition was set at 250 °F for 1 second with the pressure at 35 psi (lb/in^2). The seals were slit using scissors along the fold line of each sample. The samples were conditioned according to ASTM D 618 at 23 ± 2°C/50% ± 5% RH not less than 40 hours before testing. Seal strength was determined according to ASTM D 882-91 using an INSTRON Universal Testing Machine Model 2401 (Canton, MA) with a load cell of 1 kN and crosshead speed of 50.8 cm/min. The test conditions were at 23 ± 2 °C/ 50% ± 5% RH.

2.3.7. Water vapor permeability

The MOCON PERMATRAN-W 3/31 system (MOCON/Modern Controls, Inc., Minneapolis, MN) was used to determine the water vapor transmission rate. The rate of

permeation was calculated at steady state (no further change in the permeation rate). To test films, the film sample was prepared by putting the sample on an aluminum mask (MOCON/Modern Controls, Inc., Minneapolis, MN) having a test area of 5 cm^2. The aluminum mask with the film sample was placed into the cell chamber. The sample was calibrated at the test condition for two hours before starting the test process. The test condition was 37.8 °C (100 °F) and 90% relative humidity (RH) on one side and 0% on the other side, which is in accordance with ASTM D 895, Standard Test Method for Water Vapor Permeability of Packages. The test process was automatically started after two hours of calibration and the test was ended after steady state was reached.

2.3.8. Oxygen permeability

An Oxtran 100 (MOCON/Modern Controls, Inc., Minneapolis, MN) was used to measure oxygen gas permeability. The test was performed at 23°C and 0% relative humidity (RH). The film sample was put on the aluminum mask (MOCON/Modern Controls, Inc., Minneapolis, MN) having a test area of 5 cm^2. The film was calibrated on the machine for 2 – 3 hours before testing. The test ended after steady state was reached. The result was obtained from the chart recorder (MOCON/Modern Controls, Inc., Minneapolis, MN).

2.3.9. Surface energy

ACCU DYNE TEST marker pens (Diversified Enterprises, Claremont, NH) were used to measure the surface energy of films. This test parallels ASTM D 2578-84. The films were conditioned at 23 ± 2°C/50% ± 5% RH according to ASTM 618-81 for at least 40 hours before testing. The test was also performed at 23 ± 2°C/50% ± 5% RH. First, the sample was placed on a clean and smooth area. Then the selected ACCU DYNE

TEST marker pen was pressed firmly down against the sample in three parallel passes and only the result from the third pass was observed (the first and second passes were ignored). If the ink swath beaded up or tore apart or shrank into a thin line within one second or less, it meant that the dyne level (interpreted as surface energy) used was reading higher surface energy than the actual surface energy of the film sample. Then, the test would be repeated with a next lower dyne level marker (retesting was conducted at a different location on the sample). If the ink held or stood for one to three seconds before losing its integrity, that indicated that the dyne level of the marker closely matched the surface energy of the film sample.

2.3.10. Scanning electron microscopy (SEM)

The environmental scanning electron microscope (ESEM), model 2020 configured with a lanthium hexaboride (LaB6) filament, manufactured by ElectroScan (FEI company, Hillsboro, Oregon) was used to observe the films three-dimensional structure. The acceleration voltage ranged between 10 and 20 kV, while the water vapor pressure ranged between 2 and 3 Torr. The specimens were examined in their natural state (no conductive coating).

2.3.11. Differential scanning calorimetry (DSC)

Differential Scanning Calorimeter (TA Instruments, New Castle, DE) was used to determine the heat seal temperature range of Saran^{R} F-310 without antimicrobial agents (control film) and film containing 3% w/v sorbic acid. The heating rate was used 10 °C /min and the weight of the samples was 3 mg. Nitrogen gas flow rate was 50 cc/min.

2.4. RESULTS AND DISCUSSION

2.4.1. Antimicrobial properties

Antimicrobial-free film (control film; no antimicrobials) showed no antimicrobial activity. Films containing nisin, and adjusted to pH 5.2 showed antimicrobial activity at a minimum concentration of 1.% w/v (Table 2.1). Films containing 0.5% to 2.5% (w/v) lactoferrin either with or without a pH adjusted to 5.2 showed no antimicrobial activity. Films containing potassium sorbate in solutions of 0.01 N lactic acid and in distilled water showed antimicrobial activity. Films containing potassium sorbate solution in 0.01 N lactic acid showed antimicrobial activity at a concentration of 2% w/v, while film containing potassium sorbate solution in distilled water had antimicrobial activity at minimum concentration of 5% w/v (Table 2.2). Therefore, a potassium sorbate solution of 0.01 N lactic acid was selected for all remaining experiments with potassium sorbate. Film containing potassium sorbate could be made only with adjustment to pH 5.2, otherwise the film could not be cast and peeled off. Films containing sodium diacetate in solution of 0.01 N lactic acid or in distilled water, with or without adjustment to pH 5.2 showed no antimicrobial activity. Film containing lactoferrin either with or without adjustment to pH 5.2 showed no antimicrobial activity. Film containing sorbic acid with and without adjustment to pH 5.2 showed antimicrobial activity at a minimum concentration of 1.5% (w/v) (Table 2.3). Sorbic acid appeared to be the most compatible with the Saran^{R} F-310 solution among the five antimicrobial agents considered, based on the apparent solubility of the substances in the Saran F-310 solution. In addition, the Saran^{R} F-310 films containing sorbic acid had the best physical appearance. PVDC films containing nisin, potassium sorbate or sorbic acid had the most antimicrobial activity.

Table 2.1. Antimicrobial activity of Saran^{R} F-310 containing nisin against 4 strains of *L. monocytogenes*

Film / Concentration (w/v)	Diameter of Inhibition Zone (mm)			
	CWD 95	CWD 1503	CWD201	CWD 246
Nisin (adjusted to pH 5.2)				
0.5%	0	0	0	0
1%	21.8 ± 0.3^{a}	22.3 ± 0.6^{a}	18.67 ± 0.6^{a}	19.33 ± 1.2^{a}
2%	23.7 ± 0.6^{a}	23.0 ± 1.0^{a}	19.83 ± 0.3^{ab}	21.67 ± 1.2^{ab}
2.5%	26.5 ± 0.5^{b}	26.7 ± 1.5^{b}	21.00 ± 1.0^{a}	23.67 ± 0.6^{b}

Mean ± standard deviation. Means in the same column with different superscripts were significantly different ($p < 0.05$).

Table 2.2. Antimicrobial activity of Saran^{R} F-310 containing potassium sorbate against 4 strains of *L. monocytogenes*

Film	Diameter of Inhibition Zone (mm)			
	CWD 95	CWD 1503	CWD201	CWD 246
Potassium sorbate (in 0.01 N lactic & adjusted to pH 5.2)				
0.5% w/v	0	0	0	0
1% w/v	0	0	0	0
1.5% w/v	0	0	0	0
2% w/v	20.3 ± 0.6^{a}	20.7 ± 0.6^{a}	18.3 ± 0.6^{a}	18.7 ± 0.6^{a}
3% w/v	21.7 ± 0.6^{a}	22.0 ± 1.0^{a}	19.0 ± 1.0^{a}	20.3 ± 0.6^{a}
Potassium sorbate (in distilled water & adjusted to pH 5.2)				
0.5% w/v	0	0	0	0
1% w/v	0	0	0	0
2% w/v	0	0	0	0
3% w/v	0	0	0	0
5% w/v	24.3 ± 1.2	24.3 ± 0.6	21.3 ± 0.6	23.0 ± 1.0

Mean ± standard deviation. Means in the same column with different superscripts were significantly different ($p < 0.05$) for the same type of films.

Table 2.3. Antimicrobial activity of Saran^{R} F-310 containing sorbic acid against 4 strains of *L. monocytogenes*

Film / Concentration (w/v)	Diameter of Inhibition Zone (mm)			
	CWD 95	CWD 1503	CWD201	CWD 246
Sorbic acid (unadjusted pH)				
0.5%	0	0	0	0
1%	0	0	0	0
1.5%	24.8 ± 0.3^{a}	22.3 ± 0.6^{a}	20.3 ± 1.2^{a}	20.3 ± 0.3^{a}
2%	29.0 ± 1.0^{b}	29.2 ± 1.0^{b}	21.7 ± 0.6^{a}	24.7 ± 0.6^{b}
3%	32.8 ± 0.8^{c}	32.3 ± 1.2^{c}	25.7 ± 1.2^{b}	25.8 ± 1.0^{b}
Sorbic acid (adjusted to pH 5.2)				
0.5%	0	0	0	0
1%	0	0	0	0
1.5%	22.2 ± 0.3^{a}	21.7 ± 0.6^{a}	20.8 ± 0.3^{a}	20.5 ± 0.5^{a}
2%	26.7 ± 1.5^{b}	26.7 ± 0.6^{b}	21.7 ± 1.2^{a}	23.0 ± 1.0^{a}
3%	32.2 ± 1.0^{c}	32.7 ± 0.6^{c}	25.2 ± 0.3^{b}	26.7 ± 1.5^{b}

Mean ± standard deviation. Means in the same column with different superscripts were significantly different ($p < 0.05$).

Increasing the concentration of nisin, potassium sorbate or sorbic acid in the film increased the diameter of the *L. monocytogenes* inhibition zones for for all 4 strains ($p < 0.05$) (Table 2.4). Films containing nisin had antimicrobial activity at a concentration of 1% w/v (Figure 2.4). Films containing 1%, 2% and 2.5% nisin had inhibition zone diameters ranging from 18.7 to 26.7 mm. Increasing the concentration of nisin in the film disc increased the diameter of the inhibition zones ($p<0.05$). Ko et al. (2001) reported that incorporation of nisin in whey protein isolates, soy protein isolates, egg albumin or wheat gluten films inhibited the growth of *L. monocytogenes*, and the greater the concentration of nisin, the greater the level of inhibition in all films tested. Hoffman et al. (2001) showed that corn zein film containing nisin and lauric acid decreased the number of *Listeria monocytogenes* by more than 4 logs in 48 hours.

Films containing potassium sorbate had antimicrobial activity at a minimum concentration of 2% (w/v), with the inhibition zones ranging from 18.33 to 22 mm for 2% and 3% (w/v) respectively (Figure 2.5). Increasing the concentration of potassium sorbate did not result in increased level of inhibition for any of the four strains of *L. monocytogenes* ($p > 0.05$). El-Shenawy and Marth (1988) showed that adding 0.2-0.3% (w/v) potassium sorbate to trypticase soy broth (pH 5) inhibited the growth of *L. monocytogenes*. Han (1996) developed an antimicrobial film by incorporating potassium sorbate into low density polyethylene (LDPE) during the extrusion method and verified the antimicrobial activity against *Saccharomyces cerevisiae*.

PVDC copolymer films containing 1.5-3.0% (w/v) sorbic acid adjusted to pH 5.2, or unadjusted (pH 3.5) were also inhibitory to all four *L. monocytogenes* strains (Figure 2.6), producing inhibition zones measuring 20.5-32.7 and 20.3- 32.8 mm in diameter,

Table 2.4. Antimicrobial activities of polyvinylidene copolymer containing sorbic acid, potassium sorbate and nisin against 4 strains of *Listeria monocytogenes*

Film Type	Conc (% w/v)	Diameter of Inhibition Zone (mm)			
		Strains			
		CWD 95	CWD 1503	CWD201	CWD 246
Control (antimicrobial free)	0	0^{a}	0^{a}	0^{a}	0^{a}
Sorbic acid (pH 5.2)	1.5%	22.2 ± 0.3^{bf}	21.7 ± 0.6^{b}	20.8 ± 0.3^{be}	20.5 ± 0.5^{bd}
	2%	26.7 ± 1.5^{c}	26.7 ± 0.6^{c}	21.7 ± 1.2^{bf}	23.0 ± 1.0^{beh}
	3%	32.2 ± 1.0^{d}	32.7 ± 0.6^{d}	25.2 ± 0.3^{c}	26.7 ± 1.5^{cef}
Sorbic acid (unadjusted pH)	1.5%	24.8 ± 0.3^{cf}	22.3 ± 0.6^{b}	20.3 ± 1.2^{bde}	20.3 ± 0.3^{dg}
	2%	29.0 ± 1.0^{e}	29.7 ± 1.0^{c}	21.7 ± 0.6^{bf}	24.7 ± 0.6^{efh}
	3%	32.8 ± 0.8^{d}	32.3 ± 1.2^{d}	25.7 ± 1.2^{c}	25.8 ± 1.0^{fh}
Potassium sorbate	2%	20.3 ± 0.6^{b}	20.7 ± 0.6^{b}	18.3 ± 0.6^{de}	18.7 ± 0.6^{d}
	3%	21.7 ± 0.6^{bf}	22.0 ± 1.0^{b}	19.0 ± 1.0^{eg}	20.3 ± 0.6^{dg}
Nisin	1%	21.8 ± 0.3^{bf}	22.3 ± 0.6^{b}	18.7 ± 0.6^{e}	19.3 ± 1.2^{dg}
	2%	23.7 ± 0.6^{f}	23.0 ± 1.0^{b}	19.8 ± 0.3^{ef}	21.7 ± 1.2^{bgh}
	2.5%	26.5 ± 0.5^{c}	26.7 ± 1.5^{c}	21.0 ± 1.0^{bfg}	23.7 ± 0.6^{h}

Mean ± standard deviation. Means in the same column with different superscripts were significantly different ($p < 0.05$).

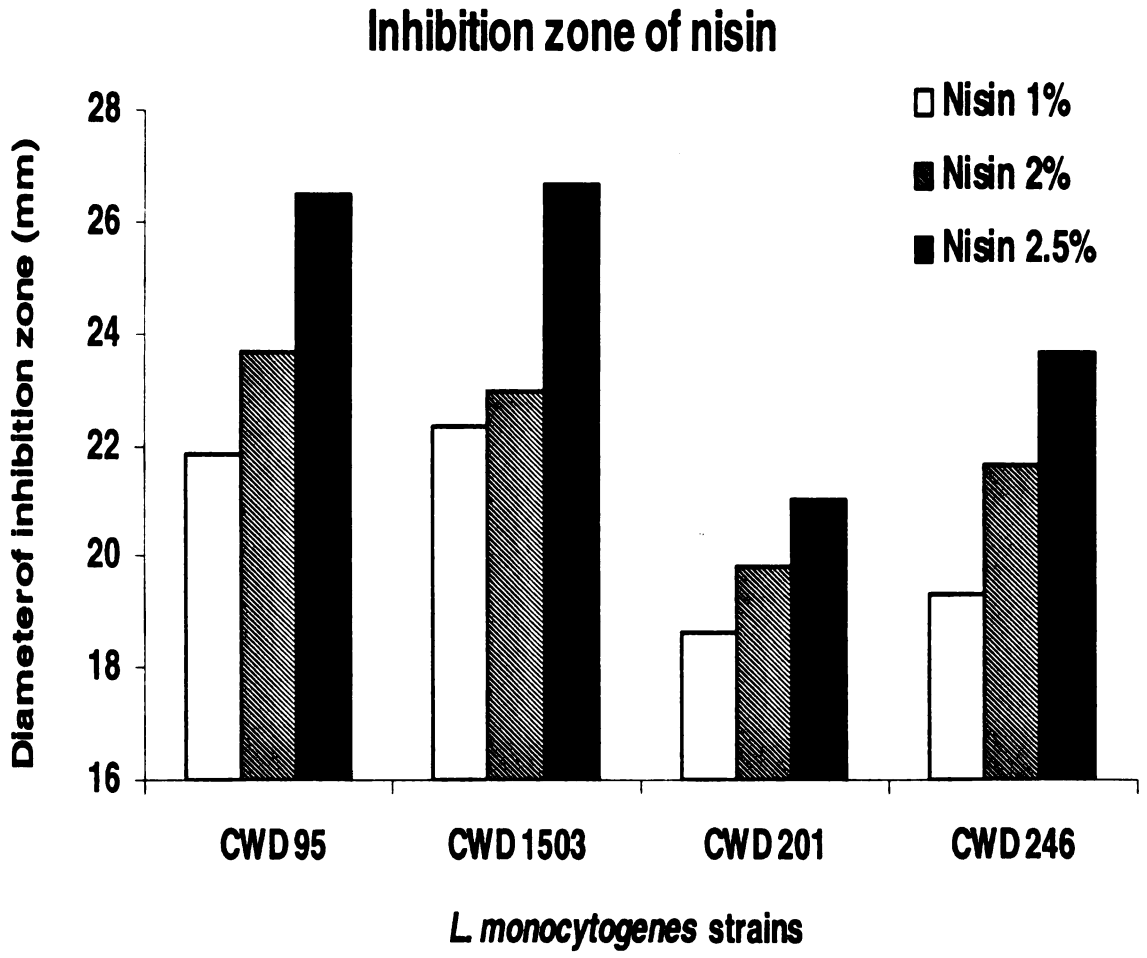

Figure 2.4. Comparison of the inhibition zones of films containing 1%, 2% or 2.5% (w/v) nisin.

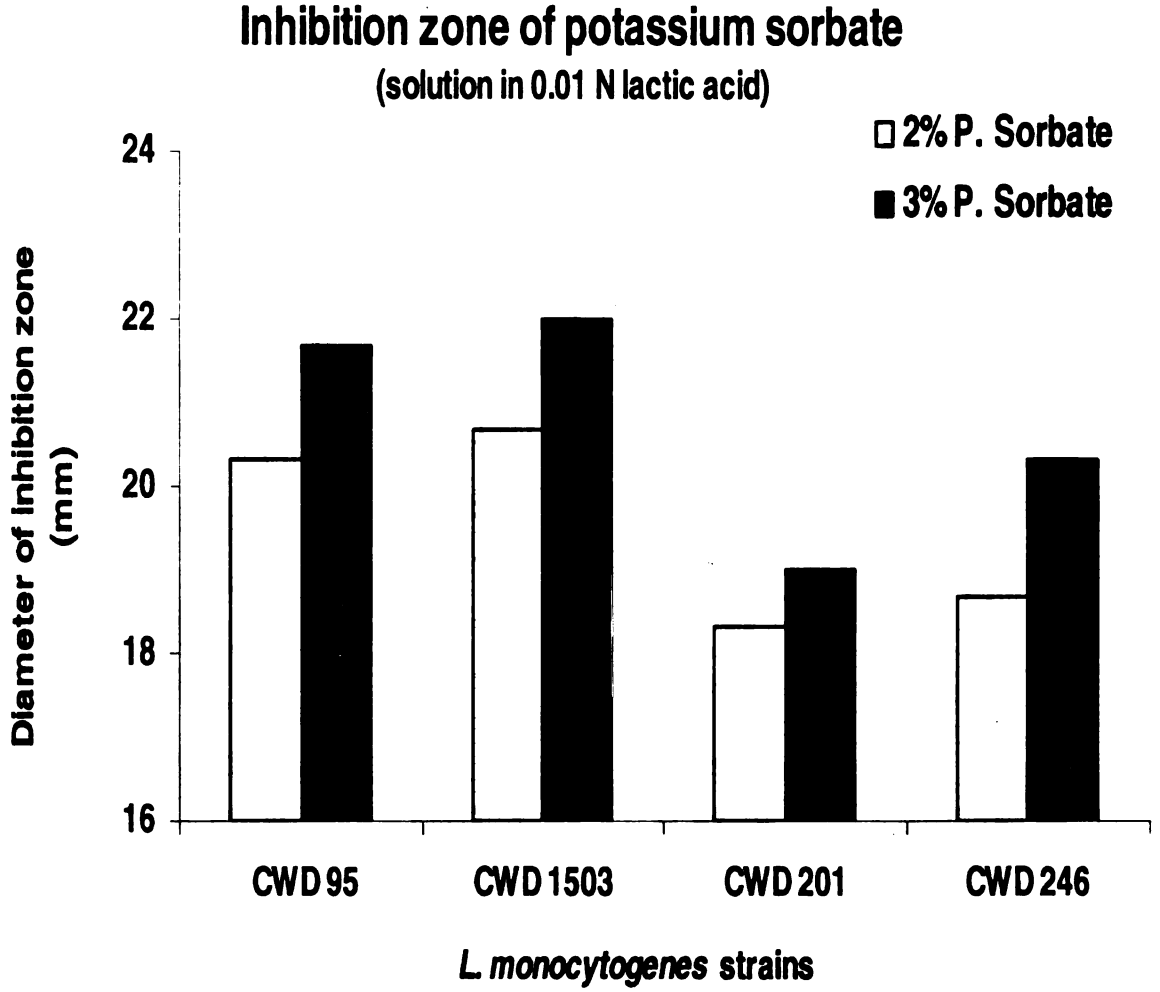

Figure 2.5. Comparison of the inhibition zones of films containing 2% or 3% (w/v) potassium sorbate.

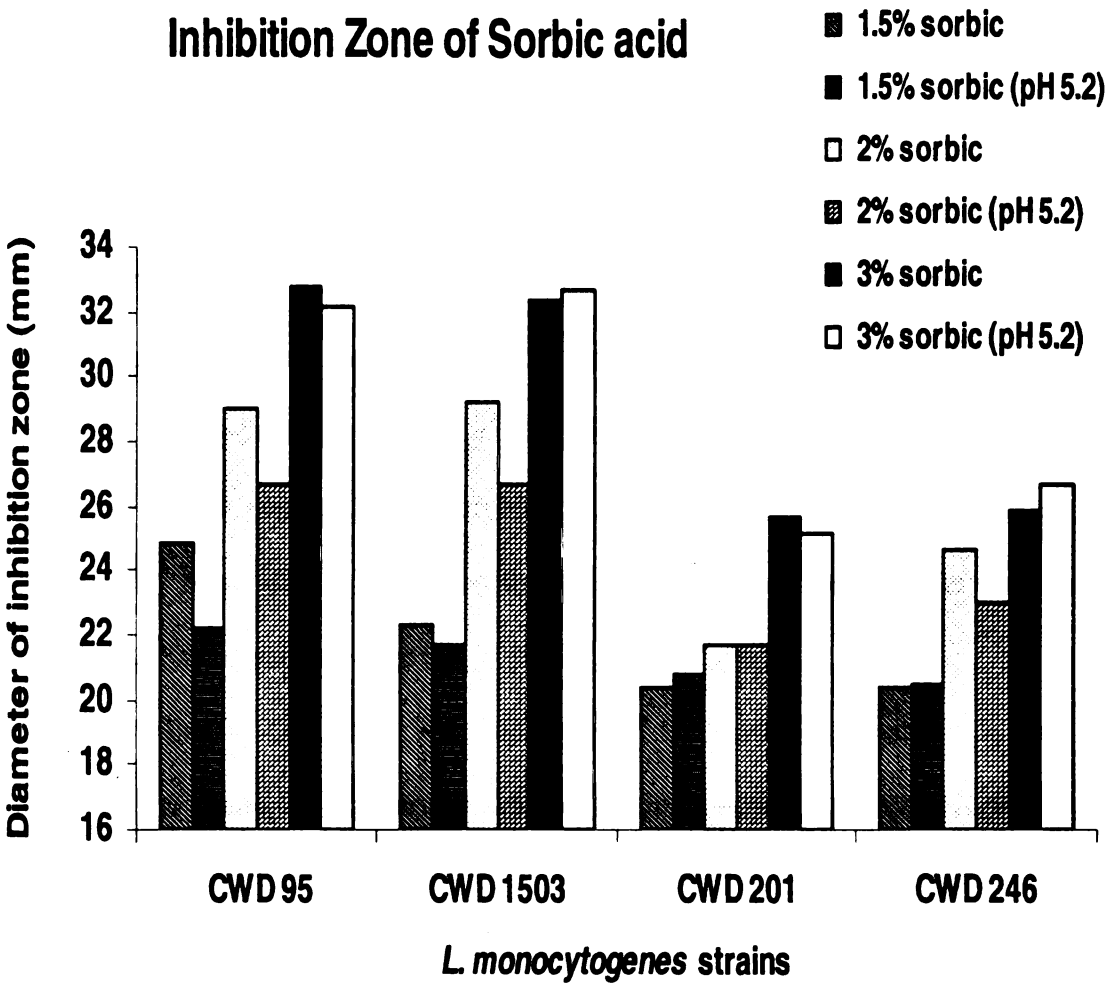

Figure 2.6. Comparison of the inhibition zones of films containing 1.5%, 2% or 3% (w/v) sorbic acid with and without adjusted to pH 5.2.

respectively. pH 5.2 was selected due to sorbic acid is most effective in the undissociated form (pKa 4.75) because its increased ability to penetrate the cytoplasmic membrane of bacteria. Film containing sorbic acid had the best antimicrobial activity against four strains of *L. monocyrogenes* (Figure 2.7). McDade et al. (1999) reported that growth of *L. monocytogenes* was inhibited on frankfurters, which were coated with whey protein film-forming solution containing sorbic acid/propionic acid (pH 5.2) and stored at 4 °C. Cagri at al. (2001) reported that whey protein isolate-based edible films containing 0.5-1.5% (w/v) sorbic acid had antimicrobial activity against *Listeria monocytogenes* on trypticase soy agar pH 5.2, and also inhibited *L. monocytogenes* on luncheon meats (Cagri et al., 2002a) and hot dogs (Cagri et al., 2002b).

According to Guilbert (1986), diffusion of antimicrobial agents from film discs depends on the shape, size, polarity of the molecule, chemical structure of the film, and degree of molecule cross-linking. The shape of the molecule, linear, branched or cyclic, may impact the diffusion rate (Micheals et al., 1962).

2.4.2 Antimicrobial properties of a coating film

The Saran^{R} F-310 solution containing sorbic acid as an antimicrobial agent was coated on PET films (0.5 mil), and tested for its antimicrobial activity against *Listeria monocytogenes*. Only the films containing 3% (w/v) sorbic acid with a 0.75 mil coating thickness had antimicrobial activity against *L. monocytogenes* CWD 95, with a hair line around the film disc. Films containing 3% (w/v) sorbic acid with coating thicknesses of 0.2 and 0.5 mil, and films containing 1.5% and 2% (w/v) sorbic acid with coating thicknesses ranging from 0.2 – 0.75 mil did not have antimicrobial activity.

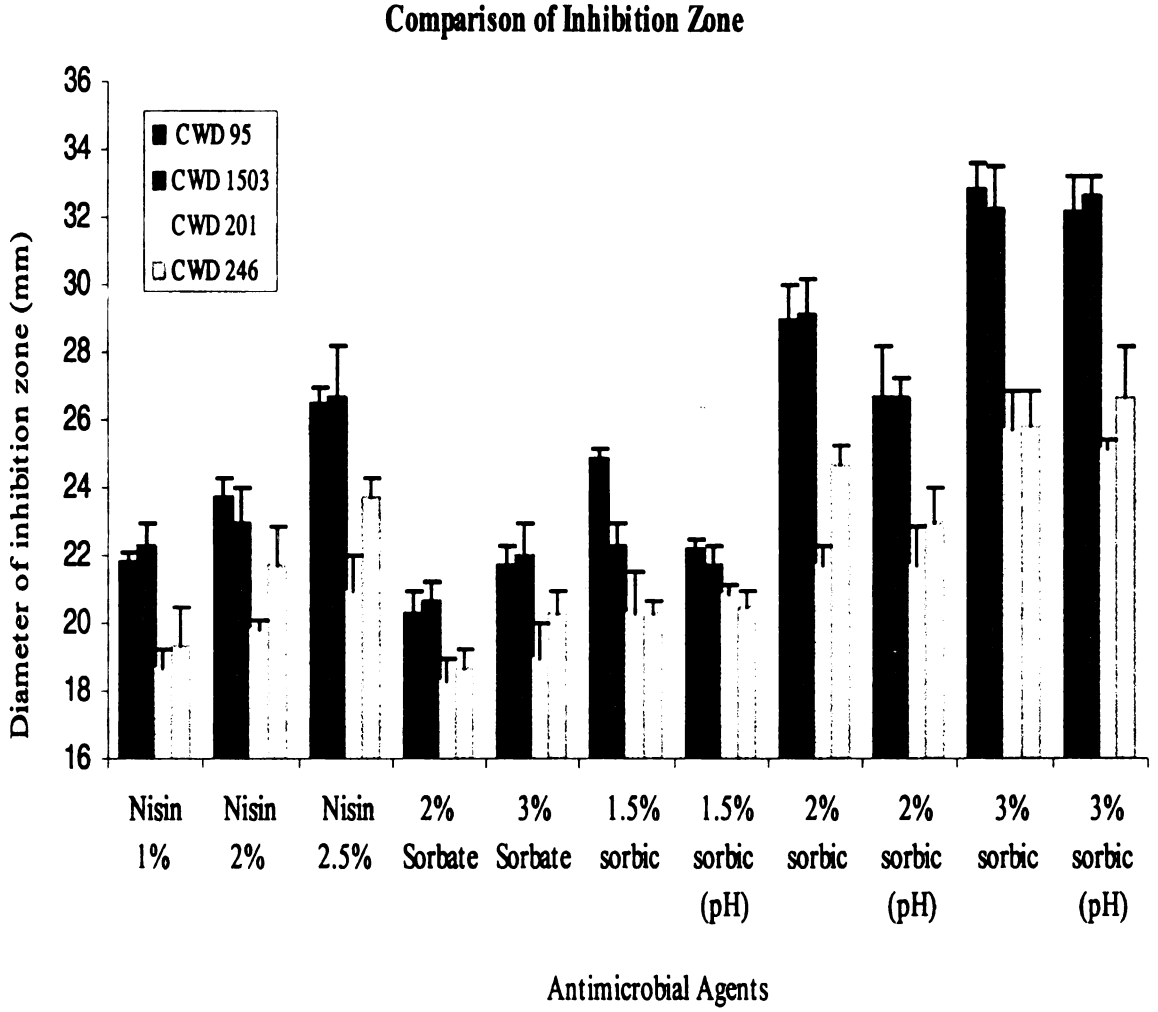

Figure 2.7. Comparison of the inhibition zones of films containing sorbic acid with and without pH adjusted to 5.2, potassium sorbate or nisin against four strains of *Listeria monocytogenes*. Mean ± standard deviation.

One of possibility could be the thickness of the coating. The thicker the coating of the film is, the greater the amount of antimicrobial agent would be in the film. Guilbert (1986) stated that measurement of antimicrobial activity using clear inhibition zones surrounding film discs (where the growth of the pathogen was inhibited) depended on the diffusion of antimicrobials from the film discs, which also depended on the size and shape of the films.

2.4.3 Mechanical properties

The average tensile strength of films containing sorbic acid with and without pH adjustment to 5.2 decreased from 3010 to 2843 lb/in^2 and 4076 to 2389 lb/in^2 when the concentration of sorbic acid in the film was increased from 1.5% to 3% (w/v), respectively (Table 2.5). Increasing the sorbic acid concentration showed no significant difference in the tensile strength for films with pH adjusted to 5.2, and no significant difference for films without pH adjustment to 5.2 up to 2.0% (w/v)($p > 0.05$). Tensile strength values of films containing 1.5 and 2.0% (w/v) sorbic acid without pH adjustment to 5.2 were not significantly different from the tensile values of the control film ($p > 0.05$), while the other films containing sorbic acid were significantly different from the control film ($p < 0.05$). When the concentration of potassium sorbate increased from 2% to 3% (w/v) and the concentration of nisin increased from 1% to 2.5% (w/v), tensile strength values decreased from 1922 to 1258 lb/in^2 and 1043 to 796 lb/in^2, respectively. Both were significantly different from the control film ($p < 0.05$). Increasing the potassium sorbate concentration made a significant difference in the tensile strength ($p < 0.05$), while increasing the concentration of nisin did not significantly alter tensile strength ($p > 0.05$).

Table 2.5. Tensile strength, percent elongation and toughness of Saran^R F-310 control film and films containing sorbic acid, nisin and potassium sorbate

Film/Concentration (% w/v)	Tensile strength (lb/in^2)	Elongation (%)	Toughness (lb/in^2)
Control	4936.4 ± 809.8^{a}	266.7 ± 20.8^{a}	11069 ± 1688^{a}
Sorbic acid (pH 5.2)			
1.5%	3010.3 ± 458.5^{b}	153.3 ± 11.5^{bcf}	4413 ± 794^{b}
2%	2985.5 ± 380.9^{b}	146.7 ± 70.2^{bcf}	3624 ± 949^{bh}
3%	2842.5 ± 150.7^{b}	286.7 ± 23.1^{a}	6613 ± 185^{c}
Sorbic acid (unadjusted pH)			
1.5%	4075.9 ± 138.4^{a}	206.7 ± 61.6^{abe}	6610 ± 1161^{c}
2%	4010.5 ± 347.1^{a}	66.7 ± 5.8^{cd}	2095 ± 113^{dg}
3%	2389.0 ± 234.3^{bc}	31.7 ± 2.9^{d}	1103 ± 168^{e}
Potassium sorbate			
2%	1922.0 ± 84.7^{c}	26.7 ± 5.8^{d}	538 ± 27^{f}
3%	1258.2 ± 110.5^{d}	146.7 ± 40.4^{ce}	1780 ± 89^{d}
Nisin			
1%	1042.9 ± 22.8^{ed}	240.0 ± 17.3^{aef}	2135 ± 83^{dg}
2%	952.1 ± 16.0^{e}	395.0 ± 22.0^{g}	3025 ± 162^{bg}
2.5%	796.2 ± 18.1^{e}	426.7 ± 6.0^{g}	2811 ± 18^{gh}

Mean ± standard deviation. Means in the same column with different superscripts were significantly different ($p < 0.05$).

Films containing 1.5%, 2% or 3% (w/v) sorbic acid with pH adjusted to 5.2 exhibited average elongation at break values of 153%, 147% and 287% respectively, and 207%, 67% and 32%, respectively, for films without pH adjustment (Table 2.5). Both were significantly different from the control film (elongation value 267%) ($p < 0.05$) except for films containing 3% and 1.5%. (w/v) sorbic acid with pH adjusted to 5.2 and without pH adjustment, respectively. Films containing 2% and 3% (w/v) potassium sorbate had percent elongations of 27% and 147%, respectively, which was significantly different from the control film ($p < 0.05$). Films containing nisin at concentrations of 1%, 2% or 2.5% (w/v) had average percent elongation values of 240%, 395% and 426.7% respectively. Only films containing 2.0 or 2.5% (w/v) nisin were significantly different from the control film ($p < 0.05$). Increasing the concentration of nisin from 2% to 2.5% (w/v) did not significantly change the percent elongation ($p > 0.05$).

The toughness of the films was also evaluated (Table 2.5). The toughness of the control film was 11069 lb/in^2. Films adjusted to pH 5.2 and containing 1.5%, 2% or 3% (w/v) sorbic acid exhibited average toughness of 4413, 3624 and 6613 lb/in^2, respectively, and 6610, 2095 and 1103 lb/in^2, respectively for unadjusted pH film. Films containing 2% or 3% (w/v) potassium sorbate had average toughness values of 538 and 1780 lb/in^2 respectively. For nisin films containing 1%, 2% or 2.5% (w/v) nisin, average toughness was 2135, 3025 and 2811 lb/in^2, respectively. The toughness of all films containing sorbic acid, potassium sorbate or nisin was significantly different from their antimicrobial-free counterparts ($p < 0.05$).

Han and Floros (1997) showed that antimicrobial low-density polyethylene (LDPE) films containing potassium sorbate up to 3% (w/w) did not affect the tensile

strength, elongation, and modulus properties of LDPE films because the incorporated potassium sorbate was considered to be captured in the void volume of the amorphous polymer structure, and therefore should not affect the polymer structure. However, if the amorphous area of LDPE was saturated with potassium sorbate, the tensile strength of the antimicrobial LDPE could be affected adversely. In this study, the antimicrobial agents nisin, potassium sorbate or sorbic acid were considered to be held in the void volume of the polymer structure (See Fig 2.8-2.11 micrographs by scanning electron microscopy). These may cause concentration stress points in the polymer structure, and result in decreasing the tensile strength and toughness of the films. Antimicrobials may function as plasticizers to increase the flexibility and movement of the polymer chains, yet decrease the tensile strength and toughness of the film. Chen et al. (1996) reported that incorporation of 4% potassium sorbate in methycellulose/chitosan antimicrobial films did not significantly change film tensile strength, but the films had poor elongation compared to films containing no antimicrobial substance. Incorporation of antimicrobial agents in whey protein isolate-based edible films generally produced films with lower tensile strength and greater elongation (Kester and Fennema, 1986). Addition of antimicrobials into the films in order to produce antimicrobial films generally produced films with lower tensile strength compared to films without antimicrobials for corn zein film (Aydt et al., 1991), soy protein film (Gennadios and Weller, 1991) and whey protein-based edible film (Cagri et al., 2001).

2.4.4 Seal strength

Polyvinylidene copolymer films containing antimicrobials (sorbic acid) could be sealed using the heat-sealing machine the same as the control film (no antimicrobial).

Both film with and without antimicrobials could be sealed at the same temperature (Table 2.6). Films containing 1.5%, 2% or 3% (w/v) sorbic acid exhibited average seal strength values ranging from 1293 to 670 lb/in^2, respectively. The antimicrobial-free film had average seal strength of 1757 lb/in^2.

Incorporation of an antimicrobial agent, sorbic acid, into the film caused a reduction in the seal strength of the seal. The higher the amount of sorbic acid added into the film, the weaker the seal was.

2.4.5 Water vapor permeability:

Water vapor permeability (WVP) is an important property of plastic films, polyvinylidene chloride film is a good moisture barrier. Films containing 1.5%, 2% or 3% (w/v) sorbic acid with pH adjusted to 5.2 exhibited average WVP values of 1.39, 1.45 and 2.00 cc.mil/m^2.day.mmHg respectively (Table 2.7), and were significantly different ($p < 0.05$) from the control film. A WVP value of 0.54 cc.mil/m^2.day.mmHg was determined for the control film. Increasing the concentration of sorbic acid from 1.5% to 3% (w/v) did not significantly alter water vapor permeability ($p > 0.05$). Average WVP of films containing 1.5%, 2.0% or 3.0% (w/v) sorbic acid (unadjusted pH) were 0.71, 0.50, 0.62 cc.mil/m^2.day.mmHg respectively, and were not significantly different from the control film ($p > 0.05$). Increasing the concentration of sorbic acid from 1.5% to 3% (w/v) did not significantly alter water vapor permeability ($p > 0.05$). Films containing 2% or 3% (w/v) potassium sorbate had average WVP values of 3.69 and 3.83 cc.mil/m^2.day.mmHg respectively, significantly different from the control ($p < 0.05$). Increasing the concentration of potassium sorbate from 2% to 3% (w/v) did not significantly change the WVP ($p > 0.05$).

Table 2.6. Seal strength of Saran^{R} F-310 film and films containing 1.5%, 2.0% or 3.0% (w/v) sorbic acid

Film/Concentration (% w/v)	Seal strength (lb/in^2)
Control	1756.6 ± 446.4^{a}
Sorbic acid	
1.5%	1292.5 ± 187.8^{a}
2%	963.5 ± 139.9^{b}
3%	670.2 ± 149.2^{c}

Table 2.7. Water vapor transmission rate and water vapor permeability of Saran^R F-310 control film and Saran^R F-310 films containing sorbic acid, nisin, and potassium sorbate

Films/Concentration (% w/v)	WVTR (gm/100 in^2/day)	Permeability (g.mil/100 in^2.day.mmHg)	Permeability (g.mil/m^2.day.mmHg)
Control	1.37	0.04 ± 0.01^{a}	0.54 ± 0.01^{a}
sorbic acid (pH 5.2)			
1.5%			
	3.03	0.09 ± 0.01^{bcd}	1.39 ± 0.14^{bcd}
2%	3.52	0.09 ± 0.01^{bc}	1.45 ± 0.19^{bc}
3%	3.59	0.13 ± 0.02^{b}	2.00 ± 0.25^{b}
sorbic acid			
(unadjusted pH)			
1.5%	2.06	0.05 ± 0.01^{ac}	0.71 ± 0.02^{ac}
2%	1.63	0.03 ± 0.01^{ad}	0.50 ± 0.05^{ad}
3%	1.42	0.04 ± 0.01^{a}	0.62 ± 0.05^{a}
Potassium sorbate			
2%	3.03	0.24 ± 0.03^{e}	3.69 ± 0.42^{e}
3%	3.07	0.25 ± 0.04^{e}	3.83 ± 0.62^{e}
Nisin	N/A	N/A	N/A

Mean ± standard deviation. Means in the same column with different superscripts were significantly different ($p < 0.05$).

Adding potassium sorbate to the film solution increased water vapor permeability (WVP) because both are hydrophilic compounds, which may increase the solubility of water in the films. Films containing nisin could not be tested for their water vapor permeability. Films failed during the calibration process during testing. This could be because the nisin crystals inside the film caused stress concentration and resulted in micro voids when the film was exposed to high pressure during sample conditioning in the permeability testing (see Fig 2.11 scanning electron micrograph). McHugh et al. (1994) stated that adding sorbic acid to film might increase the hydrophilic character and the water solubility coefficient of the film. In this study, films containing potassium sorbate had the highest water vapor permeability. This could be because potassium sorbate created pits in the polymer structure, increasing the permeability of moisture (see Fig 2.10 scanning electron micrograph). Antimicrobials may function as plasticizers to increase the distance between polymer chains or weaken chain packing resulting in a looser polymer structure, which increases water permeability of film.

2.4.6 Oxygen permeability:

Films containing 1.5, 2.0 or 3.0% (w/v) sorbic acid with pH adjusted to 5.2 showed average oxygen permeability values of 33.5, 57.4 and 86.2 cc.mil/ m^2.day.atm respectively (Table 2.8). Film containing 1.5% (w/v) sorbic acid had lower oxygen permeability (OP) than the control film (53.3 cc.mil/ m^2.day.atm), and film containing 2.0% (w/v) sorbic acid was not significantly different from the control film ($p > 0.05$). Average O_2 permeabilities of non-pH adjusted films containing 1.5, 2.0 or 3.0% (w/v) sorbic acid were much higher: 171, 210, and 522 cc.mil/ m^2.day.atm, and were significantly different from the control film ($p < 0.05$). Increasing the concentration of

Table 2.8. Oxygen transmission rate (OTR) and oxygen permeability of Saran^R F-310 control film and Saran^R F-310 films containing sorbic acid, nisin, and potassium sorbate

Films/Concentration (% w/v)	OTR ($cc/m^2/day$)	Oxygen Permeability ($cc.mil/ m^2.day.atm$)
Control	61.3 ± 16.2	53.3 ± 2.6[a]
sorbic acid (pH 5.2)		
1.5%	33.8 ± 0.3	33.5 ± 2.1[b]
2%	44.2 ± 0.9	57.4 ± 1.2[a]
3%	66.3 ± 1.1	86.2 ± 1.5[c]
sorbic acid (unadjusted pH)		
1.5%	76.7 ± 5.8	171.0 ± 8.7[d]
2%	103.3 ± 5.8	210.0 ± 10.0[e]
3%	270.0 ± 10.0	521.7 ± 9.6[f]
Potassium sorbate		
2%	2 ± 0.01	4.5 ± 0.1[g]
3%	2000 ± 0.01	4600 ± 0.01[h]
Nisin	N/A	N/A

Mean ± standard deviation. Means in the same column with different superscripts were significantly different ($p < 0.05$).

sorbic acid from 1.5% to 3.0% (w/v) resulted in significantly different oxygen permeability ($p < 0.05$). PVDC films containing 2.0% (w/v) potassium sorbate had an average OP value of 4.53 cc.mil/ m^2.day.atm, with films prepared with 3.0% potassium sorbate exhibiting an average that was about 1000-fold higher. Again films containing nisin could not be tested for O_2 permeability due to the aforementioned problem. The results showed that the films containing antimicrobial agents had higher oxygen permeability than control films, except for films containing 1.5% (w/v) sorbic acid adjusted to pH 5.2 and films containing 2% (w/v) potassium sorbate. Statistical analysis showed that incorporation of different antimicrobial agents and increased concentration resulted in statistically significant differences in oxygen permeability ($p < 0.05$).

2.4.7 Surface energy

The ability of a film to anchor inks, coating or adhesives is directly related to its surface energy. If the substrate surface energy does not significantly exceed the surface tension of the fluid that is to cover it, wetting will be impeded and a poor bond will result. All films containing sorbic acid, potassium sorbate or nisin (at all concentrations) exhibited the same surface energy, 38 dynes/cm, which was the same as the control film (without the antimicrobial agents). Therefore, the surface energy of the films was not affected by addition of antimicrobial agents.

2.4.8 Scanning electron microscopy:

The three-dimensional structures of the films were determined using scanning electron microscopy. These are shown in Figures 2.8 – 2.11. Films without antimicrobial agents (control films) had a homogeneous structure (Figure 2.8). For film containing sorbic acid (Figure 2.9 A, B) the sorbic acid was distributed in the film

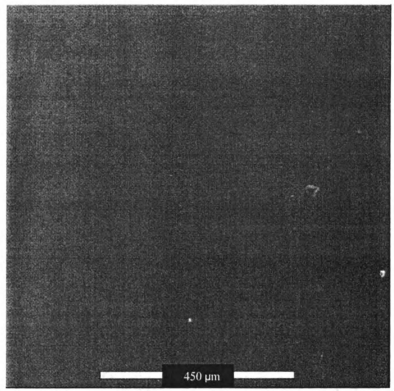

Figure 2.8. SEM micrographs of the surface structure of polyvinylidene chloride copolymer films containing no antimicrobial (control film).

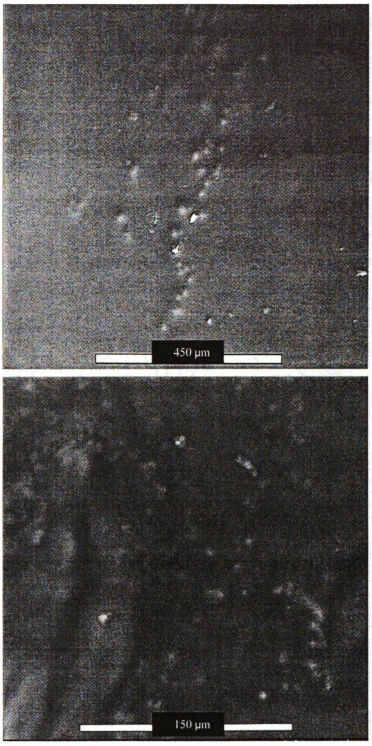

Figure 2.9. SEM micrographs of the surface structure of polyvinylidene chloride copolymer films (Top) containing 1.5% (w/v) sorbic acid, (Bottom) 3% (w/v) sorbic acid

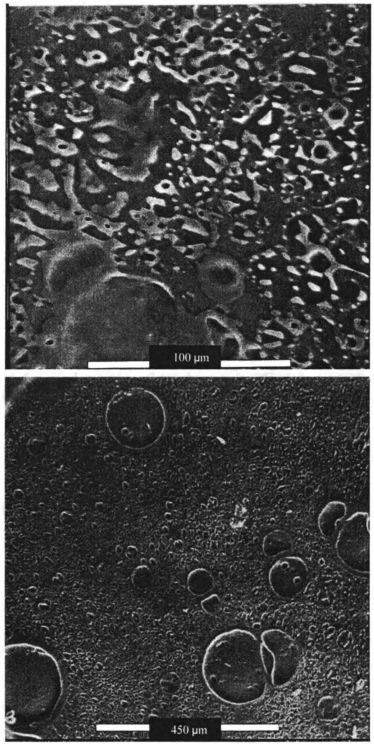

Figure 2.10. SEM micrographs of the surface structure of polyvinylidene chloride copolymer films containing potassium sorbate.

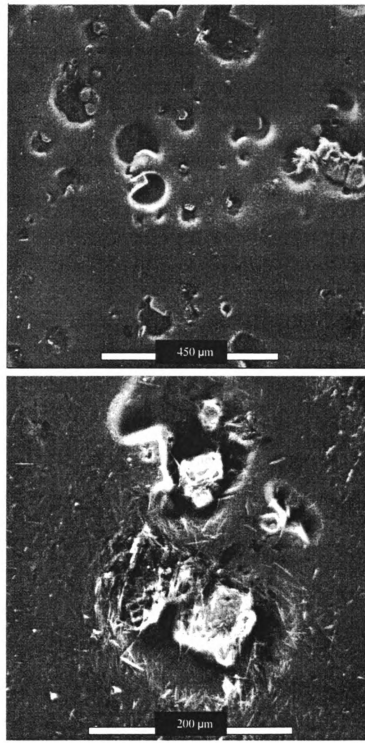

Figure 2.11. Scanning electron microscopy photomicrograph of the structure of polyvinylidene chloride copolymer films containing nisin (Top) nisin spread in film (Bottom) interphase between film matrix and nisin

structure, and created a non-homogeneous interphase (between the polyvinylidene copolymer phase and the sorbic acid phase) in the polymer structure, which resulted in decreased mechanical properties. Addition of potassium sorbate created sieves/holes (widely distributed) in the film structure (Figure 2.10 A, B). This porous morphology caused reduction in tensile strength and toughness of the film, and also produced higher permeability of the films. Incorporation of nisin into the polyvinylidene chloride copolymer also created a non-homogeneous structure (Figure 2.11 A, B). The nisin crystals, which are much larger than sorbic acid, resulted in decreased in tensile strength, decreased toughness and failure of water vapor and oxygen permeability testing.

2.4.9. Differential scanning calorimeter (DSC)

DSC was used to determine the sealing temperature of films with and without sorbic acid. For film without sorbic acid, the sealing temperature started from approximately at 125 °C (Figure 2.12). The sealing temperature for film containing sorbic acid started approximately at 120 °C (Figure 2.13). Thus, adding sorbic acid to the film slightly shifted the heat seal conditions for the film.

2.5. CONCLUSION

Polyvinylidene chloride copolymer films containing 1.5 to 3.0% (w/v) sorbic acid, 2.0 to 3.0% (w/v) potassium sorbate, or 1.0 to 2.5% (w/v) nisin inhibited the growth of *L. monocytogenes* on TSAYE in a disc diffusion assay. Films containing lactoferrin or sodium diacetate did not show inhibition against *L. monocytogenes*. Water vapor and oxygen barrier properties decreased when antimicrobials other than sorbic acid were added. Tensile strength of the antimicrobial films decreased except for film containing

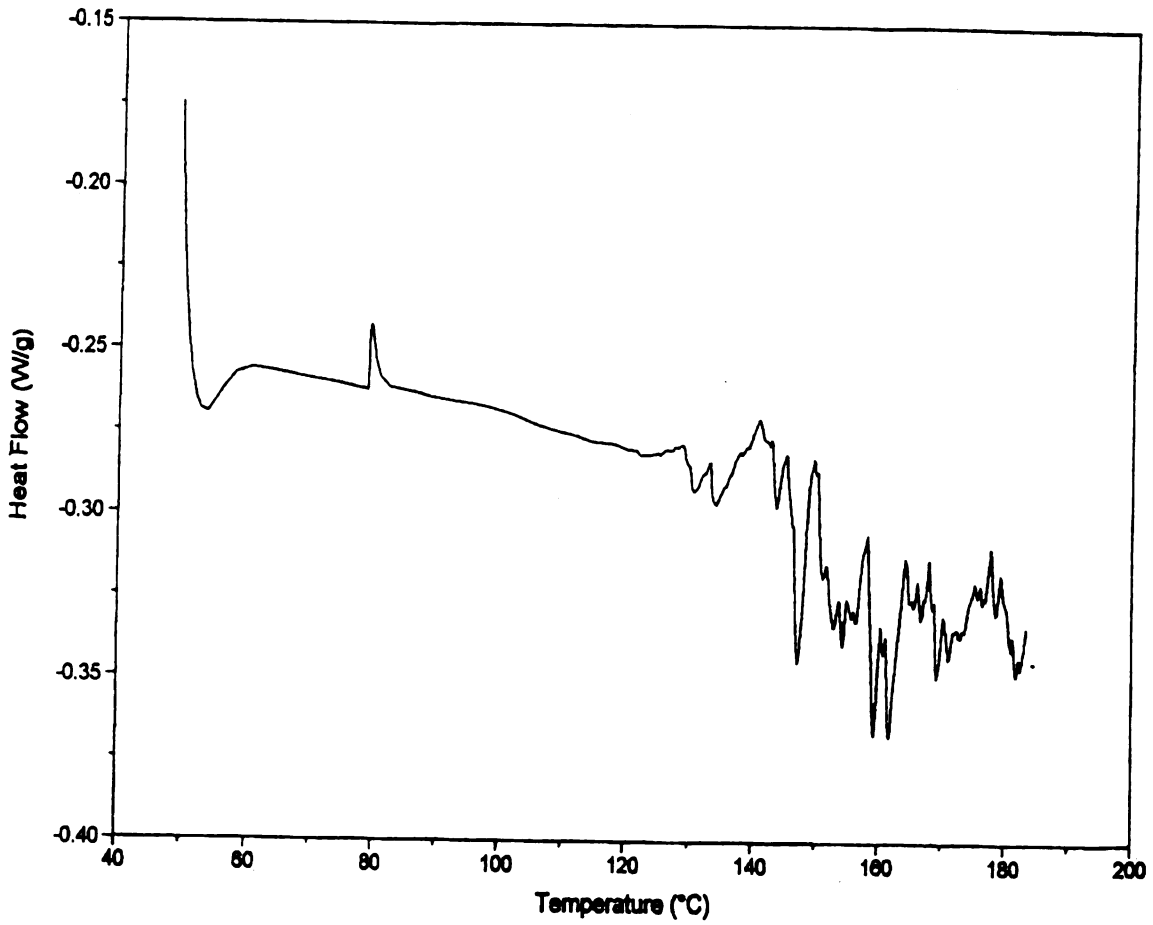

Figure 2.12. Differential Scanning Calorimetry (DSC) profile of polyvinylidene chloride copolymer (Saran F-310) film containing no antimicrobial

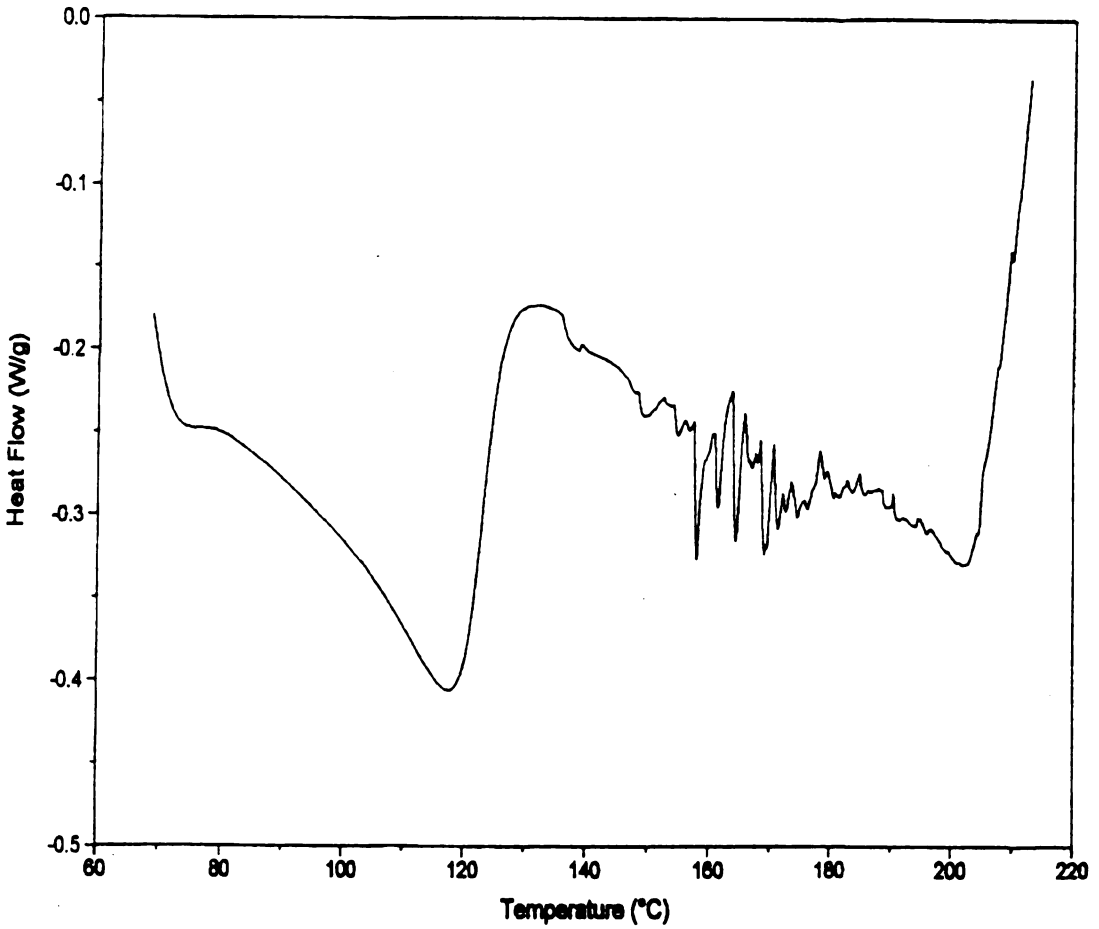

Figure 2.13. Differential Scanning Calorimetry (DSC) profile of polyvinylidene chloride copolymer (Saran F-310) film containing 3.0% sorbic acid

1.5% or 2.0% sorbic acid and toughness decreased with incorporation of antimicrobial agents. Films containing sorbic acid exhibited the most favorable antimicrobial, barrier and mechanical properties with sorbic acid relatively evenly distributed in the polymer structure. Films containing sorbic acid either without pH adjustment (pH 3.5) or with pH adjusted to 5.2 were most antimicrobial effective against *L. monocytogenes*. PVDC (Saran^{R} F-310) containing 3% (w/v) sorbic acid at a 0.75 mil (0.00075 inch) coating thickness on polyethylene terephthalate film (PET) also had antimicrobial activity against *L. monocytogenes*.

These antimicrobial packaging films may eventually prove to be useful in inhibiting both pathogenic and spoilage organisms on the surface of cooked/ready-to-eat or processed foods that may be exposed to contamination after processing.

CHAPTER 3.

INACTIVIATION OF LISTERIA MONOCYTOGENES ON BEEF BOLOGNA AND CHEDDAR CHEESE USING ANTIMICROBIAL POLYVINYLIDENE CHLORIDE FILM

3.1 ABSTRACT

This study investigated the ability of an antimicrobial polyvinylidene chloride (PVDC) copolymer film to inactivate *Listeria monocytogenes* and extend the shelf life of beef bologna and Cheddar cheese. Sorbic acid-containing PVDC (Saran^R F-310) copolymer films were made by a solvent-casting method using methyl ethyl ketone. PVDC copolymer film solutions containing 0, 1.5 and 3.0% (w/v) sorbic acid were cast on glass plates and dried at 86°C for 5 minutes. These films were aseptically cut and placed between 3-mm thick slices of commercially produced beef bologna which were previously surface inoculated with *L monocytogenes* at a level of 10^5 or 10^3 CFU/g. Locally produced Cheddar cheese was cut into slices measuring 3 × 3 × 2.5 cm, surface inoculated to contain 10^5 or 10^3 *L. monocytogenes* CFU/g, wrapped with 0, 1.5 or 3.0% (w/v) sorbic acid films and stored at 4°C. Both products were examined at appropriate intervals for numbers of *L. monocytogenes*, mesophilic aerobic bacteria, lactic acid bacteria, and yeast/mold using Modified Oxford Agar, Plate Count Agar, MRS Lactobacillus Agar, and Rose Bengal Agar, respectively.

Films containing 1.5 and 3.0% (w/v) sorbic acid decreased *L. monocytogenes* populations 4.4 and 4.5 logs, respectively on bologna slices initially inoculated to contain 10^5 CFU/g, and 6.5 and 7.2 logs on bologna initially inoculated to contain 10^3 CFU/g, after 28 days of refrigerated storage; whereas numbers of *Listeria* increased 3.8 and 5.8 logs using antimicrobial-free film on bologna initially inoculated with 10^5 and 10^3 CFU/g of *Listeria*, respectively. When Cheddar cheese was stored for 35 days, films containing 1.5 and 3.0% (w/v) sorbic acid decreased *Listeria* population 0.8 and 1.3 logs on cheese initially containing 10^5 CFU/g, and 0.6 and 1.2 logs on cheese initially containing 10^3

CFU/g. *Listeria* populations remained constant on Cheddar cheese wrapped in sorbic acid-free films. These films, which also inhibited mesophilic bacteria, lactic acid bacteria and yeast/mold, may be useful in enhancing the safety and shelf life of refrigerated ready-to-eat foods.

3.2. INTRODUCTION

Post-processing contamination of ready-to-eat (RTE) foods by foodborne pathogens and spoilage microorganisms is of great concern in the food industry. Ryser and Marth (1987) reported that *L. monocytogenes* can survive more than 1 year in Cheddar cheese and persist during manufacture and storage in cottage cheese (Hicks and Lund 1991, Ryser et al., 1985, Piccin and Shelef, 1995). Outbreaks have been reported in both domestic and imported cheese (Ryser and Marth, 1988).

In 2000, there were 2,298 cases of listeriosis reported in US, which cost approximately $ 1 million per case, making listeriosis the costliest foodborne disease per case (USDA-FISS, 2001). Use of antimicrobial films may provide manufactures another means of reducing bacterial pathogens on foods. Weng and Chen (1997) incorporated antimicrobial agents within packaging materials to minimize growth of surface contaminants. Antimicrobial additives such as sorbic acid are increasingly used as one means to improve food preservation (Gianakopoulos and Guilbert, 1986).

Sorbic acid and its salt (such as potassium sorbate) are two primary food preservatives (Sofos and Busta, 1981; Liewen and Marth, 1985; Luck, 1990; Weng and Chen, 1997) in many food products including intermediate moisture foods (Troller and Christian, 1978; Torres and Karel, 1985), poultry products (Robach and Ivey, 1978;

Cunningham, 1979), cheese and meat products (Zamora and Zaritzky, 1987a, b). Sorbic anhydride was used as antimicrobial additive in polyethylene film to produce an antimicrobial food packaging film (Weng and Chen, 1997, Weng and Hotchkiss, 1993). Whey protein isolate films containing sorbic acid or p-aminobenzoic acid reportedly inhibited *L. monocytogenes* as well as *Escherichia coli* O157:H7 and *Salmonella* Typhimerium DT104 on aerobically packaged slices of bologna and summer sausage (Cagri et al., 2002).

In a previous study, polyvinylidene chloride (PVDC) copolymer films containing 1.5 to 3% (w/v) sorbic acid were found to inhibit the growth of *L. monocytogenes* on acidified (pH 5.2) trypticase soy agar containing 0.6% yeast extract (Limjaroen et al., 2002). The objective of this study was to assess the antimicrobial activity of sorbic acid-containing PVDC films for activity against *L. monocytogenes* and several groups of spoilage organisms while in direct contact with bologna and Cheddar cheese.

3.3. MATERIALS AND METHODS

3.3.1. Target Organism

Listeria monocytogenes strain CWD 95 D Gallup, the most sorbic acid resistant of four strains previously tested by Cagri et al. (2001), was obtained from Michigan State University Food Microbiology culture collection. The strain was maintained at – 70 °C in trypticase soy broth containing 10% (v/v) glycerol (Difco Laboratories, Detroit, MI). Cultures of *L. monocytogenes* were prepared by transferring the *Listeria* strain from –70 °C storage into TSB + 0.6% yeast extract (TSB-YE) (Difco Laboratories, Detroit, MI). Following 18 – 24 hours of incubation at 35 °C, a second transfer was similarly prepared

prior to use. The initial culture had a population of 10^9 CFU/ml and was serially diluted in 0.1% peptone water to inoculate beef bologna and Cheddar cheese.

3.3.2. Products

Beef bologna (diameter ~ 9.6 cm) and Cheddar cheese (dimension ~ 3 × 5 cm) were obtained from a local supermarket and the MSU dairy store, respectively. Beef bologna contained beef, salt, sodium lactate, flavor, dextrose, hydrolyzed yeast, sodium phosphate, sodium diacetate, sodium erythorbate, sodium nitrite, and extracts of paprika as reported by the manufacture. Cheddar cheese contained pasteurized milk, cheese culture, salt, enzymes and color. The pre-sliced bologna (~3 mm thick) (pH value of 6.12) was cut into 10-g squares shapes (7.5 ×7.5 cm). Cheddar cheese (pH~5.0) was cut into 25-g pieces measuring 3 x 3 x 2.5 cm.

3.3.3. Film preparation

Polyvinylidene chloride (PVDC) copolymer (Dow Chemical, Midland, MI) resin (18% w/v) was slowly dissolved in methyl ethyl ketone (J.T Baker, Phillipsburg, NJ) at room temperature with continuous agitation. Thereafter, 0%, 1.5% or 3% (w/v) of sorbic acid (Sigma Chemical Co., St. Louis, MO) was added to the solution (pH 3.5), which was cast on glass plate (9 × 13 inches) and dried in an Oven (Fisher Scientific, Pittsburgh, PA) at 86 ± 0.5 °C for 5 minutes. After drying, the films were peeled from the plates.

3.3.4. Product inoculation of Cheddar cheese and beef bologna and storage

Listeria monocytogenes (0.1 ml) was spread on the top and bottom surface of each piece of Cheddar cheese, bologna using a sterile glass rod to obtain inoculum level of 10^5 CFU/g and 10^3 CFU/g. The inoculated cheese and bologna slices were air dried. Cheddar cheese samples were wrapped in film (8.75 × 12.5 cm) containing 0% (without

antimicrobial agent), 1.5% or 3% (w/v) sorbic acid, which was previously sterilized by exposure to UV light for 20 minutes. For bologna, the inoculated slices were placed between PVDC films (3.5 × 3.5 inch) containing 0, 1.5 or 3.0% (w/v) sorbic acid and stacked 4 slices high in 150 mm-diameter sterile Petri dishes. The samples were stored aerobically at 4 °C. All experiments were replicated three times.

3.3.5. Microbiological analysis

3.3.5.1. *Cheddar cheese*

Samples were taken for microbiological analysis immediately after inoculation and again following 4, 7, 10, 14, 21 and 35 days of refrigerated storage. For analysis, a 25-g sample was diluted in 225 ml of warm 2% trisodium citrate (Sigma), homogenized in a stomacher (Tekmar Co., Cincinnati, OH) for 3 minutes, and serially diluted in 0.1% peptone water. The population of *L. monocytogenes*, mesophilic aerobic bacteria, lactic acid bacteria, molds and yeast were determined by plating appropriate dilutions on Modified Oxford Agar (Difco), Plate Count Agar (Difco), MRS Lactobacillus Agar (Difco) and Rose Bengal Agar for both mold and yeast (Difco), respectively, as outlined in the FDA Bacteriological Analytical Manual (FDA 1998). All experiments were replicated three times.

3.3.5.2. *Bologna*

Samples were taken for microbiological analysis immediately after inoculation and again following 4, 7, 10, 14, 21 and 28 days of refrigerated storage. A 10-g sample was diluted in 90 ml of 0.1% peptone water, homogenized in a stomacher (Tekmar Co., Cincinnati, OH) and serially diluted in 0.1% peptone water. Appropriate dilutions were

plated to determine the numbers of *L. monocytogenes*, mesophilic aerobic bacteria, lactic acid bacteria, molds and yeast. All experiments were replicated three times.

3.3.6. Statistical analysis

Two-way analysis of variance (ANOVA) using SAS Statistical Analysis System (SAS Institute Inc., 1990) was performed to analyze the results at the 95% confidence level ($p = 0.05$) using the Tukey-Kramer adjustment.

3.4. RESULTS AND DISCUSSION

3.4.1. Antimicrobial activity on Cheddar cheese inoculated to contain 10^5 and 10^3 *L. monocytogenes* CFU/g.

3.4.1.1. *L. monocytogenes*

Cheddar cheese was initially inoculated to contain 10^5 *L. monocytogenes* CFU/g and examined during 35 days of storage. The population distributions of *L. monocytogenes* during 35 day were shown in Table 3.1 and 3.2. Populations of *L. monocytogenes* decreased 0.78 and 1.31 logs (Table 3.3) on cheese wrapped with PVDC film containing 1.5% and 3.0% (w/v) sorbic acid, respectively (Figure 3.1) after 35 days of stored at 4 °C. In contrast, cell numbers on cheese wrapped with antimicrobial-free film remained unchanged throughout 35 days storage (Table 3.3). The reduction in number of *L. monocytogenes* using PVDC films containing 1.5% sorbic acid was not significantly different ($p > 0.05$) from 3.0% (w/v) sorbic acid. Both films containing 1.5% or 3.0% (w/v) sorbic acid were not significantly different ($p > 0.05$) from the control film for Cheddar cheese initially inoculated to contain 10^5 *L. monocytogenes* CFU/g on the surface of the cheese. For cheese inoculated to contain 10^3 CFU/g

Table 3.1. Inhibition of *L. monocytogenes*, mesophilic bacteria and lactic acid bacteria on Cheddar cheeses with an initial inoculum of 10^5 CFU/g using PVDC copolymer films containing 0%, 1.5% and 3% (w/v) sorbic acid.

Antimicrobial (%w/v)	Day	*L. monocytogenes*	Mesophilic bacteria	Lactic acid bacteria	Mold	Yeast
0% sorbic acid	0	5.8 ± 0.07^{a}	6.8 ± 0.30^{ag}	7.0 ± 0.06^{a}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	4	5.6 ± 0.13^{abd}	6.9 ± 0.53^{a}	7.2 ± 0.12^{b}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	7	5.7 ± 0.02^{ac}	6.5 ± 0.25^{a}	7.5 ± 0.13^{c}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	10	5.7 ± 0.04^{ac}	8.0 ± 0.15^{bc}	8.1 ± 0.12^{dg}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	14	5.7 ± 0.01^{ac}	8.1 ± 0.15^{b}	8.3 ± 0.23^{d}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	21	5.7 ± 0.01^{ac}	8.1 ± 0.13^{bc}	8.1 ± 0.08^{dg}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	35	5.7 ± 0.02^{ac}	8.1 ± 0.15^{bc}	6.5 ± 0.14^{ef}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
1.5% sorbic acid	0	5.7 ± 0.07^{ac}	6.8 ± 0.19^{aeh}	6.8 ± 0.19^{ae}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	4	5.5 ± 0.04^{bdf}	6.9 ± 0.28^{aeh}	7.0 ± 0.06^{ab}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	7	5.7 ± 0.06^{ac}	7.0 ± 0.12^{adeh}	7.8 ± 0.01^{cgh}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	10	5.6 ± 0.03^{ab}	7.6 ± 0.12^{cdef}	8.1 ± 0.05^{dg}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	14	5.6 ± 0.06^{bcdf}	7.3 ± 0.17^{defh}	7.7 ± 0.25^{c}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	21	5.4 ± 0.09^{df}	7.3 ± 0.19^{dfh}	7.7 ± 0.14^{c}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	35	5.0 ± 0.05^{e}	7.3 ± 0.17^{dfh}	6.3 ± 0.22^{fi}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$

Table 3.1. (Con't)

Antimicrobial (%w/v)	Day	*L. monocytogenes*	Mesophilic bacteria	Lactic acid bacteria	Mold	Yeast
3.0% sorbic acid	0	5.7 ± 0.08^{ac}	6.6 ± 0.25^{a}	6.9 ± 0.17^{ab}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	4	5.5 ± 0.04^{bdf}	6.8 ± 0.16^{ag}	7.0 ± 0.03^{ab}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	7	5.5 ± 0.04^{df}	6.7 ± 0.08^{ah}	7.7 ± 0.01^{cfg}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	10	5.4 ± 0.02^{df}	7.7 ± 0.20^{cf}	7.9 ± 0.06^{gh}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	14	5.4 ± 0.04^{f}	7.5 ± 0.32^{fg}	8.3 ± 0.06^{d}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	21	5.1 ± 0.07^{g}	7.2 ± 0.16^{ge}	8.0 ± 0.11^{dh}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	35	4.4 ± 0.15^{h}	7.2 ± 0.12^{gh}	6.0 ± 0.12^{i}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$

Unit = log CFU/g. Mean ± standard deviation. Mean in the same column with different superscripts were significantly different ($p<0.05$).

Table 3.2. Inhibition of *L. monocytogenes*, mesophilic bacteria and lactic acid bacteria on Cheddar cheeses with an initial inoculum of 10^3 CFU/g using PVDC copolymer films containing 0%, 1.5% and 3.0% sorbic acid.

Antimicrobial	Day	*L. monocytogenes*	Mesophilic bacteria	Lactic acid bacteria	Mold	Yeast
0% sorbic acid	0	3.6 ± 0.11^{ab}	3.8 ± 0.05^{a}	6.5 ± 0.08^{a}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	7	3.7 ± 0.02^{a}	5.8 ± 0.07^{bg}	7.7 ± 0.08^{b}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	14	3.7 ± 0.02^{a}	6.2 ± 0.03^{c}	8.1 ± 0.35^{be}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	21	3.6 ± 0.02^{ab}	7.3 ± 0.03^{d}	7.3 ± 0.68^{ac}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	35	3.1 ± 0.03^{abd}	6.3 ± 0.06^{e}	7.1 ± 0.07^{cd}	1.82 ± 0.17	$< 1.0 \pm 0.0$
1.5% sorbic acid	0	3.5 ± 0.11^{ab}	3.8 ± 0.06^{a}	6.5 ± 0.07^{a}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	7	3.7 ± 0.04^{a}	5.6 ± 0.06^{b}	7.7 ± 0.26^{bd}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	14	3.4 ± 0.04^{ab}	5.6 ± 0.03^{b}	7.4 ± 0.09^{bce}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	21	3.1 ± 0.07^{bd}	7.0 ± 0.32^{f}	7.1 ± 0.42^{ac}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	35	2.5 ± 0.06^{ec}	6.3 ± 0.04^{ec}	7.0 ± 0.30^{ac}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
3.0% sorbic acid	0	3.5 ± 0.08^{ab}	3.8 ± 0.05^{a}	6.5 ± 0.04^{a}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	7	3.5 ± 0.14^{ab}	5.6 ± 0.02^{b}	7.5 ± 0.27^{bc}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	14	3.4 ± 0.11^{ab}	5.6 ± 0.04^{b}	7.0 ± 0.07^{ac}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	21	2.7 ± 0.13^{cd}	6.3 ± 0.003^{ce}	5.9 ± 0.33^{f}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$
	35	2.3 ± 0.62^{e}	6.0 ± 0.33^{g}	5.3 ± 0.30^{g}	$< 1.0 \pm 0.0$	$< 1.0 \pm 0.0$

Unit = log CFU/g. Mean ± standard deviation. Mean in the same column with different superscripts were significantly different ($p<0.05$).

Table 3.3. Population change (log CFU/g) of *L. monocytogenes* due to PVDC copolymer films containing 0%, 1.5% or 3.0% (w/v) sorbic acid on Cheddar cheese after 35 days, and bologna slices after 28 days of refrigerated storage.

Food Types	Film Types	*L. monocytogenes*	
		10^5 CFU/g	10^3 CFU/g
Cheese	0% sorbic acid	-0.03 ± 0.04^a	-0.45 ± 0.13^a
	1.5% sorbic acid	-0.78 ± 0.03^a	-0.62 ± 0.04^b
	3.0% sorbic acid	-1.31 ± 0.15^a	-1.15 ± 0.59^c
Bologna	0% sorbic acid	3.88 ± 0.18^a	5.78 ± 0.12^a
	1.5% sorbic acid	-4.43 ± 0.14^b	-6.46 ± 0.13^b
	3.0% sorbic acid	-4.51 ± 0.29^b	-7.15 ± 0.32^c

Mean ± standard deviation. Means in the same column with different superscripts were significantly different ($p<0.05$).
Minus (-) represent decrease population.

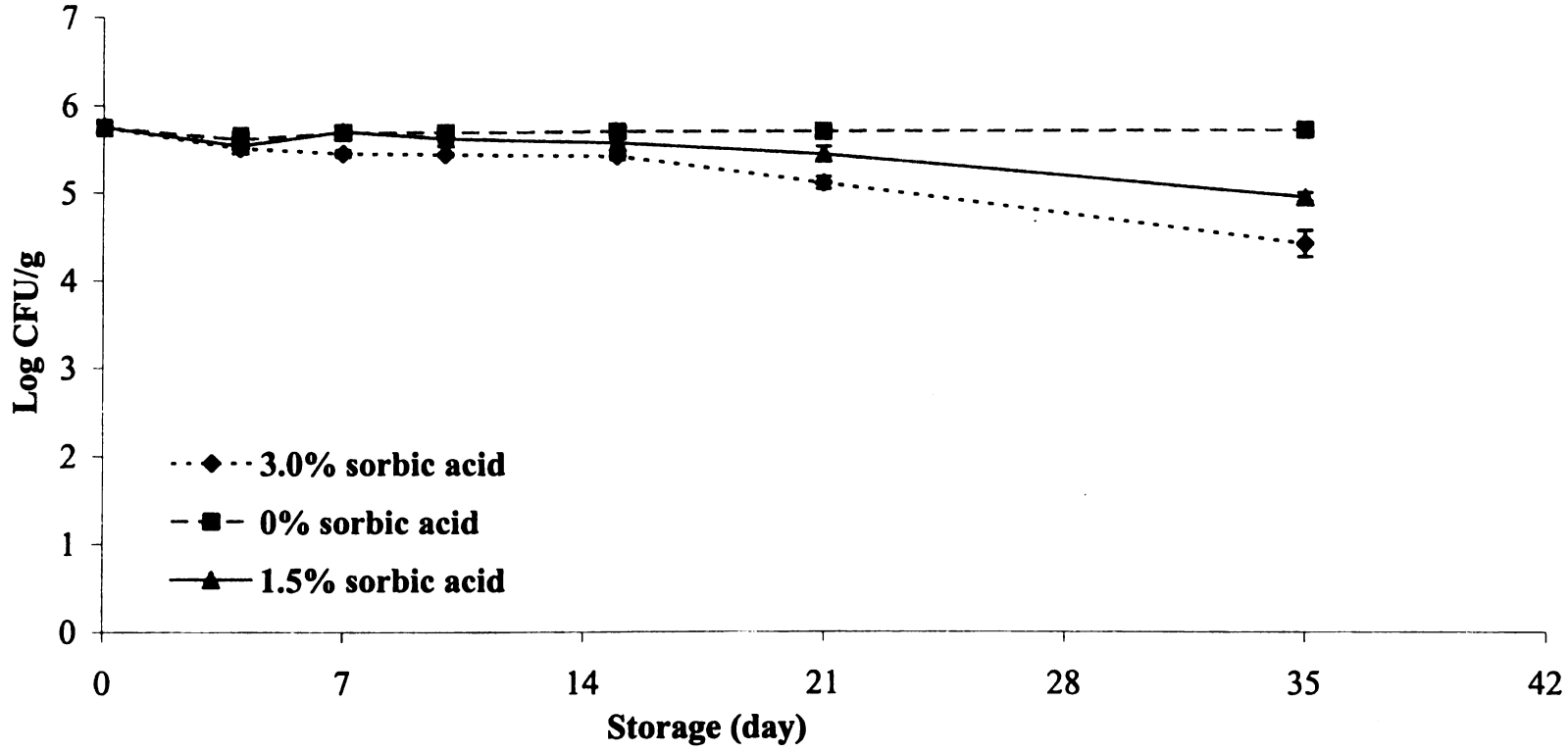

Figure 3.1. *L. monocytogenes* on Cheddar cheese with an initial inoculum of 10^5 CFU/g *L. monocytogenes*.

L. monocytogenes, *Listeria* was inhibited as much as 0.62 and 1.15 logs when the cheeses were wrapped with PVDC films containing 1.5% and 3.0% (w/v) sorbic acid, respectively (Figure 3.2), while the antimicrobial-free film wrapped cheese had a slightly decreased level after 35 days of refrigerated storage. The log reduction of *Listeria* of PVDC copolymer films containing 1.5% (w/v) was significantly different ($p < 0.05$) from film containing 3.0% (w/v) sorbic acid and significantly different ($p < 0.05$) from the control film for Cheddar cheese initially inoculated to contain 10^3 *L. monocytogenes* CFU/g. The low pH of the cheese (pH ~ 5.09) may have cause consistent number of *L. monocytogenes* in cheese for the film without antimicrobial agent during 35 days. Although sorbic acid is primarily used as a mold inhibitor in cheese products, it also possesses antibacterial activity against *Listeria* spp (Ryser and Marth, 1988). Piccinin and Shelef (1995) reported that potassium sorbate in cheese reduced the growth of *L. monocytogenes* after 24 days of storage at 5 °C. Larson et al. (1999) found that addition of 1.0% potassium sorbate or 1.0% sodium benzoate decreased survival of *L. monocytogenes* in cheese brines Number of *L. monocytogenes* decreased in cheese containing 0.3% sorbic acid or 0.3% sodium propionate, which was acidified to pH 5.0 to 5.1 using lactic and/or acetic acid (Ryser and Marth, 1988). More recently, PVDC copolymer films containing 1.5% to 3.0% (w/v) sorbic acid inhibited *L. monocytogenes* on laboratory media (Paweena et al., 2002).

3.4.1.2. Mesophilic Aerobic Bacteria (MAB)

The population of mesophilic aerobic bacteria (MAB), decreased 0.8 and 0.9 logs after 35 days storage (Figure 3.3) using a PVDC copolymer film containing1.5% and

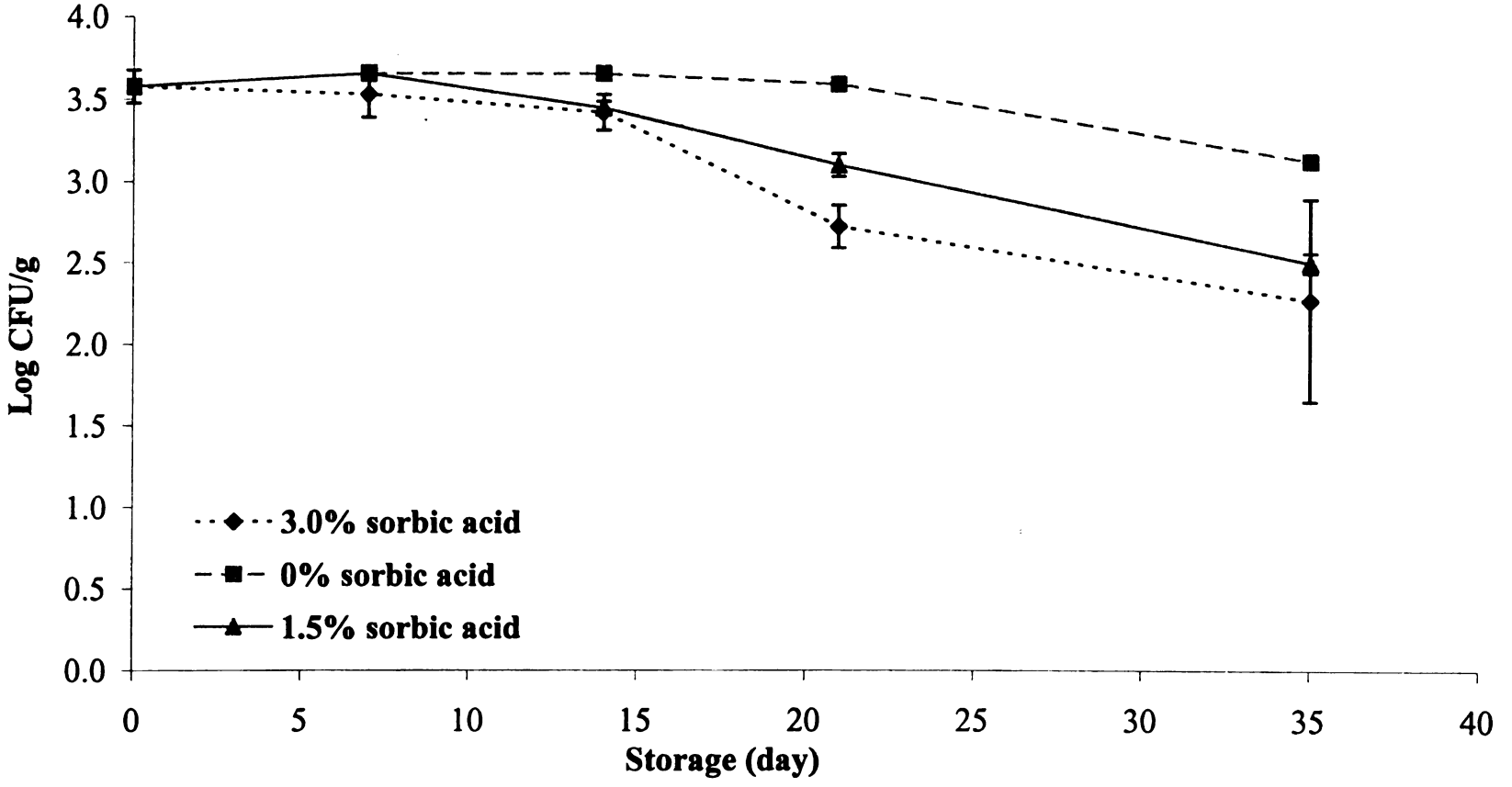

Figure 3.2. *L. monocytogenes* on Cheddar cheese with an initial inoculum of 10^3 CFU/g *L. monocytogenes*

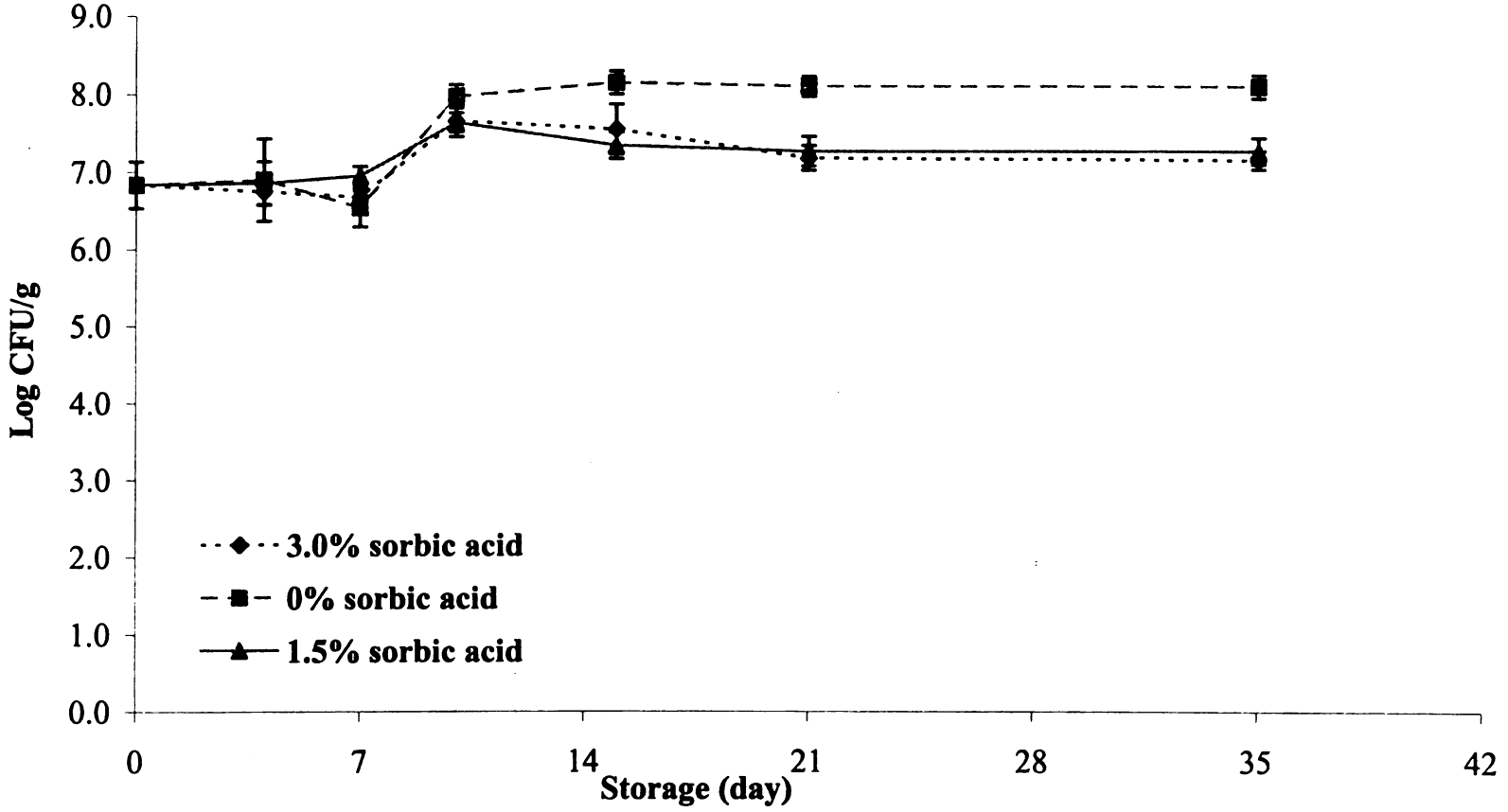

Figure 3.3. Mesophilic aerobic bacteria on Cheddar cheese with an initial inoculum of 10^5 CFU/g *L. monocytogenes*.

3.0% (w/v) sorbic acid, respectively compared to the antimicrobial-free film (Table 3.4). For cheeses initially containing 10^3 *L. monocytogenes* CFU/g, MAB decreased about 0.05 and 0.4 logs (Figure 3.4) using PVDC copolymer films containing 1.5% and 3.0% (w/v) sorbic acid, respectively as compared to cheeses wrapped with antimicrobial-free film. In contrast, the number of MAB on cheeses wrapped with films containing no antimicrobial agent increased in cheeses initially inoculated to contain 10^5 or 10^3 *L. monocytogenes* CFU/g after 35 days of storage at 4 °C. The reduction in mesophilic aerobic bacteria due to contact with PVDC copolymer films containing 1.5% sorbic acid was not significantly different ($p > 0.05$) from 3.0% (w/v) sorbic acid film, and both were significantly different ($p < 0.05$) from the control film for cheese initially inoculated to contain 10^5 or 10^3 *L. monocytogenes* CFU/g. The polyvinylidene chloride copolymer showed the potential to extend the shelf life of Cheddar cheese.

3.4.1.3. Lactic Acid Bacteria (LAB)

The populations of lactic acid bacteria on cheese inoculated to contain 10^5 or 10^3 *L. monocytogenes* CFU/g were also examined during 35 days of refrigerated storage. The number of lactic acid bacteria decreased slightly, 0.2 and 0.5 log using films containing 1.5% and 3.0% (w/v) sorbic acid, respectively, on cheese initial inoculated to contain *L. monocytogenes* 10^5 CFU/g compared to the control film (Figure 3.5, Table 3.5). The reduction in LAB for PVDC copolymer films containing 1.5% (w/v) sorbic acid was not significantly different ($p > 0.05$) from 3.0% (w/v) sorbic acid, and was also not significantly different ($p > 0.05$) from the antimicrobial-free film. A 0.2 and 2 log reduction (Figure 3.6) in LAB was observed after 35 days for cheeses that were surface inoculated with 10^3 *L. monocytogenes* CFU/g, and wrapped with films containing 1.5

Table 3.4. Population change (log CFU/g) of Mesophilic Aerobic Bacteria due to PVDC copolymer films containing 0%, 1.5% or 3.0% (w/v) sorbic acid on Cheddar cheese after 35 days, and bologna slices after 28 days of refrigerated storage.

Food Types	Film Types	Mesophilic Aerobic Bacteria	
		10^5 CFU/g	10^3 CFU/g
Cheese	0% sorbic acid	1.33 ± 0.33^a	2.47 ± 0.09^a
	1.5% sorbic acid	-0.83 ± 0.33^b	-0.05 ± 0.10^b
	3.0% sorbic acid	-0.93 ± 0.26^b	-0.38 ± 0.41^b
Bologna	0% sorbic acid	3.64 ± 0.03^a	5.71 ± 0.09^a
	1.5% sorbic acid	-4.25 ± 0.13^b	-6.44 ± 0.26^b
	3.0% sorbic acid	-4.31 ± 0.23^b	-6.88 ± 0.22^b

Mean ± standard deviation. Mean in the same column with different superscripts were significantly different ($p<0.05$).
Minus (-) represent decrease population.

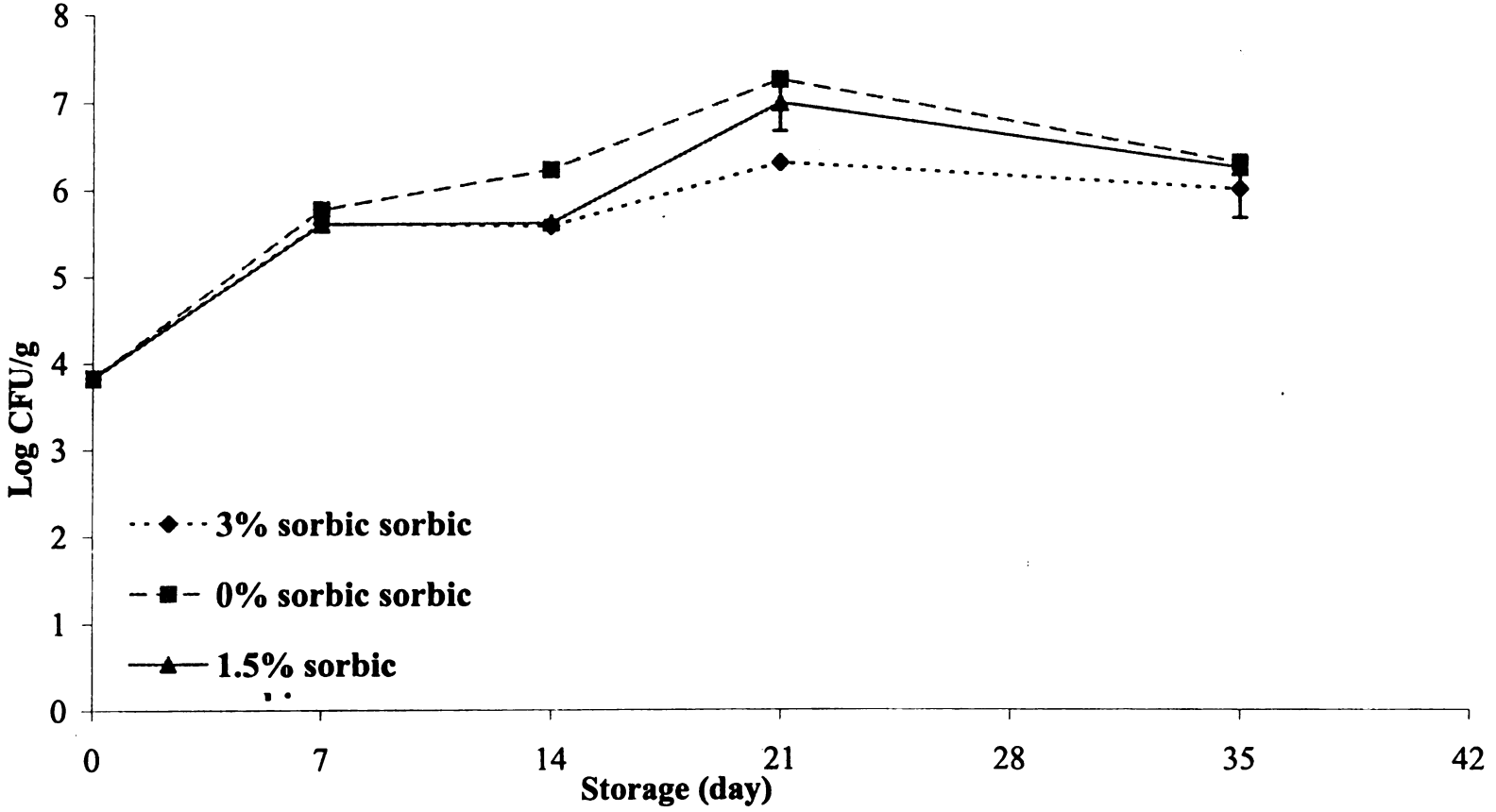

Figure 3.4. Mesophilic aerobic bacteria on Cheddar cheese with an initial inoculum of 10^3 CFU/g *L. monocytogenes*

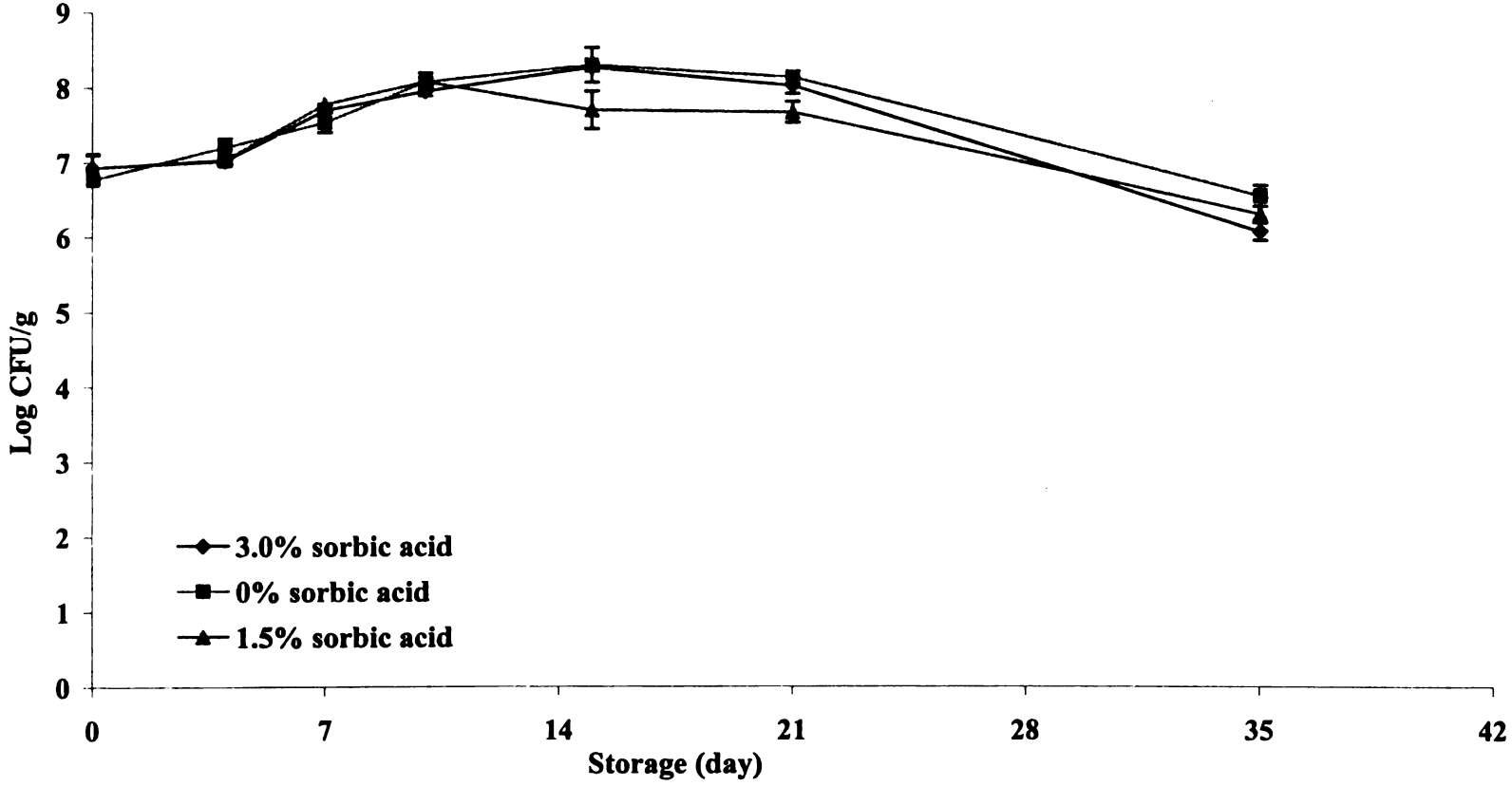

Figure 3.5. Lactic acid bacteria on Cheddar cheese with an initial inoculum of 10^5 CFU/g *L. monocytogenes*.

Table 3.5. Population change (log CFU/g) of Lactic Acid Bacteria (LAB) due to PVDC copolymer films containing 0%, 1.5% or 3.0% (w/v) sorbic acid on Cheddar cheese after 35 days, and bologna slices after 28 days of refrigerated storage.

Food Types	Film Types	Lactic Acid Bacteria	
		10^5 CFU/g	10^3 CFU/g
Cheese	0% sorbic acid	-0.46 ± 0.17^a	0.63 ± 0.07^a
	1.5% sorbic acid	-0.24 ± 0.11^a	-0.20 ± 0.32^b
	3.0% sorbic acid	-0.47 ± 0.07^a	-1.90 ± 0.17^c
Bologna	0% sorbic acid	8.87 ± 0.05^a	8.73 ± 0.04^a
	1.5% sorbic acid	-4.40 ± 0.46^b	-6.16 ± 0.06^b
	3.0% sorbic acid	-4.46 ± 0.26^c	-6.50 ± 0.22^b

Mean ± standard deviation. Mean in the same column with different superscripts were significantly different ($p<0.05$).
Minus (-) represent decreased population.

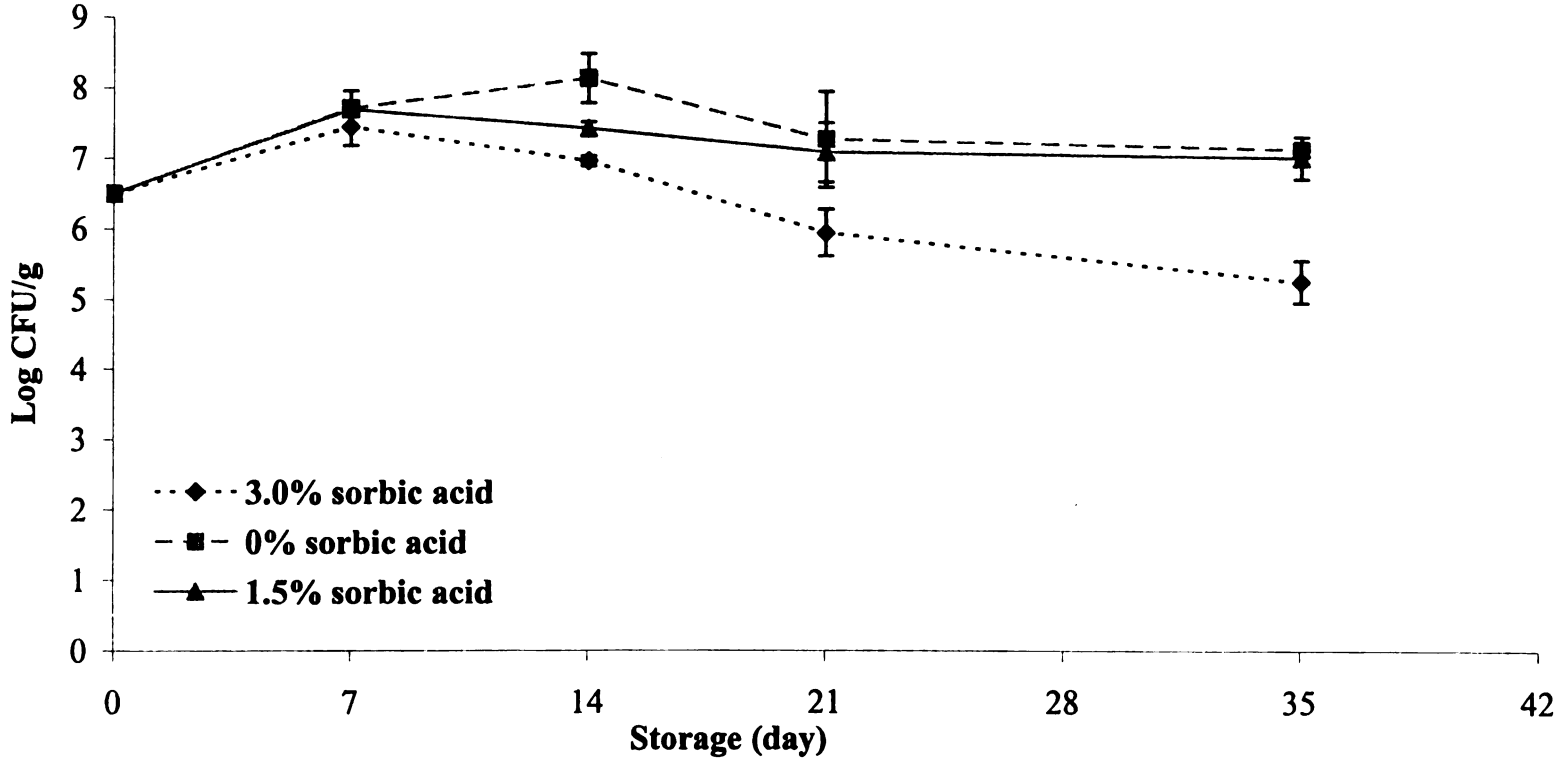

Figure 3.6. Lactic acid bacteria on Cheddar cheese with an initial inoculum of 10^3 CFU/g *L. monocytogenes*.

and 3.0% (w/v) sorbic acid, respectively. In contrast, cheese wrapped with the antimicrobial-free film had a 0.6 log increase (Table 3.5). Defects in cheese, such as undesirable flavors, gas formation, or white surface haze can result from growth of nonstarter lactic acid bacteria (Somers et al., 2001). Swearingen et al. (2001) reported that nonstarter lactic acid bacteria, *Lactobacillus* spp., *Streptococcus thermophillus* and *Lactococcus* spp., found in 3 month ripened Cheddar cheese affected the flavor and quality. During Cheddar cheese ripening, nonstarter lactic acid bacteria levels normally exceed 10^7 CFU/g, which in some instances can be significantly higher than the starter levels in cheese (Peterson and Marshall, 1990, Fox et al., 1993, Ryan et al., 1996), and can play a significant role in proteolysis and flavor development during ripening (McSweeney et al., 1994 and Ryan et al., 1996).

3.4.1.4. Mold and Yeast

No mold or yeast growth on Cheddar cheese was found, for either *L. monocytogenes* inoculum level. When cheese wrapped in films containing either 1.5% or 3.0% (w/v) sorbic acid was refrigerated for 35 days. Mold growth appeared only on cheeses initially surface inoculated to contain 10^3 *L. monocytogenes* CFU/g, when wrapped with antimicrobial-free films at day 35 of storage. Sorbic acid is well known as a yeast and mold inhibitor in cheese food (Marth and Ryser, 1988). Weng and Chen (1997) also reported that polyethylene containing sorbic anhydride inhibited *Penicillium* spp. and *Aspergillus niger* on laboratory media.

3.4.2. Antimicrobial activity on bologna inoculated to contain 10^5 and 10^3 *L. monocytogenes* CFU/g.

3.4.2.1. *L. monocytogenes*

The populations of *L. monocytogenes* on bologna for both inoculum levels were examined immediately after inoculation and again following 4, 7, 10, 14, 21 and 28 days of storage (Table 3.6 and 3.7). The number of *L. monocytogenes* on bologna slices, which was initially surface inoculated to contain 10^5 *L. monocytogenes* CFU/g, decreased 4.4 and 4.5 logs using PVDC copolymer containing 1.5% and 3.0% (w/v) sorbic acid, respectively, after 28 days at refrigerated storage; whereas *Listeria* increased 3.9 logs using film without sorbic acid (Figure 3.7). The log reduction of *Listeria* due to contact with PVDC copolymer films containing 1.5% sorbic acid was significantly different ($p < 0.05$) from film containing 3.0% (w/v) sorbic acid, and both were significantly different ($p < 0.05$) from the antimicrobial-free film. For bologna slices with an initial inoculum level of 10^3 CFU/g of *L. monocytogenes*, *Listeria* was inhibited up to 6.5 and 7.2 logs (Table 3.7) using films containing 1.5% and 3.0% (w/v) sorbic acid, respectively compared to the control film (Figure 3.8). *Listeria* on bologna in contact with the antimicrobial-free films increased 5.8 logs after 28 days storage at 4 °C. The log reduction in numbers of *L. monocytogenes* for film containing 1.5% sorbic acid was not significantly different ($p > 0.05$) from 3.0% (w/v) sorbic acid, and both were significantly different ($p < 0.05$) from the film containing 0% sorbic acid. In a previous study, PVDC copolymer films containing 1.5% to 3.0% (w/v) sorbic acid inhibited the growth of *L. monocytogenes* on laboratory media (Limjaroen et al., 2002). Inhibition of *L. monocytogenes* was found on sliced bologna using 0.26% potassium sorbate during 28

Table 3.6. Inhibition of *L. monocytogenes*, mesophilic bacteria and lactic acid bacteria on bologna which were inoculated with 10^5 CFU/g using PVDC copolymer films containing 0%, 1.5% and 3% (w/v) sorbic acid.

Antimicrobial (%w/v)	Day	*L. monocytogenes*	Mesophilic bacteria	Lactic acid bacteria
0% sorbic acid	0	5.2 ± 0.15^{agi}	5.2 ± 0.09^{a}	$< 1.0 \pm 0.0^{a}$
	4	6.1 ± 0.50^{b}	6.0 ± 0.52^{b}	4.0 ± 0.51^{bh}
	7	6.5 ± 0.05^{c}	6.4 ± 0.02^{c}	6.5 ± 0.03^{c}
	10	7.5 ± 0.01^{d}	7.5 ± 0.03^{d}	7.1 ± 0.44^{c}
	14	7.5 ± 0.03^{d}	8.5 ± 0.19^{e}	7.7 ± 0.85^{cd}
	21	8.9 ± 0.13^{e}	8.8 ± 0.16^{e}	8.0 ± 0.84^{d}
	28	9.0 ± 0.06^{e}	8.8 ± 0.10^{e}	8.9 ± 0.06^{e}
1.5% sorbic acid	0	5.2 ± 0.14^{ag}	5.2 ± 0.15^{a}	$< 1.0 \pm 0.0^{a}$
	4	5.5 ± 0.15^{a}	5.1 ± 0.38^{af}	3.5 ± 0.09^{bg}
	7	4.8 ± 0.08^{fhik}	5.3 ± 0.48^{af}	4.8 ± 0.02^{f}
	10	5.0 ± 0.11^{fg}	4.9 ± 0.20^{afi}	5.0 ± 0.18^{f}
	14	4.9 ± 0.03^{fgh}	4.7 ± 0.24^{fgi}	5.1 ± 0.09^{f}
	21	4.7 ± 0.18^{fhk}	4.7 ± 0.22^{fi}	4.9 ± 0.17^{f}
	28	4.6 ± 0.11^{hk}	4.6 ± 0.15^{fi}	4.7 ± 0.49^{fb}

Table 3.6. (Con't)

Antimicrobial (%w/v)	Day	*L. monocytogenes*	Mesophilic bacteria	Lactic acid bacteria
3.0% sorbic acid	0	5.1 ± 0.12^{gij}	5.1 ± 0.10^{ag}	$< 1.0 \pm 0.0^{a}$
	4	4.9 ± 0.10^{gh}	3.8 ± 0.12^{h}	2.8 ± 0.30^{g}
	7	4.7 ± 0.05^{hfk}	4.8 ± 0.07^{afi}	4.7 ± 0.10^{f}
	10	4.9 ± 0.04^{hg}	5.0 ± 0.21^{afi}	4.8 ± 0.03^{f}
	14	4.8 ± 0.07^{jhfk}	4.8 ± 0.13^{afi}	4.8 ± 0.03^{f}
	21	4.7 ± 0.13^{hfk}	4.7 ± 0.15^{afi}	4.6 ± 0.10^{f}
	28	4.6 ± 0.25^{k}	4.5 ± 0.25^{i}	4.5 ± 0.36^{fh}

Unit = log CFU/g. Mean ± standard deviation. Mean in the same column with different superscripts were significantly different ($p<0.05$).

Table 3.7. Inhibition of *L. monocytogenes*, mesophilic bacteria and lactic acid bacteria on bologna which were inoculated with 10^3 CFU/g using PVDC copolymer films containing 0%, 1.5% and 3% (w/v) sorbic acid.

Antimicrobial (%w/v)	Day	*L. monocytogenes*	Mesophilic bacteria	Lactic acid bacteria
0% sorbic acid	0	3.0 ± 0.2^{ab}	3.0 ± 0.2^{afh}	$< 1.0 \pm 0.0^{a}$
	4	3.2 ± 0.1^{b}	4.0 ± 0.2^{ab}	$< 1.0 \pm 0.0^{a}$
	7	4.2 ± 0.2^{c}	4.2 ± 0.2^{b}	4.0 ± 0.24^{b}
	10	5.2 ± 0.2^{c}	4.4 ± 0.1^{b}	4.5 ± 0.01^{b}
	14	6.0 ± 0.2^{d}	5.1 ± 0.1^{c}	5.8 ± 0.17^{c}
	21	8.0 ± 0.1^{e}	7.4 ± 0.1^{d}	7.9 ± 0.09^{d}
	28	8.8 ± 0.1^{f}	8.7 ± 0.1^{e}	8.7 ± 0.06^{e}
1.5% sorbic acid	0	3.0 ± 0.1^{a}	3.0 ± 0.2^{fh}	$< 1.0 \pm 0.0^{a}$
	4	2.9 ± 0.1^{ag}	3.0 ± 0.1^{f}	$< 1.0 \pm 0.0^{a}$
	7	2.6 ± 0.1^{aghi}	2.5 ± 0.4^{fgi}	$< 1.0 \pm 0.0^{a}$
	10	2.6 ± 0.1^{aghi}	2.6 ± 0.2^{fg}	2.9 ± 0.52^{f}
	14	2.5 ± 0.1^{ghi}	2.6 ± 0.1^{fg}	2.6 ± 0.18^{fg}
	21	2.3 ± 0.1^{hij}	2.5 ± 0.2^{fg}	2.6 ± 0.14^{fg}
	28	2.4 ± 0.1^{ih}	2.3 ± 0.3^{gi}	2.6 ± 0.14^{f}

Table 3.7. (Con't)

Antimicrobial (%w/v)	Day	*L. monocytogenes*	Mesophilic bacteria	Lactic acid bacteria
3.0% sorbic acid	0	3.1 ± 0.1^{ab}	3.0 ± 0.2^{fh}	$< 1.0 \pm 0.0^{a}$
	4	2.8 ± 0.1^{ah}	3.0 ± 0.1^{fh}	$< 1.0 \pm 0.0^{a}$
	7	2.4 ± 0.1^{hgi}	2.5 ± 0.2^{ghi}	$< 1.0 \pm 0.0^{a}$
	10	2.3 ± 0.2^{ij}	2.5 ± 0.2^{gi}	1.8 ± 0.17^{fg}
	14	2.3 ± 0.1^{ij}	2.2 ± 0.2^{gi}	2.5 ± 0.14^{f}
	21	1.9 ± 0.3^{jk}	1.8 ± 0.2^{i}	2.2 ± 0.26^{g}
	28	1.7 ± 0.3^{k}	1.9 ± 0.3^{i}	2.3 ± 0.24^{fg}

Unit = log CFU/g. Mean ± standard deviation. Mean in the same column with different superscripts were significantly different ($p<0.05$).

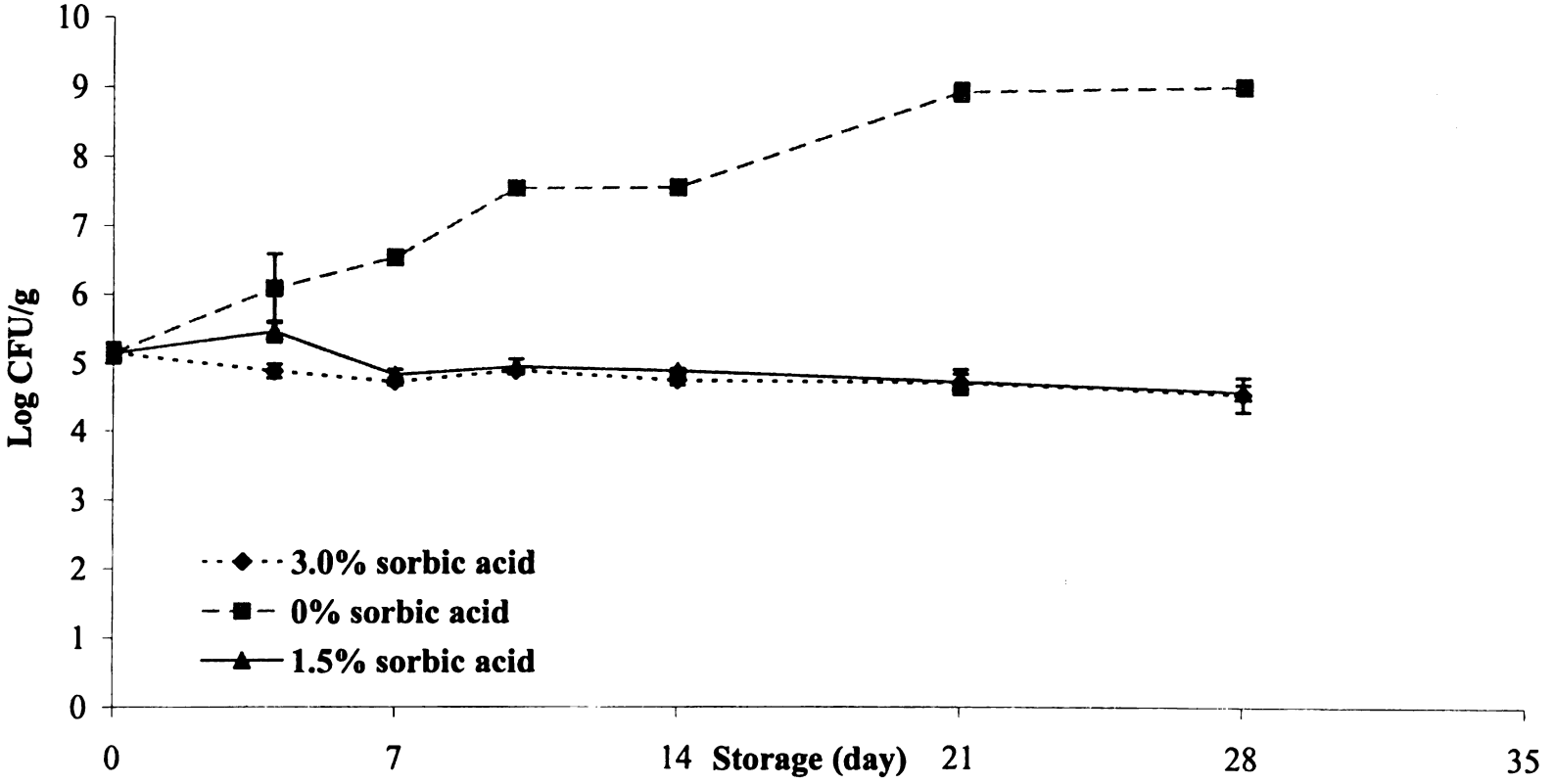

Figure 3.7. *L. monocytogenes* on bologna with an initial inoculum of 10^5 CFU/g *L. monocytogenes* using different film types.

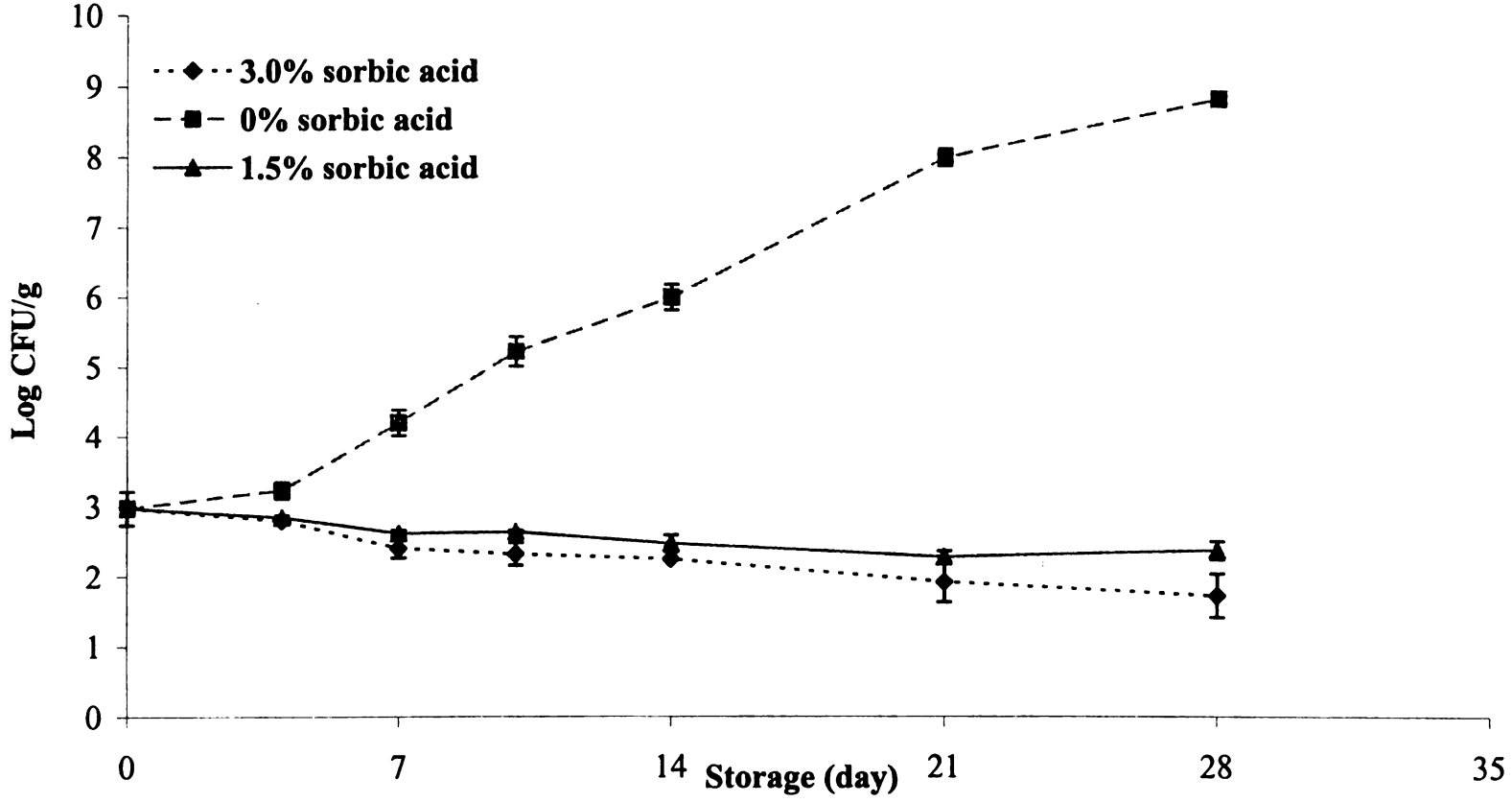

Figure 3.8. *L. monocytogenes* on bologna with an initial inoculum of 10^3 CFU/g *L. monocytogenes* using different film types.

days of refrigerated storage (Wederquist et al, 1994). Cagri et al. (2002) reported that low pH whey protein isolate films containing 0.5% to 1.0% (w/v) p-aminobenzoic acid and/or sorbic acid inhibited growth of *L. monocytogenes* about 3.4-4.1 logs on bologna and summer sausage slices during 21 days of refrigerated storage.

3.4.2.2. Mesophilic Aerobic Bacteria (MAB)

Numbers of mesophilic aerobic bacteria (MAB) after 28 days of storage on bologna slices, which were initially surface inoculated with 10^5 *L. monocytogenes* CFU/g decreased 4.3 and 4.3 logs (Figure 3.9) due to contact with films containing 1.5% and 3.0% (w/v) sorbic acid, respectively, compared to a 3.6 log increase for the antimicrobial-free film. Population of MAB decreased by 6.4 and 6.9 (Figure 3.10) logs on bologna slices initially contaminated with 10^3 *L. monocytogenes* CFU/g due to contact with PVDC copolymer films containing 1.5% and 3.0% (w/v) sorbic acid, respectively. Numbers of MAB on bologna increased 5.7 logs after 28 days of storage using sorbic acid-free film (Figure 3.10). Log reductions in MAB on bologna inoculated with 10^5 or 10^3 *L. monocytogenes* CFU/g due to films containing 1.5% or 3.0% (w/v) sorbic acid were significantly different from the antimicrobial-free film ($p < 0.05$). Cagri et al. (2002) reported that whey protein isolate film containing sorbic acid reduced MAB populations about 5.0 logs on bologna slices after 21 days of storage at 4 °C.

3.4.2.3. Lactic Acid Bacteria (LAB)

Populations of lactic acid bacteria were also examined during 28 days of refrigerated storage (Figure 3.11 and 3.12). Lactic acid bacteria decreased 4.4 and 4.5 logs on bologna slices initially surface inoculated with 10^5 *L. monocytogenes* CFU/g using films containing 1.5% and 3.0% (w/v), respectively, compared to the control film,

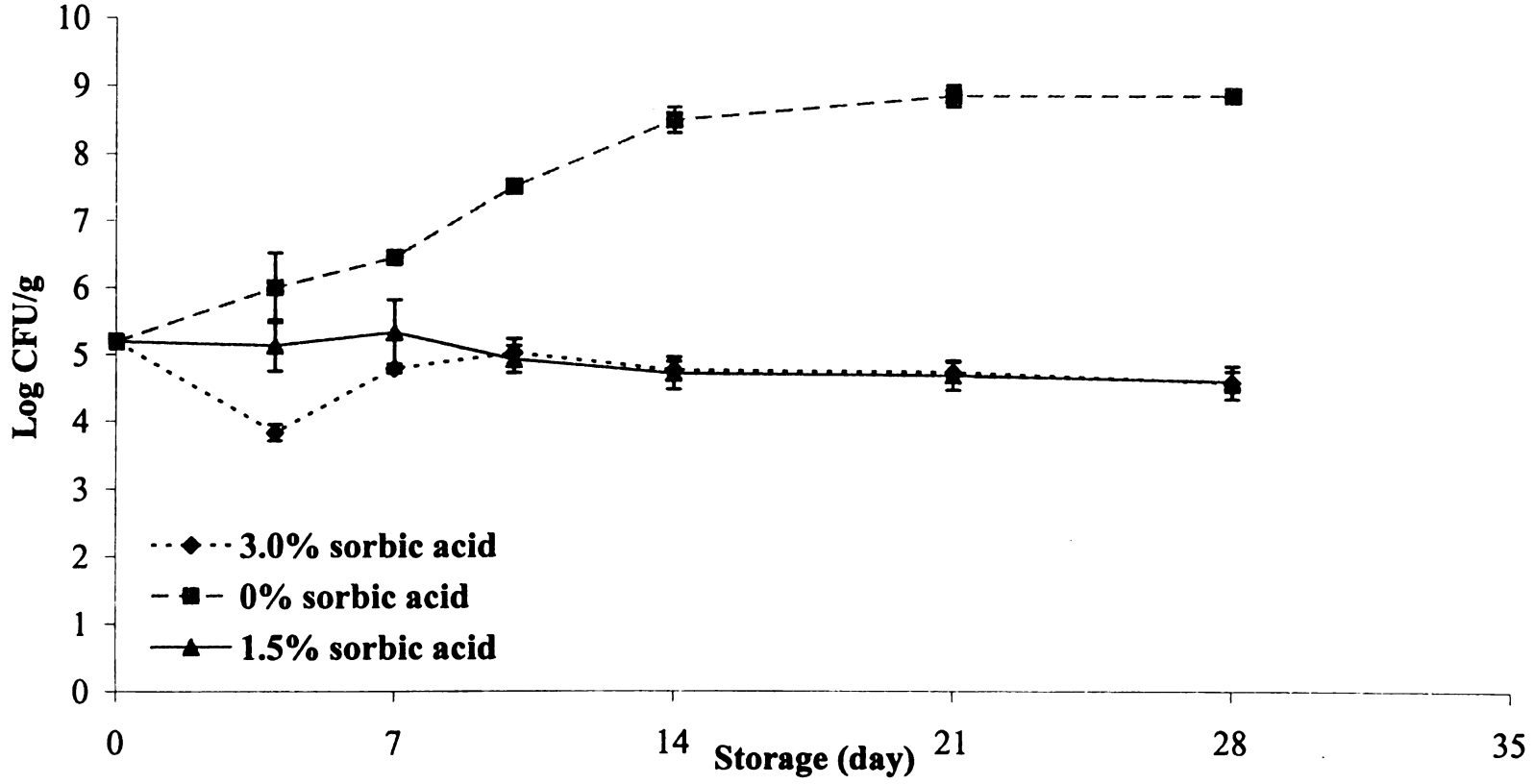

Figure 3.9. Mesophilic aerobic bacteria on bologna with an initial inoculum of 10^5 CFU/g *L. monocytogenes* using different film types.

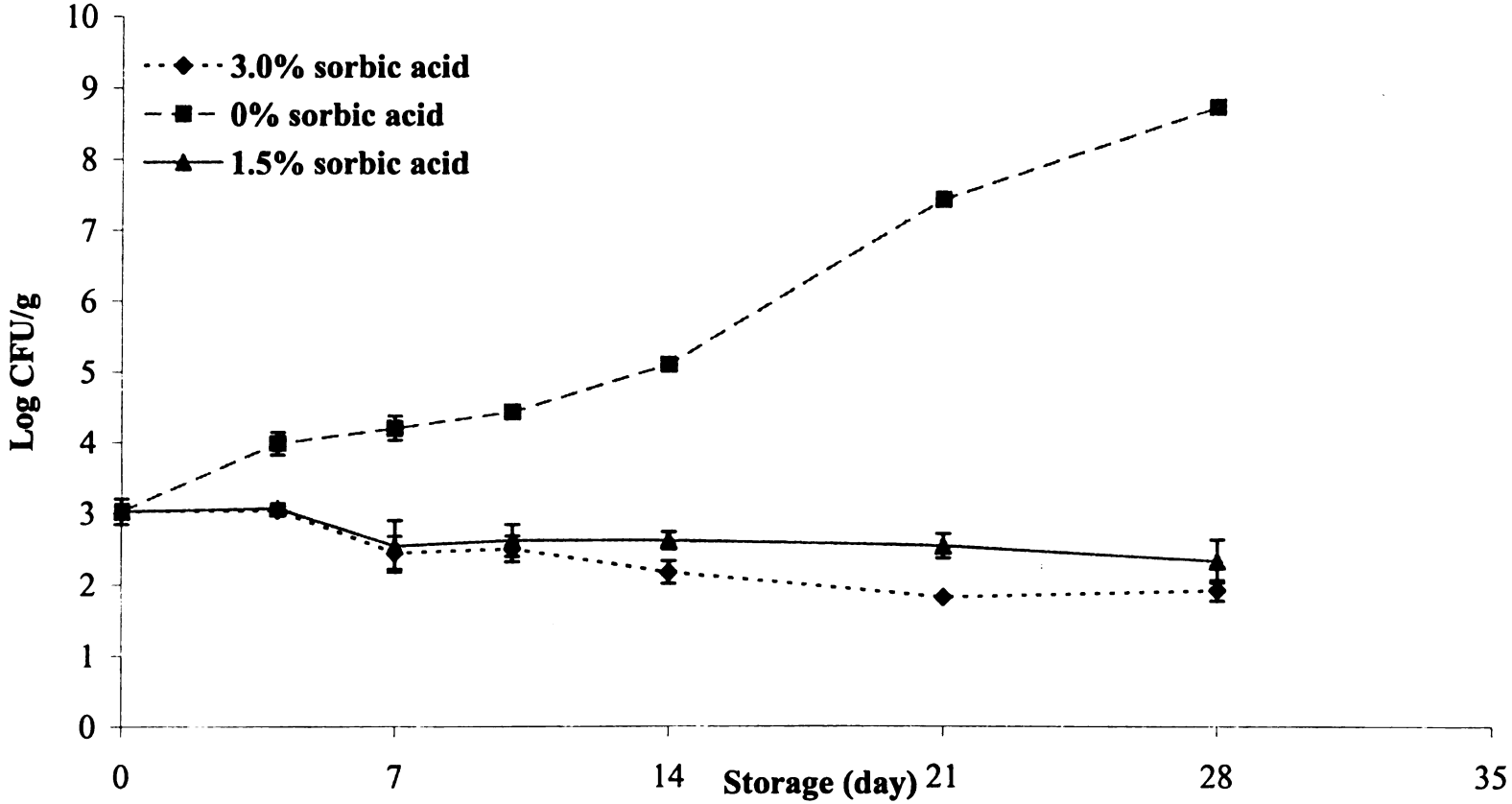

Figure 3.10. Mesophilic aerobic bacteria on bologna with an initial inoculum of 10^3 CFU/g *L. monocytogenes* using different film types.

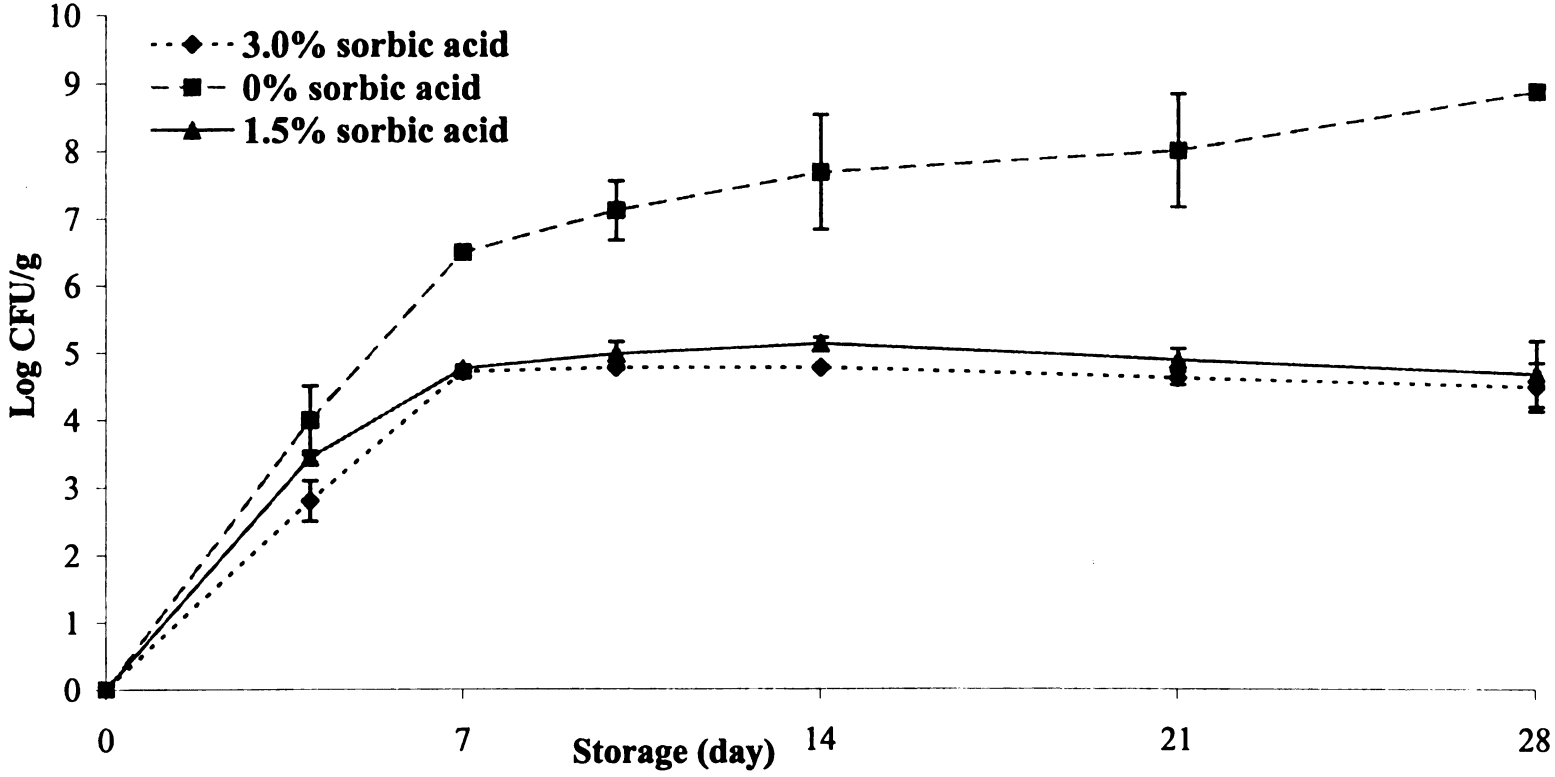

Figure 3.11. Lactic acid bacteria on bologna with an initial inoculum of 10^5 CFU/g *L. monocytogenes* using different film types.

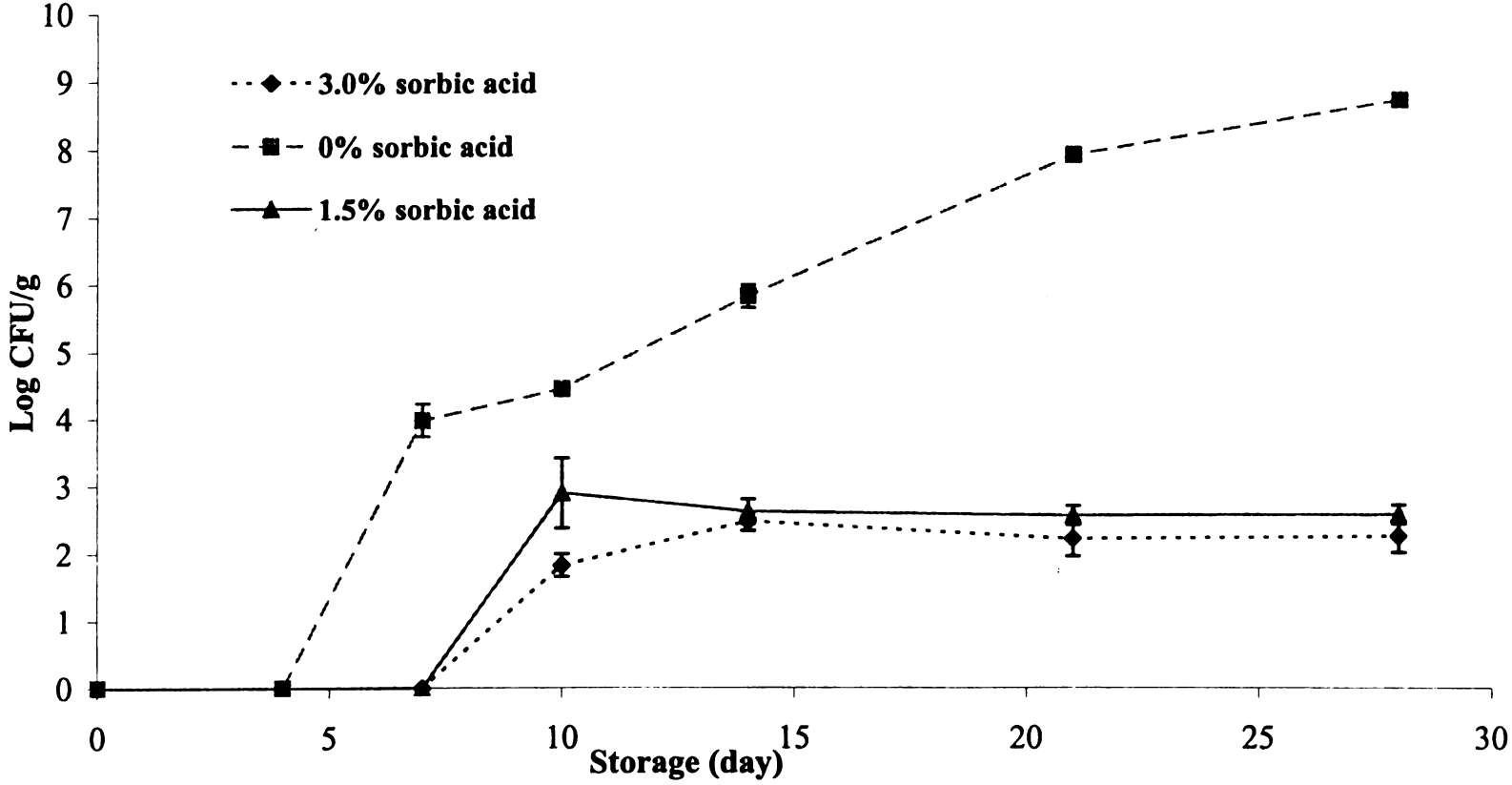

Figure 3.12. Lactic acid bacteria on bologna with an initial inoculum of 10^3 CFU/g *L. monocytogenes* using different film types.

where the number of LAB increased 8.9 logs. The log reduction in LAB using PVDC copolymer films containing 1.5% sorbic acid was significantly different ($p < 0.05$) from film containing 3.0% (w/v) sorbic acid and both were significantly different ($p < 0.05$) from the antimicrobial-free film. For bologna, which initially contained 10^3 *L. monocytogenes* CFU/g, the population of LAB decreased 6.2 and 6.5 logs due to PVDC copolymer films containing 1.5% and 3.0% (w/v) sorbic acid, respectively. LAB populations increased 8.7 logs on bologna slices after 28 days when wrapped with antimicrobial-free film. The log reduction in LAB due to contact with PVDC copolymer film containing 1.5% sorbic acid was not significantly different ($p > 0.05$) from 3.0% (w/v) sorbic acid but both were significantly different ($p < 0.05$) from the control film. Holly (1997) reported that for bologna slices stored at 7 °C lactic acid bacteria (LAB), dominated were most plentiful with *Lactobacillus sake* dominating.

3.4.2.4. Mold and Yeast

No mold or yeast growth ($< 1.0 \pm 0.0$ log CFU/g) occurred on bologna slices, for either inoculum level of *L. monocytogenes* CFU/g, regardless of the level of sorbic acid after 28 days of refrigerated storage. Matamoros et al. (1999) reported that sorbic acid salt and potassium sorbate inhibited the growth of *Penicillium digitatum*, *Penicillium glabrum* and *Penicillium italium* in potato dextrose agar. Cagri et al. (2002) reported that whey protein isolate films containing sorbic acid inhibited mold growth on bologna during 21 days storage at 4 °C.

3.5. CONCLUSION

PVDC copolymer films containing 1.5 or 3.0% sorbic acid reduced *L. monocytogenes* population by 0.1 – 1 on Cheddar cheese and 4 – 7 logs on bologna slices after 35 and 28 days of refrigerated storage, respectively. Minimal reductions in the numbers of mesophilic and lactic acid bacteria were observed for Cheddar cheese, whereas these same groups of potential spoilage organisms decreased 4–6 logs on bologna slices wrapped in PVDC film containing 1.5 – 3.0 % sorbic acid.

CHAPTER 4.

MIGRATION OF SORBIC ACID FROM POLYVINYLIDENE CHLORIDE ANTIMICROBIAL FILM TO CHEDDAR CHEESE AND BOLOGNA

4.1. ABSTRACT

The migration of sorbic acid from the polyvinylidene antimicrobial films to Cheddar cheese and beef bologna was determined using High Performance Liquid Chromatography (HPLC). The sorbic acid migration to Cheddar cheese and bologna was monitored over a 28 day storage period, and the sorbic acid concentration remaining in the film was determined. The migration rate constant was also determined using linear regression with experimental data based on a first order reaction. The migration of sorbic acid at the end of 28 days of storage to Cheddar cheese was 6.5% wt/wt, and to bologna was 15% wt/wt. The rate constant for Cheddar cheese was 0.007 per day, and for bologna was 0.040 per day. This indicates that sorbic acid could migrate from films to Cheddar cheese and bologna to inhibit microorganisms.

4.2. INTRODUCTION

Sorbic acid is widely used as a preservative in food products such as cheese and meat products. Sorbic acid inhibits the growth of mold, yeast and some bacteria. Sorbic acid is commonly used as an antimicrobial agent in antimicrobial films. A polyvinylidene chloride (PVDC) copolymer film containing sorbic acid was shown to inhibit the growth of *Listeria monocytogenes* on laboratory media (Limjaroen et al., 2002a), and on Cheddar cheese and beef bologna (Limjaroen et al., 2002b). The antimicrobial film was positioned in direct contact with the food. The antimicrobial agent migrates to the surface of the packaging material and then to the food to inhibit microbial growth. The release rates and migration amounts of the antimicrobial agents from the packaging material to food is very important (Han, 2000). Diffusion between the packaging material and the food, and partitioning at the interface are the main migration phenomena involved. In other cases,

the antimicrobial is effective against microorganisms on the food surface without migration of active agents to the food.

In this chapter, the migration/release of sorbic acid from the PVDC antimicrobial film to food over storage time at 4°C was determined. The effect of food on migration/release of sorbic acid was also determined. Two types of food were used in this study, Cheddar cheese and beef bologna. The migration or loss of sorbic acid from the PVDC film was verified as well.

4.3. MATERIALS AND METHODS

4.3.1. Products

Beef bologna (diameter ~ 9.6 cm) and Cheddar cheese (dimensions ~ 3 × 5 cm) commercially produced were obtained from a local supermarket and the MSU dairy store, respectively. The pre-sliced bologna (~3 mm thick), which had a pH value of 6.1 when purchased, was cut into 10-g squares (3 × 3 inches). Cheddar cheese (pH~5.0) was cut into 25-g pieces measuring 3 x 3 x 2.5 cm.

4.3.2. Film preparation

Polyvinylidene chloride (PVDC) copolymer (Saran F-310, Dow Chemical, Midland, MI) resin was slowly dissolved in methyl ethyl ketone (J.T Baker, Phillipsburg, NJ) (18% w/v) at room temperature with continuous agitation until completely dissolved. 3% (w/v) sorbic acid (Sigma Chemical Co., St. Louis, MO) was then incorporated into the solution. The film solution (pH 3.5) was cast onto a glass plate (9 × 13 inches) and dried in a Oven (Fisher Scientific, Pittsburgh, PA) at 86 ± 0.5 °C for 5 minutes. After

drying, the films were peeled from the plates. The films were then positioned in direct contact with the food or without food for the control.

4.3.3. Sample preparation

For cheese, 25-g pieces were wrapped with film (3.5 × 5 inch) containing 3% (w/v) sorbic acid. For bologna, 10-g slices were placed between PVDC films (3.5 × 3.5 inch) containing 3% (w/v) sorbic acid and stacked 3 slices high in 150 mm-diameter sterile Petri dishes. The samples were stored at 4°C for 28 days. The samples were evaluated after 0, 1, 2, 3, 4, 5, 6, 7, 14, 21 and 28 days of refrigerated storage. PVDC films containing 3.0% (w/v) sorbic acid samples (control film, no food contact) were also analyzed for sorbic acid content over this storage period. The samples were stored at 4°C. All experiments were replicated three times.

4.3.4. Migration test

4.3.4.1. Standard calibration curve

Standard solutions of sorbic acid were first prepared by dissolving 0.1 gram in 100 ml HPLC grade methanol in a volumetric flask. The solution was then serially diluted to 1, 2.5, 5, 10, 25, 50 and 80 parts per million (ppm) to prepare a series of standard solutions of known sorbic acid concentration for a standard calibration curve. The standard curve is shown in Figure 4.1.

4.3.4.2. Extraction procedure and HPLC evaluation

The film samples, unwrapped from Cheddar cheese and un-stacked from bologna, and the control film (no food contact) were cut into small pieces. 0.5 grams of the samples were immersed in 50 ml HPLC grade methanol and vortexed thoroughly, and stored at room temperature for 7 days. During the 7 days storage, the sample solutions

were thoroughly vortexed once a day. For analysis, the sample solution was filtered through a 0.45 μm microfilter (Fisher Scientific, Pittsburgh, PA), and then serially diluted to 1:100 using HPLC grade methanol. Sorbic acid was analyzed using a Waters' HPLC system (Waters Corporate, Milford, MA) with UV detector, and C 18 column with inside dimension of 150 × 5 mm (Waters). The mobile-phase solution was methanol-water (1:1), injection volume 10 μl, flow rate 1.0 ml/min. The sorbic acid concentration was determined using a UV detector at 254 nm. The retention time was 1.4 minutes (see Figure 4.2). The concentration of sorbic acid was determined by the following equation:

$$\% \text{ sorbic acid (wt/wt)} = \frac{R_s \times C.F. \times V_{total}}{V_{inj} \times Wt_{polymer}}$$

where R_s = detector response value for the sample (area unit)

C. F. = calibration factor from slope of standard calibration curve (g/Au)

V_{total} = total volume containing analyte (ml)

V_{inj} = injection volume of unknown sample solution

$Wt_{polymer}$ = weight of the polymer sample used for analysis (g)

4.3.4.3. Calculation of migration/release rate

The migration/release rate of sorbic acid can be calculated from the kinetic curve, which followed a first rate order relationship. A first order reaction is one in which the rate of the reaction is proportional to the concentration of only one of the reacting substances (Benson, 1960 and Han, 1984).

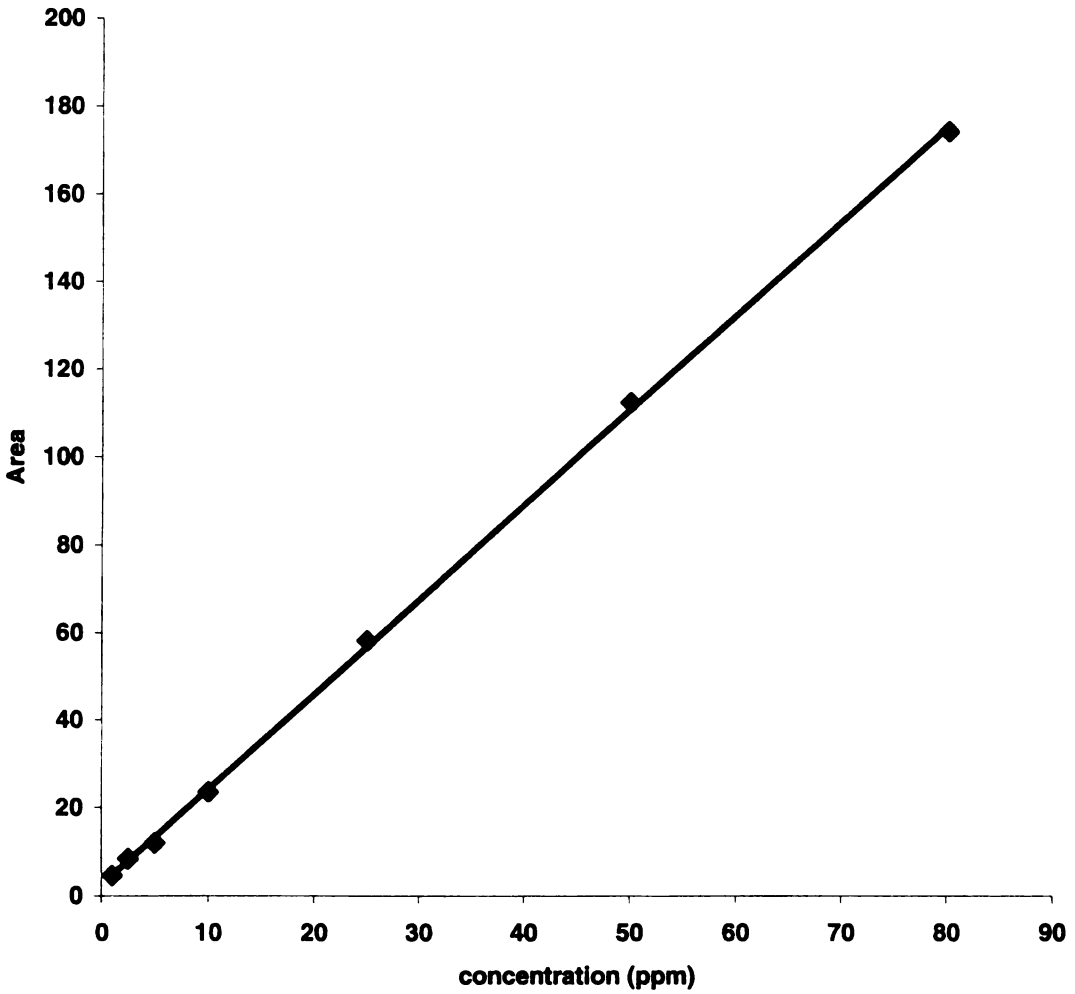

Figure 4.1. Standard curve of sorbic acid concentration in a methanol solution.

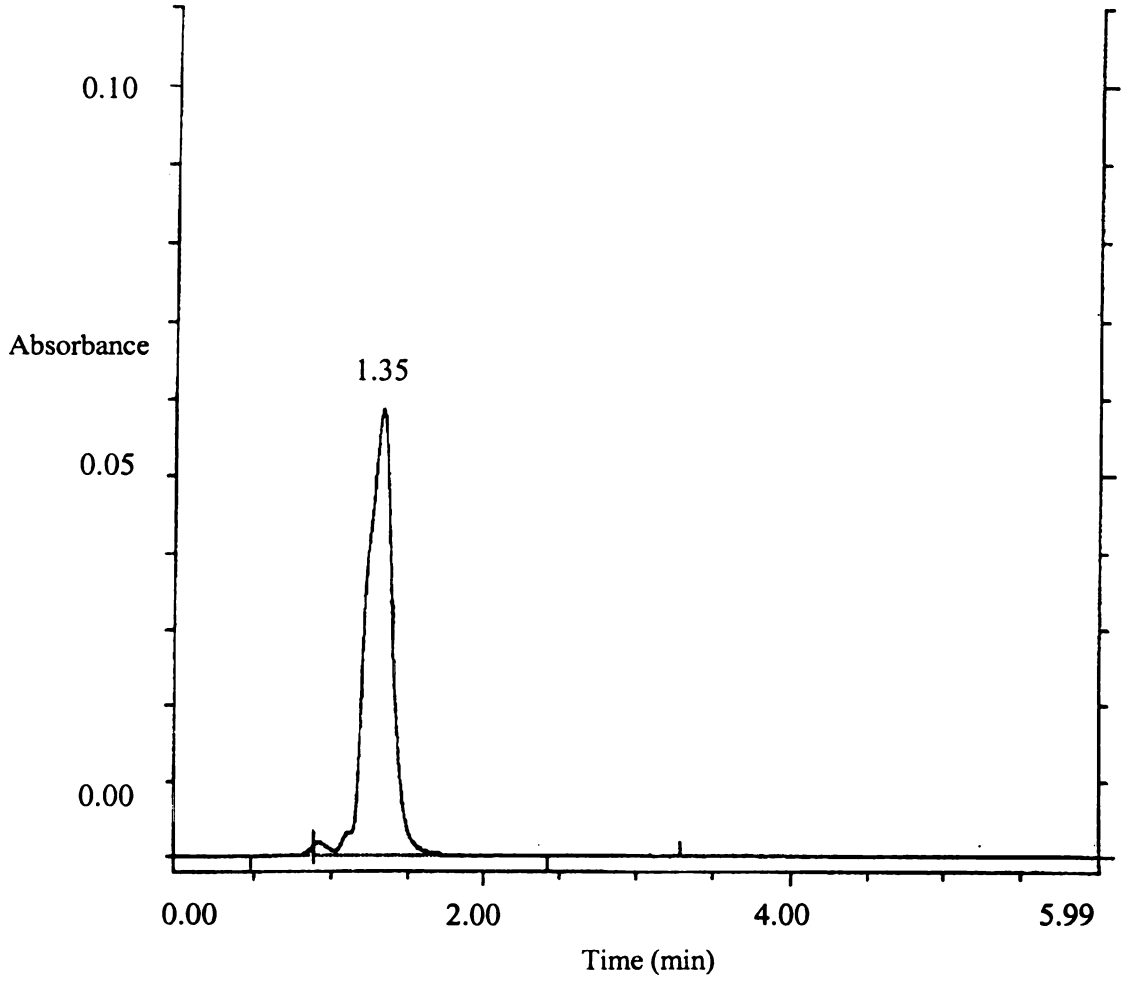

Figure 4.2. Chromatogram of sorbic acid from the polyvinylidene chloride antimicrobial film

$$\frac{dc}{dt} = -kC \qquad (1)$$

Integrated to; $\ln C/C_0 = -kt$

Where: C_0, C = Initial and final concentration of sorbic acid in the film sample, % (w/w), respectively

k = rate constant, 1/day

t = time interval, day

4.4 RESULTS AND DISCUSSION

The migration of antimicrobial agent from film to the food is an important key to inhibit the growth of pathogens, and spoilage organisms. In this study, the migration/release of sorbic acid from polyvinylidene chloride copolymer films to food products, Cheddar cheese and beef bologna, was determined. Antimicrobial activity on food products was evaluated in the previous chapter. Film with no food contact (control film) was also evaluated for loss of antimicrobial agent during 28 days of storage. The results are shown in Tables 4.1, 4.2 and 4.3, respectively. For better illustration, the results are presented graphically in Figures 4.3 and 4.4 for films with cheese, Figures 4.5 and 4.6 for films with bologna, and Figures 4.7 and 4.8 for control films.

After 7 days storage at 4 °C, the remaining sorbic acid content in film wrapped around cheese and bologna, and the control film relative to the initial control film were 64%, 50% and 92% sorbic acid, respectively. About 36% of sorbic acid migrated/loss from film to cheese and 50% sorbic acid from film to the bologna after 7 days of storage. The control film showed 8% loss (or volatilization) of sorbic acid. After 28 days of

Table 4.1. Loss of sorbic acid from polyvinylidene chloride antimicrobial film wrapped around Cheddar cheese during storage at 4 °C.

Initial sorbic acid concentration, Co (wt/wt %)	Time interval, t (days)	Sorbic acid concentration after time t, C (wt/wt %)	Relative of sorbic acid (C/Co × 100) (%)*
16.07	1	12.94	81
	2	13.75	86
	3	13.00	81
	6	12.73	79
	7	10.31	64
	14	9.69	60
	28	9.62	60

* Value represents relative percent of initial concentration of sorbic acid present in film.

Table 4.2. Loss of sorbic acid from polyvinylidene chloride antimicrobial film sandwiched between beef bologna during storage. at 4 °C

Initial sorbic acid concentration, Co (wt/wt %)	Time interval, t (days)	Sorbic acid concentration after time t, C (wt/wt %)	Relative of sorbic acid (C/Co × 100) (%)*
16.07	1	11.51	72
	2	8.19	51
	3	9.61	60
	4	9.1	57
	5	7.25	45
	7	8.07	50
	14	2.34	15
	21	1.83	11
	28	1.11	7

* Value represents relative percent of initial concentration of sorbic acid present in film.

Table 4.3. Loss of sorbic acid from polyvinylidene chloride antimicrobial film (no food contact) during storage at 4 °C.

Initial sorbic acid concentration, Co (wt/wt %)	Time interval, t (days)	Sorbic acid concentration after time t, C (wt/wt %)	Relative of sorbic acid (C/Co × 100) (%)*
16.07	0	16.07	100
	1	14.94	93
	2	15.57	97
	4	14.87	93
	5	14.27	89
	6	13.72	85
	7	14.82	92
	14	14.71	92
	21	13.53	84
	28	13.58	85

* Value represents relative percent of initial concentration of sorbic acid present in film.

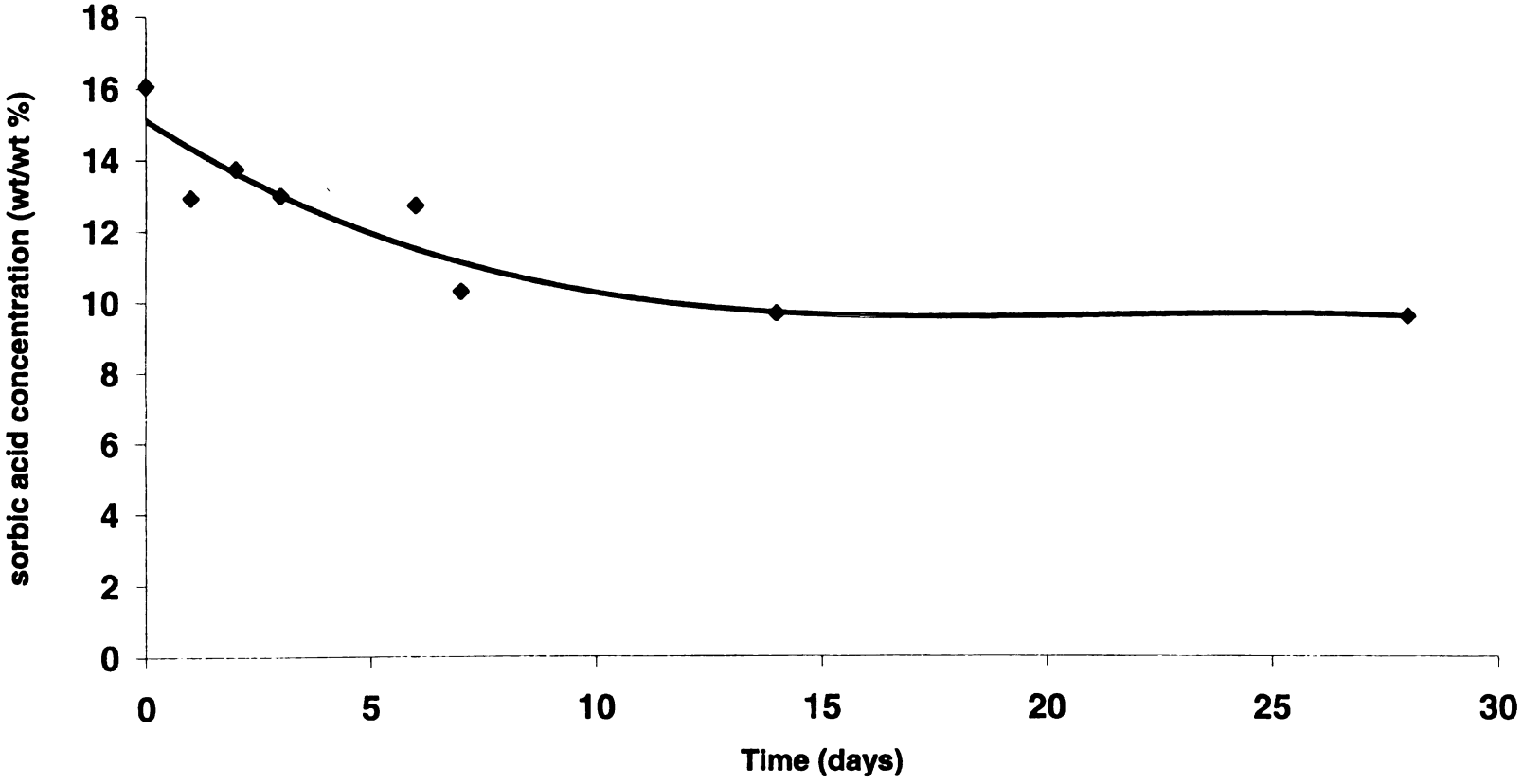

Figure 4.3. The change in concentration of sorbic acid as a function of storage time from PVDC antimicrobial film wrapped Around Cheddar cheese at 4°C.

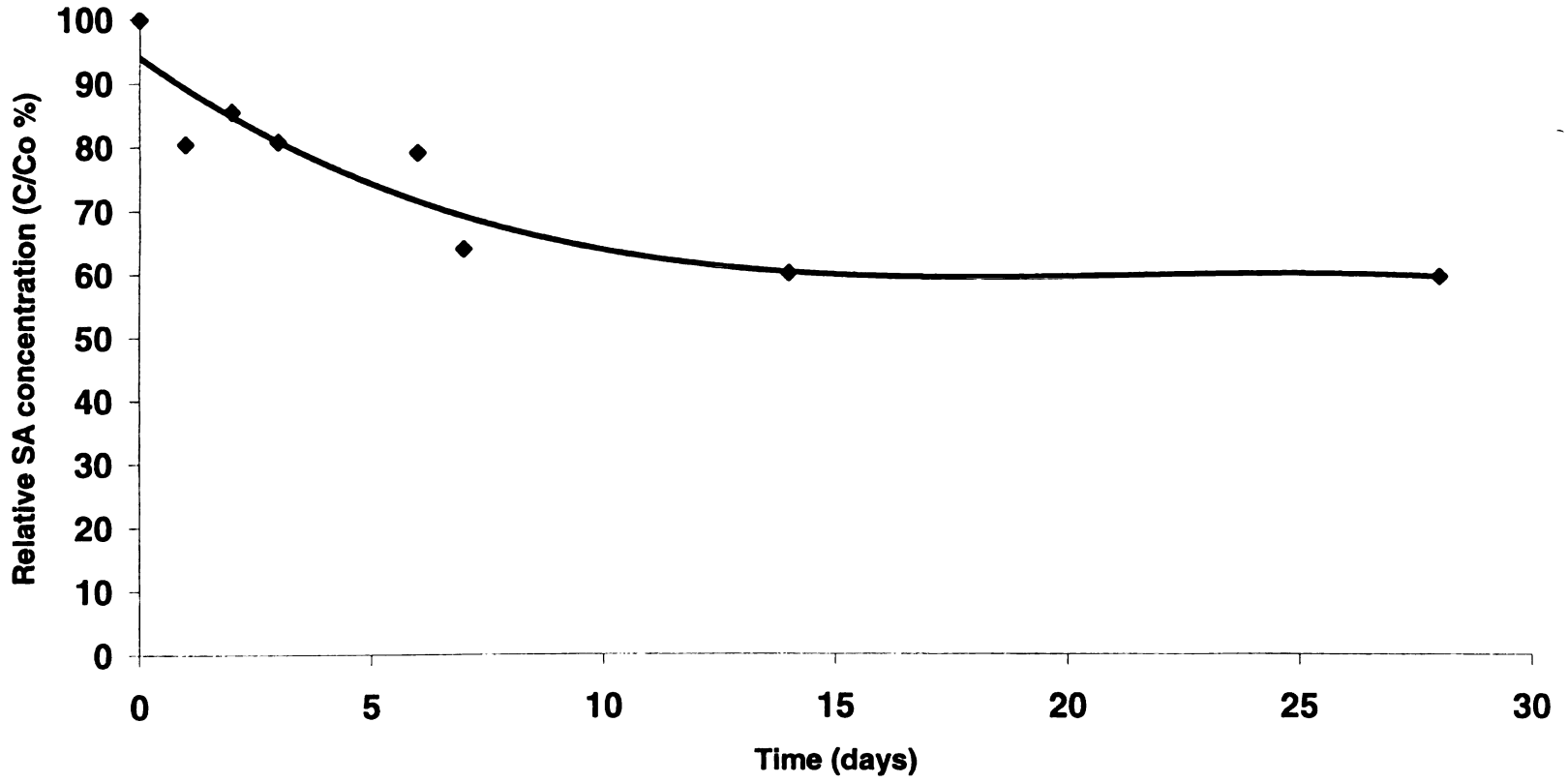

Figure 4.4. The relative concentration of sorbic acid as a function of storage time from PVDC antimicrobial film wrapped around Cheddar cheese at 4°C.

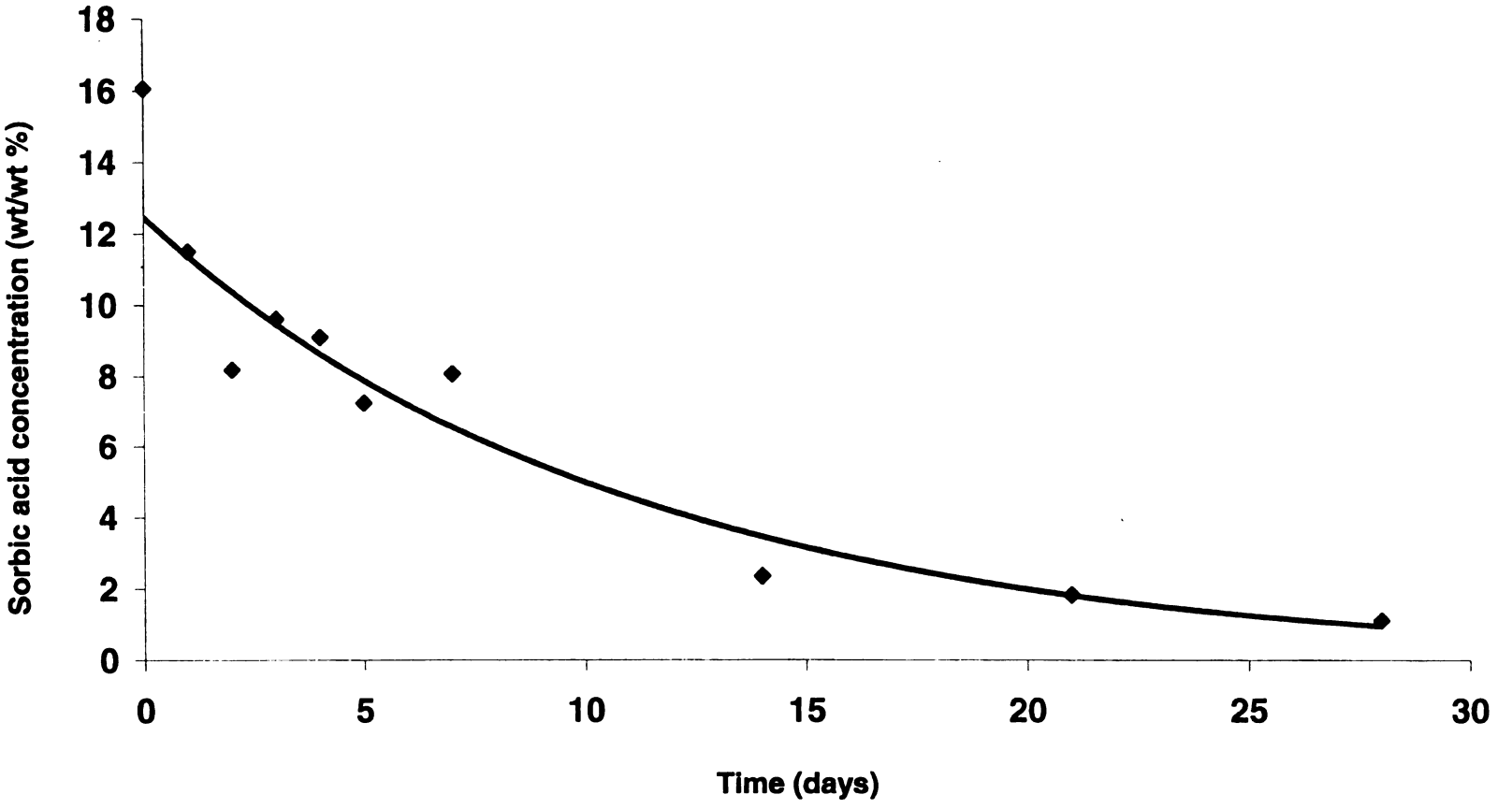

Figure 4.5. The change in concentration of sorbic acid as a function of storage time from PVDC antimicrobial film sandwiched between bologna at 4°C.

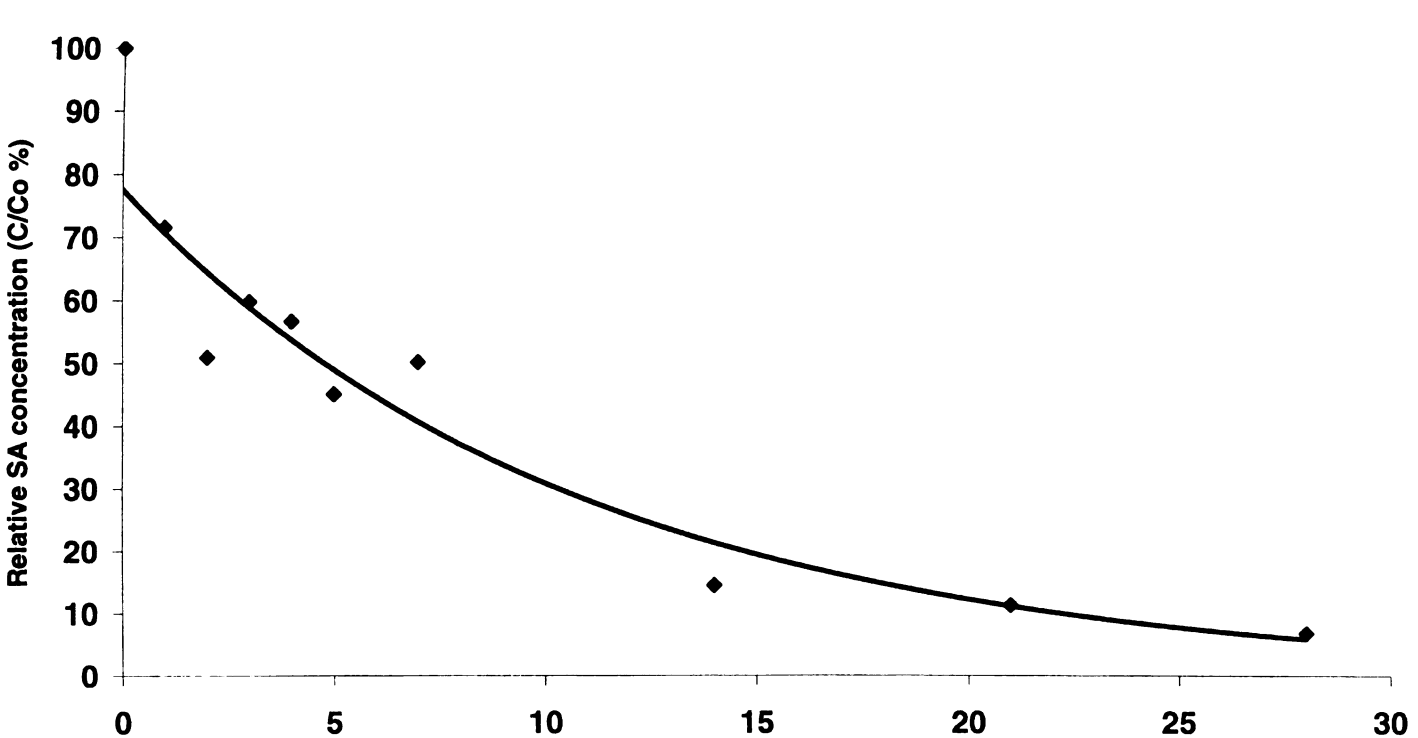

Figure 4.6. The relative concentration of sorbic acid as a function of storage time from PVDC antimicrobial film sandwiched between bologna at 4°C.

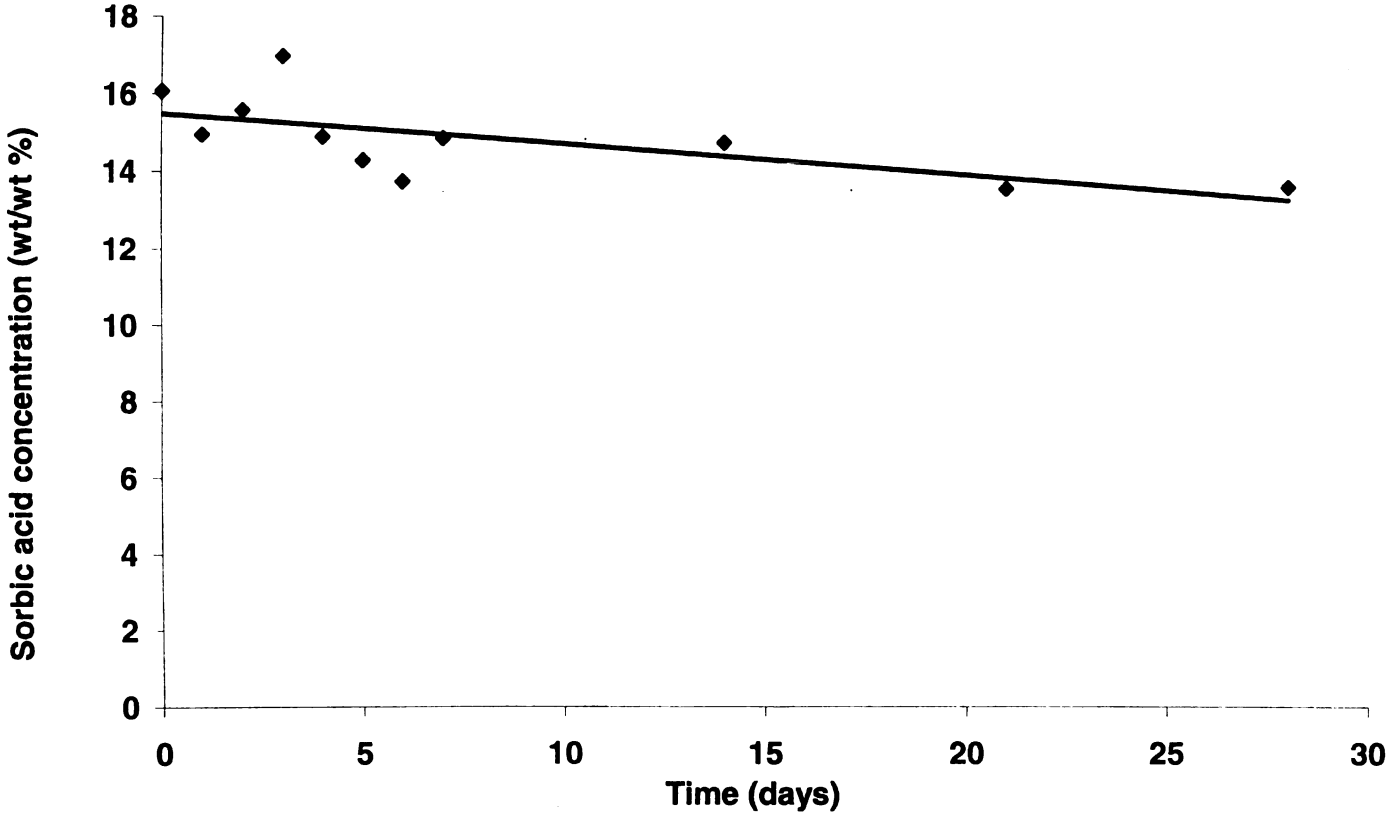

Figure 4.7. The change in concentration of sorbic acid of PVDC antimicrobial film (control film) as a function of storage time at 4°C.

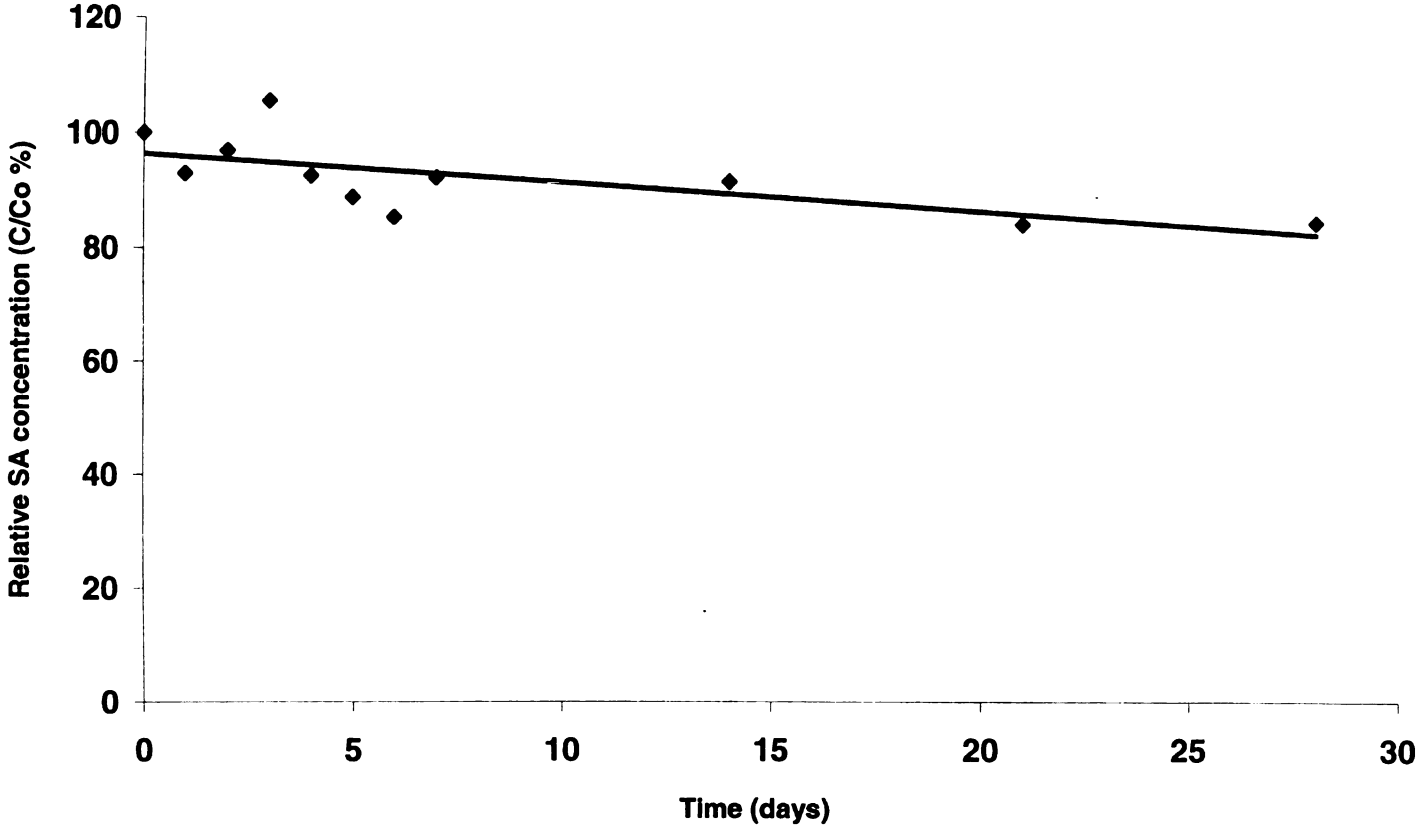

Figure 4.8. A first order rate plot of the loss of sorbic acid of PVDC antimicrobial film (control film) at 4°C.

storage, there was 60% sorbic acid remaining in the film wrapped around cheese, and 7% in the film wrapped around bologna. There was 85% remaining in the control film. The migration/loss of sorbic acid at the end of storage from films wrapped around cheese, bologna and the control films were 40%, 93% and 15%, respectively. Comparison of sorbic acid migrated/loss from the films wrapped around cheese and bologna and the control film is shown in Figures 4.9 and 4.10. The results showed that the migration of sorbic acid to bologna was higher than to cheese. Chen et al. (1996) reported that methylcellulose/chitosan films (antimicrobial film) released 39% of the sorbic acid into a glycerol/water solution in 30 minutes at 4 °C. After 6 hours of storage time, 49% of the sorbic acid was released from the antimicrobial film. Weng et al. (1998) developed a poly(ethylene-co-methacrylic acid) film (PEMA film) containing sorbic acid as an antimicrobial film for food packaging. PEMA film with NaOH or HCl treatment released 55 mg (0.49 mmol) and 0.5 mg (0.004 mmol), respectively, and PEMA film without treatment released 0.5 mg (0.004 mmol) from the initial sorbic acid concentration of 0.5 mol.

The migration/loss of sorbic acid from PVDC films can be presented on a semi-logarithmic plot where the coordinates of log C/Co vs. time gave a straight line relationship (Figures 4.11, 4.12 and 4.13). The rate constants for the migration/loss of sorbic acid were determined from their gradient, calculated using Equation (1), and are presented in Table 4.4. Migration/release rate of sorbic acid from film wrapped around cheese was 0.007 per day while film wrapped around bologna was 0.040 per day. The loss rate of control film without food contact was 0.001 per day. The comparison among films wrapped with cheese and bologna, and control film is presented in Figure 4.14.

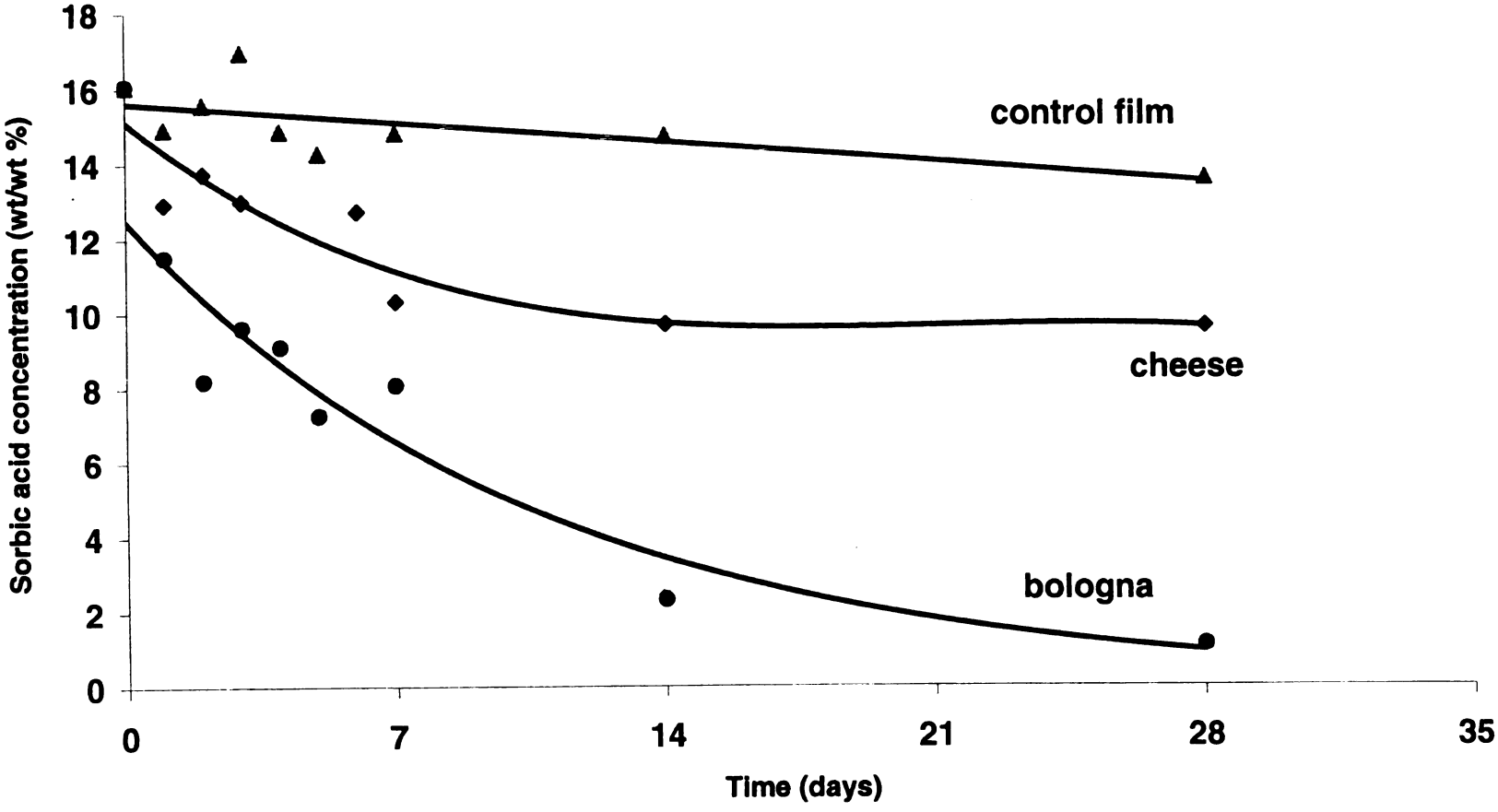

Figure 4.9. Comparison of the change in concentration of sorbic acid between PVDC antimicrobial film wrapped cheese and bologna as a function of storage time at 4°C.

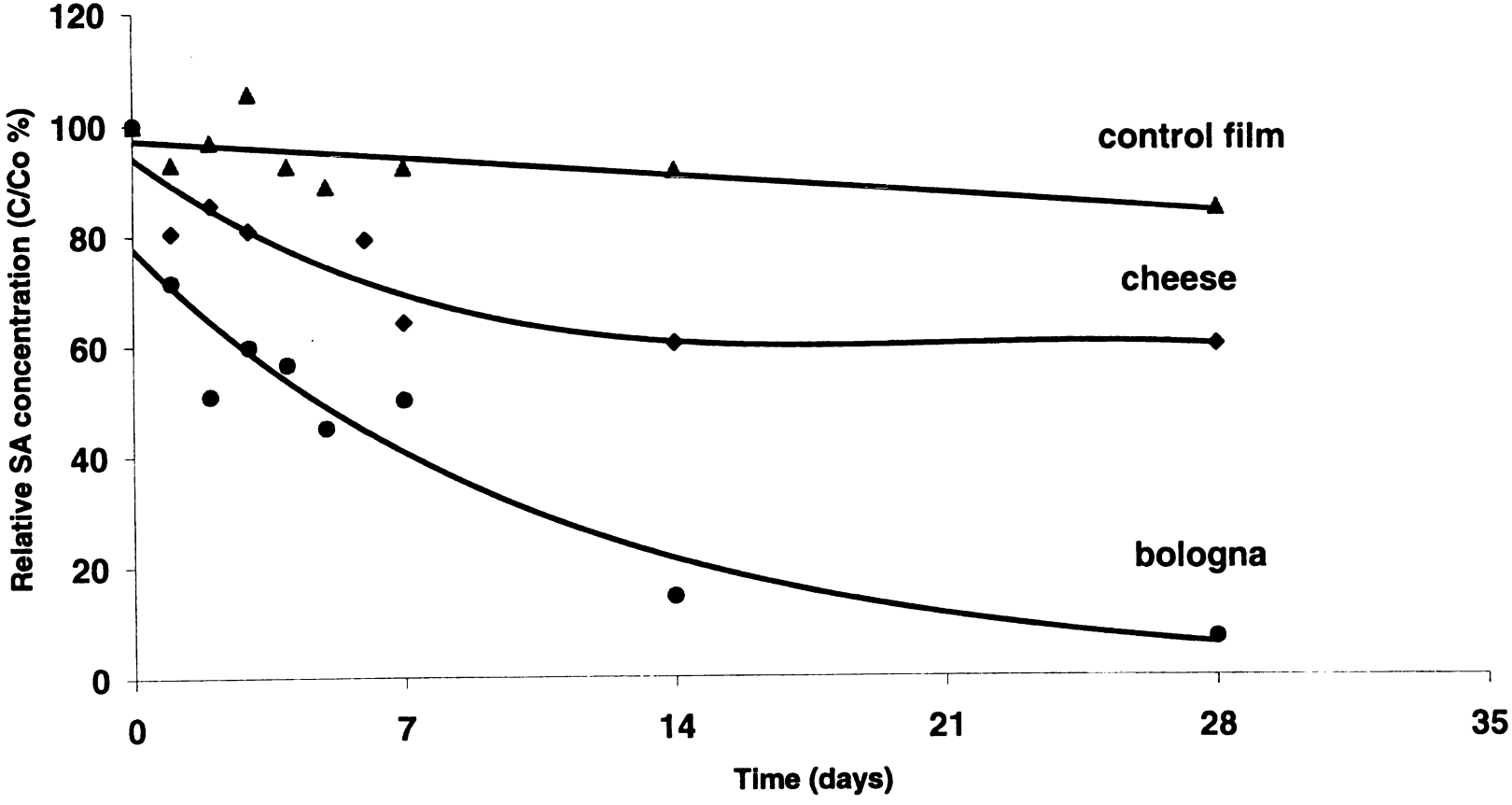

Figure 4.10. Comparison of the relative concentration of sorbic acid as a function of storage time between PVDC antimicrobial film wrapped Cheddar cheese and bologna at 4°C.

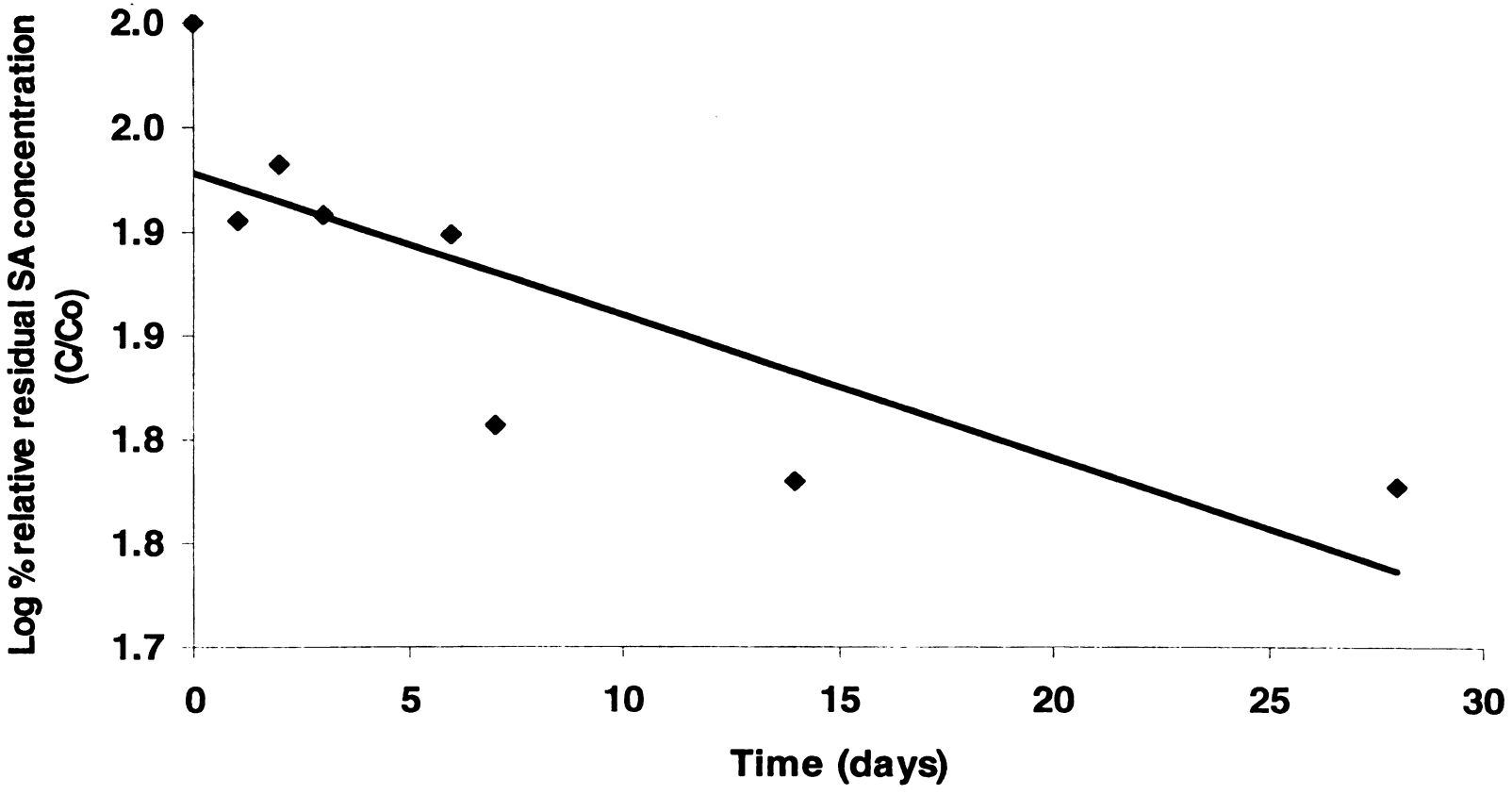

Figure 4.11. A first order rate plot of the loss of sorbic acid from PVDC antimicrobial film wrapped Cheddar cheese at 4°C.

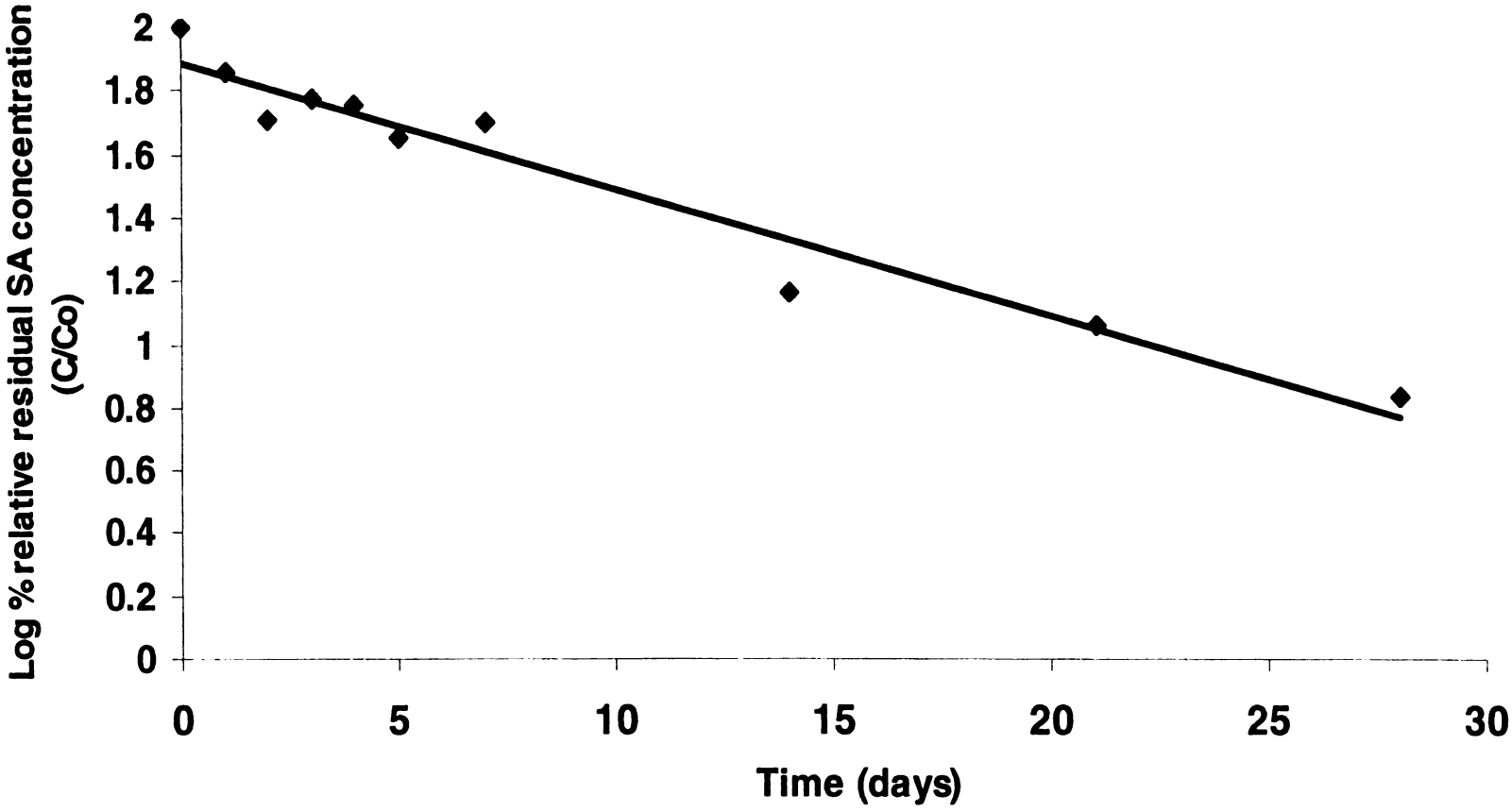

Figure 4.12. A first order rate plot of the loss of sorbic acid from PVDC antimicrobial film wrapped bologna at 4°C.

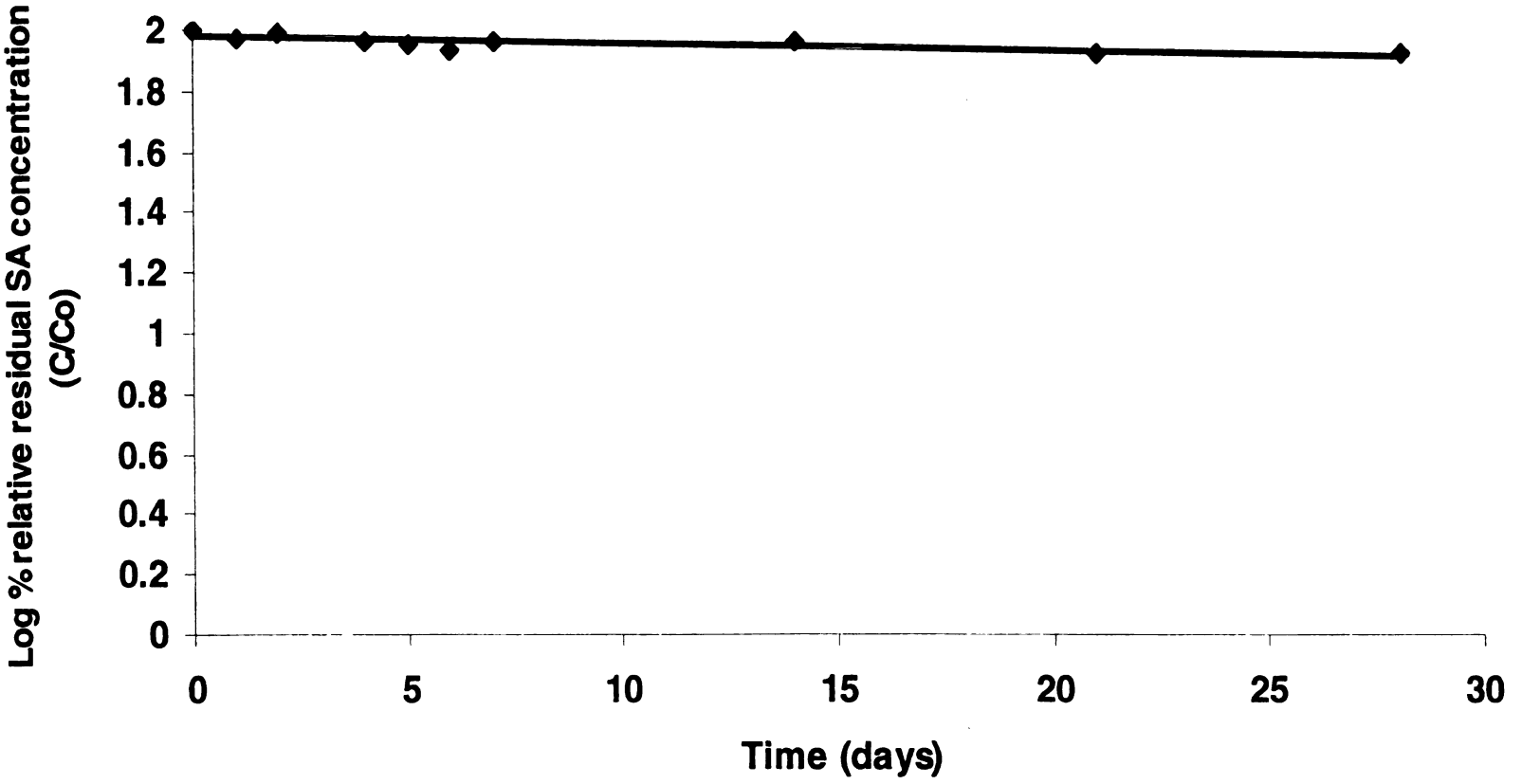

Figure 4.13. A first order rate plot of the loss of sorbic acid from PVDC control film (without wrapping food) at 4°C.

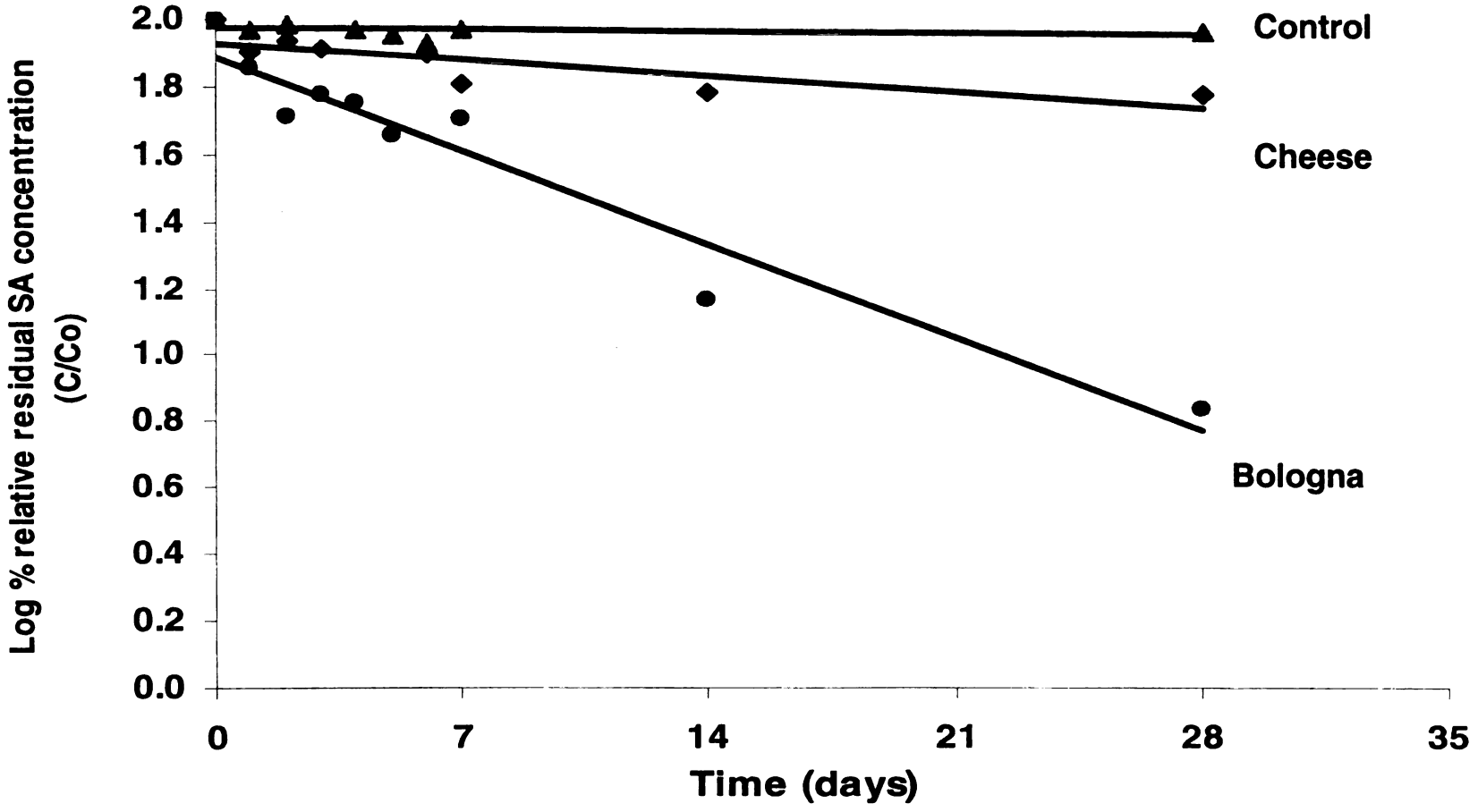

Figure 4.14. A first order rate plot of the loss of sorbic acid from PVDC antimicrobial film wrapped cheese and bologna as a function of storage time at 4°C.

Table 4.4. The rate constants of releasing/loss of sorbic acid from PVDC antimicrobial films

Food types	Rate constant of releasing/loss K (1/day)
Cheddar cheese	0.007
Beef bologna	0.040
Control film (without food)	0.001

4.5. CONCLUSION

The study showed that the sorbic acid was released from the films to food for both Cheddar cheese and beef bologna. There was 40% sorbic acid release from film wrapped around cheese at the end of 28 days of storage with a rate of 0.007 per day, 93% was released from film sandwiched between bologna with a rate of 0.040 per day. About 15% of the sorbic acid in the control film was lost at the end of 28 days of storage.

CONCLUSION

Polyvinylidene chloride copolymer (Saran^R F-310) films containing 1.5 to 3.0% (w/v) sorbic acid, 2.0 to 3.0% (w/v) potassium sorbate, or 1.0 to 2.5% (w/v) nisin showed inhibition against *Listeria monocytogenes* (CWD 95, CWD 246, CWD 201 and CWD 1503). Films containing lactoferrin or sodium diacetate did not show inhibition against *L. monocytogenes* in laboratory media, trypticase soy agar containing 0.6% yeast extract. Films containing sorbic acid had the best antimicrobial activity, barrier and mechanical properties, and distribution of the antimicrobial in the polymer structure. Polyvinylidene chloride film containing 3% sorbic acid coated on polyethylene terephthalate (PET) film at a minimum thickness of 0.75 mil or 0.00075 inch had antimicrobial activity against *Listeria monocytogenes* in laboratory media.

Subsequently, polyvinylidene chloride (PVDC) copolymer films containing 1.5 or 3.0% sorbic acid were tested on Cheddar cheese and beef bologna, with an initial surface inoculation of 10^5 or 10^3 *L. monocytogenes* CFU/g of product. On Cheddar cheese the number of *L. monocytogenes* was reduced by 0.1 – 1 log, and 4 – 7 logs on bologna slices after 35 and 28 days refrigerated storage for Cheddar cheese and bologna, respectively. Reduction in the numbers of mesophilic aerobic bacteria and lactic acid bacteria were also observed on Cheddar cheese and bologna slices.

Migration/loss of sorbic acid from PVDC copolymer film showed that 40% of the impregnated sorbic acid migrated/lost from the film wrapped around the Cheddar cheese at the end of 28 days storage with the rate of 0.007 per day. For bologna, 93% migrated/lost from the film sandwiched between bologna, at a rate of 0.040 per day.

Polyvinylidene chloride film containing sorbic acid not in contact with any food had a loss about 15% at the end of 28 days of storage.

This work shows that it may be possible to use a film such as polyvinylidene chloride polymer containing antimicrobial agent as a food wrap to reduce the risk of *Listeria* spp. contamination, while extending the shelf life of food products.

The directions of future research should include;

1) Detailed study of role(s) of the distribution of sorbic acid in the film chemical structure, the polymer chain orientation of the film and barrier property.

2) Detailed study of the relationship of the thickness of antimicrobial film and efficacy against *L. monocytogenes*. According to the study, it showed that increasing film thickness resulted in an increase in antimicrobial activity. It would be interesting to address why it behaves such way and what is responsible for such event.

APPENDIX I

STATISTIC ANALYSIS OF STORAGE FOR CHEESE AND BOLOGNA

A. *L. monocytogenes* on Cheddar cheese during storage at 4 °C for 35 days

(Initially inoculated *L. monocytogenes* 10^3 CFU/g)

Effect	Num DF	Den DF	F Value	Pr > F
Chemical	2	30	28.41	<.0001
time	4	30	57.29	<.0001
Chemical*time	8	30	5.92	0.0001

Effect	Chemical	time	Chemical	time	Adjustment	Adj P
Chemical	C		S		Tukey	0.0005
Chemical	C		SS		Tukey	<.0001
Chemical	S		SS		Tukey	0.0075
time		0		7	Tukey	0.9412
time		0		14	Tukey	0.9850
time		0		21	Tukey	0.0002
time		0		35	Tukey	<.0001
time		7		14	Tukey	0.7095
time		7		21	Tukey	<.0001
time		7		35	Tukey	<.0001
time		14		21	Tukey	0.0010
time		14		35	Tukey	<.0001
time		21		35	Tukey	<.0001
Chemical*time	C	0	C	7	Tukey	1.0000
Chemical*time	C	0	C	14	Tukey	1.0000
Chemical*time	C	0	C	21	Tukey	1.0000
Chemical*time	C	0	C	35	Tukey	0.1822
Chemical*time	C	0	S	0	Tukey	1.0000
Chemical*time	C	0	S	7	Tukey	1.0000
Chemical*time	C	0	S	14	Tukey	0.9998
Chemical*time	C	0	S	21	Tukey	0.1151
Chemical*time	C	0	S	35	Tukey	<.0001
Chemical*time	C	0	SS	0	Tukey	1.0000
Chemical*time	C	0	SS	7	Tukey	1.0000
Chemical*time	C	0	SS	14	Tukey	0.9975
Chemical*time	C	0	SS	21	Tukey	0.0001
Chemical*time	C	0	SS	35	Tukey	<.0001
Chemical*time	C	7	C	14	Tukey	1.0000
Chemical*time	C	7	C	21	Tukey	1.0000
Chemical*time	C	7	C	35	Tukey	0.0541
Chemical*time	C	7	S	0	Tukey	0.9998
Chemical*time	C	7	S	7	Tukey	1.0000

Chemical*time	C	7	S	14	Tukey	0.9750
Chemical*time	C	7	S	21	Tukey	0.0316
Chemical*time	C	7	S	35	Tukey	<.0001
Chemical*time	C	7	SS	0	Tukey	1.0000
Chemical*time	C	7	SS	7	Tukey	0.9996
Chemical*time	C	7	SS	14	Tukey	0.9173
Chemical*time	C	7	SS	21	Tukey	<.0001
Chemical*time	C	7	SS	35	Tukey	<.0001
Chemical*time	C	14	C	21	Tukey	1.0000
Chemical*time	C	14	C	35	Tukey	0.0541
Chemical*time	C	14	S	0	Tukey	0.9998
Chemical*time	C	14	S	7	Tukey	1.0000
Chemical*time	C	14	S	14	Tukey	0.9750
Chemical*time	C	14	S	21	Tukey	0.0316
Chemical*time	C	14	S	35	Tukey	<.0001
Chemical*time	C	14	SS	0	Tukey	1.0000
Chemical*time	C	14	SS	7	Tukey	0.9996
Chemical*time	C	14	SS	14	Tukey	0.9173
Chemical*time	C	14	SS	21	Tukey	<.0001
Chemical*time	C	14	SS	35	Tukey	<.0001
Chemical*time	C	21	C	35	Tukey	0.1389
Chemical*time	C	21	S	0	Tukey	1.0000
Chemical*time	C	21	S	7	Tukey	1.0000
Chemical*time	C	21	S	14	Tukey	0.9992
Chemical*time	C	21	S	21	Tukey	0.0858
Chemical*time	C	21	S	35	Tukey	<.0001
Chemical*time	C	21	SS	0	Tukey	1.0000
Chemical*time	C	21	SS	7	Tukey	1.0000
Chemical*time	C	21	SS	14	Tukey	0.9923
Chemical*time	C	21	SS	21	Tukey	0.0001
Chemical*time	C	21	SS	35	Tukey	<.0001
Chemical*time	C	35	S	0	Tukey	0.3216
Chemical*time	C	35	S	7	Tukey	0.0541
Chemical*time	C	35	S	14	Tukey	0.6599
Chemical*time	C	35	S	21	Tukey	1.0000
Chemical*time	C	35	S	35	Tukey	0.0113
Chemical*time	C	35	SS	0	Tukey	0.2654
Chemical*time	C	35	SS	7	Tukey	0.3459
Chemical*time	C	35	SS	14	Tukey	0.8092
Chemical*time	C	35	SS	21	Tukey	0.2761
Chemical*time	C	35	SS	35	Tukey	<.0001
Chemical*time	S	0	S	7	Tukey	0.9998
Chemical*time	S	0	S	14	Tukey	1.0000
Chemical*time	S	0	S	21	Tukey	0.2163
Chemical*time	S	0	S	35	Tukey	<.0001
Chemical*time	S	0	SS	0	Tukey	1.0000

Chemical*time	S	0	SS	7	Tukey	1.0000
Chemical*time	S	0	SS	14	Tukey	0.9999
Chemical*time	S	0	SS	21	Tukey	0.0004
Chemical*time	S	0	SS	35	Tukey	<.0001
Chemical*time	S	7	S	14	Tukey	0.9750
Chemical*time	S	7	S	21	Tukey	0.0316
Chemical*time	S	7	S	35	Tukey	<.0001
Chemical*time	S	7	SS	0	Tukey	1.0000
Chemical*time	S	7	SS	7	Tukey	0.9996
Chemical*time	S	7	SS	14	Tukey	0.9173
Chemical*time	S	7	SS	21	Tukey	<.0001
Chemical*time	S	7	SS	35	Tukey	<.0001
Chemical*time	S	14	S	21	Tukey	0.5109
Chemical*time	S	14	S	35	Tukey	<.0001
Chemical*time	S	14	SS	0	Tukey	1.0000
Chemical*time	S	14	SS	7	Tukey	1.0000
Chemical*time	S	14	SS	14	Tukey	1.0000
Chemical*time	S	14	SS	21	Tukey	0.0016
Chemical*time	S	14	SS	35	Tukey	<.0001
Chemical*time	S	21	S	35	Tukey	0.0202
Chemical*time	S	21	SS	0	Tukey	0.1743
Chemical*time	S	21	SS	7	Tukey	0.2351
Chemical*time	S	21	SS	14	Tukey	0.6746
Chemical*time	S	21	SS	21	Tukey	0.3977
Chemical*time	S	21	SS	35	Tukey	<.0001
Chemical*time	S	35	SS	0	Tukey	<.0001
Chemical*time	S	35	SS	7	Tukey	<.0001
Chemical*time	S	35	SS	14	Tukey	<.0001
Chemical*time	S	35	SS	21	Tukey	0.9750
Chemical*time	S	35	SS	35	Tukey	0.0601
Chemical*time	SS	0	SS	7	Tukey	1.0000
Chemical*time	SS	0	SS	14	Tukey	0.9997
Chemical*time	SS	0	SS	21	Tukey	0.0003
Chemical*time	SS	0	SS	35	Tukey	<.0001
Chemical*time	SS	7	SS	14	Tukey	1.0000
Chemical*time	SS	7	SS	21	Tukey	0.0004
Chemical*time	SS	7	SS	35	Tukey	<.0001
Chemical*time	SS	14	SS	21	Tukey	0.0030
Chemical*time	SS	14	SS	35	Tukey	<.0001
Chemical*time	SS	21	SS	35	Tukey	0.0016

B. *L. monocytogenes* on Cheddar cheese during storage at 4 °C for 35 days

(Initially inoculated *L. monocytogenes* 10^5 CFU/g)

Effect	Num DF	Den DF	F Value	Pr > F
Chemical	2	105	304.50	<.0001
time	6	105	167.61	<.0001
Chemical*time	12	105	53.89	<.0001

Effect	Chemical	time	Chemical	time	Adjustment	Adj P
Chemical	C		S		Tukey	<.0001
Chemical	C		SS		Tukey	<.0001
Chemical	S		SS		Tukey	<.0001
time		0		4	Tukey	<.0001
time		0		7	Tukey	0.0008
time		0		10	Tukey	<.0001
time		0		14	Tukey	<.0001
time		0		21	Tukey	<.0001
time		0		35	Tukey	<.0001
time		4		7	Tukey	0.0688
time		4		10	Tukey	0.6178
time		4		14	Tukey	0.9840
time		4		21	Tukey	<.0001
time		4		35	Tukey	<.0001
time		7		10	Tukey	0.8982
time		7		14	Tukey	0.3722
time		7		21	Tukey	<.0001
time		7		35	Tukey	<.0001
time		10		14	Tukey	0.9727
time		10		21	Tukey	<.0001
time		10		35	Tukey	<.0001
time		14		21	Tukey	<.0001
time		14		35	Tukey	<.0001
time		21		35	Tukey	<.0001
Chemical*time	C	0	C	4	Tukey	0.0377
Chemical*time	C	0	C	7	Tukey	0.9996
Chemical*time	C	0	C	10	Tukey	0.9987
Chemical*time	C	0	C	14	Tukey	0.9996
Chemical*time	C	0	C	21	Tukey	0.9999
Chemical*time	C	0	C	35	Tukey	1.0000
Chemical*time	C	0	S	0	Tukey	1.0000
Chemical*time	C	0	S	4	Tukey	0.0003
Chemical*time	C	0	S	7	Tukey	0.9991
Chemical*time	C	0	S	10	Tukey	0.2772
Chemical*time	C	0	S	14	Tukey	0.0071
Chemical*time	C	0	S	21	Tukey	<.0001
Chemical*time	C	0	S	35	Tukey	<.0001

Chemical*time	C	0	SS	0	Tukey	0.9994
Chemical*time	C	0	SS	4	Tukey	<.0001
Chemical*time	C	0	SS	7	Tukey	<.0001
Chemical*time	C	0	SS	10	Tukey	<.0001
Chemical*time	C	0	SS	14	Tukey	<.0001
Chemical*time	C	0	SS	21	Tukey	<.0001
Chemical*time	C	0	SS	35	Tukey	<.0001
Chemical*time	C	4	C	7	Tukey	0.5968
Chemical*time	C	4	C	10	Tukey	0.6818
Chemical*time	C	4	C	14	Tukey	0.5968
Chemical*time	C	4	C	21	Tukey	0.5389
Chemical*time	C	4	C	35	Tukey	0.2357
Chemical*time	C	4	S	0	Tukey	0.2772
Chemical*time	C	4	S	4	Tukey	0.9987
Chemical*time	C	4	S	7	Tukey	0.6539
Chemical*time	C	4	S	10	Tukey	1.0000
Chemical*time	C	4	S	14	Tukey	1.0000
Chemical*time	C	4	S	21	Tukey	0.0667
Chemical*time	C	4	S	35	Tukey	<.0001
Chemical*time	C	4	SS	0	Tukey	0.6255
Chemical*time	C	4	SS	4	Tukey	0.9518
Chemical*time	C	4	SS	7	Tukey	0.1817
Chemical*time	C	4	SS	10	Tukey	0.0919
Chemical*time	C	4	SS	14	Tukey	0.0296
Chemical*time	C	4	SS	21	Tukey	<.0001
Chemical*time	C	4	SS	35	Tukey	<.0001
Chemical*time	C	7	C	10	Tukey	1.0000
Chemical*time	C	7	C	14	Tukey	1.0000
Chemical*time	C	7	C	21	Tukey	1.0000
Chemical*time	C	7	C	35	Tukey	1.0000
Chemical*time	C	7	S	0	Tukey	1.0000
Chemical*time	C	7	S	4	Tukey	0.0262
Chemical*time	C	7	S	7	Tukey	1.0000
Chemical*time	C	7	S	10	Tukey	0.9744
Chemical*time	C	7	S	14	Tukey	0.2559
Chemical*time	C	7	S	21	Tukey	<.0001
Chemical*time	C	7	S	35	Tukey	<.0001
Chemical*time	C	7	SS	0	Tukey	1.0000
Chemical*time	C	7	SS	4	Tukey	0.0047
Chemical*time	C	7	SS	7	Tukey	<.0001
Chemical*time	C	7	SS	10	Tukey	<.0001
Chemical*time	C	7	SS	14	Tukey	<.0001
Chemical*time	C	7	SS	21	Tukey	<.0001
Chemical*time	C	7	SS	35	Tukey	<.0001
Chemical*time	C	10	C	14	Tukey	1.0000
Chemical*time	C	10	C	21	Tukey	1.0000

Chemical*time	C	10	C	35	Tukey	1.0000
Chemical*time	C	10	S	0	Tukey	1.0000
Chemical*time	C	10	S	4	Tukey	0.0377
Chemical*time	C	10	S	7	Tukey	1.0000
Chemical*time	C	10	S	10	Tukey	0.9878
Chemical*time	C	10	S	14	Tukey	0.3230
Chemical*time	C	10	S	21	Tukey	<.0001
Chemical*time	C	10	S	35	Tukey	<.0001
Chemical*time	C	10	SS	0	Tukey	1.0000
Chemical*time	C	10	SS	4	Tukey	0.0071
Chemical*time	C	10	SS	7	Tukey	<.0001
Chemical*time	C	10	SS	10	Tukey	<.0001
Chemical*time	C	10	SS	14	Tukey	<.0001
Chemical*time	C	10	SS	21	Tukey	<.0001
Chemical*time	C	10	SS	35	Tukey	<.0001
Chemical*time	C	14	C	21	Tukey	1.0000
Chemical*time	C	14	C	35	Tukey	1.0000
Chemical*time	C	14	S	0	Tukey	1.0000
Chemical*time	C	14	S	4	Tukey	0.0262
Chemical*time	C	14	S	7	Tukey	1.0000
Chemical*time	C	14	S	10	Tukey	0.9744
Chemical*time	C	14	S	14	Tukey	0.2559
Chemical*time	C	14	S	21	Tukey	<.0001
Chemical*time	C	14	S	35	Tukey	<.0001
Chemical*time	C	14	SS	0	Tukey	1.0000
Chemical*time	C	14	SS	4	Tukey	0.0047
Chemical*time	C	14	SS	7	Tukey	<.0001
Chemical*time	C	14	SS	10	Tukey	<.0001
Chemical*time	C	14	SS	14	Tukey	<.0001
Chemical*time	C	14	SS	21	Tukey	<.0001
Chemical*time	C	14	SS	35	Tukey	<.0001
Chemical*time	C	21	C	35	Tukey	1.0000
Chemical*time	C	21	S	0	Tukey	1.0000
Chemical*time	C	21	S	4	Tukey	0.0205
Chemical*time	C	21	S	7	Tukey	1.0000
Chemical*time	C	21	S	10	Tukey	0.9605
Chemical*time	C	21	S	14	Tukey	0.2166
Chemical*time	C	21	S	21	Tukey	<.0001
Chemical*time	C	21	S	35	Tukey	<.0001
Chemical*time	C	21	SS	0	Tukey	1.0000
Chemical*time	C	21	SS	4	Tukey	0.0035
Chemical*time	C	21	SS	7	Tukey	<.0001
Chemical*time	C	21	SS	10	Tukey	<.0001
Chemical*time	C	21	SS	14	Tukey	<.0001
Chemical*time	C	21	SS	21	Tukey	<.0001
Chemical*time	C	21	SS	35	Tukey	<.0001

Chemical*time	C	35	S	0	Tukey	1.0000
Chemical*time	C	35	S	4	Tukey	0.0041
Chemical*time	C	35	S	7	Tukey	1.0000
Chemical*time	C	35	S	10	Tukey	0.7609
Chemical*time	C	35	S	14	Tukey	0.0667
Chemical*time	C	35	S	21	Tukey	<.0001
Chemical*time	C	35	S	35	Tukey	<.0001
Chemical*time	C	35	SS	0	Tukey	1.0000
Chemical*time	C	35	SS	4	Tukey	0.0006
Chemical*time	C	35	SS	7	Tukey	<.0001
Chemical*time	C	35	SS	10	Tukey	<.0001
Chemical*time	C	35	SS	14	Tukey	<.0001
Chemical*time	C	35	SS	21	Tukey	<.0001
Chemical*time	C	35	SS	35	Tukey	<.0001
Chemical*time	S	0	S	4	Tukey	0.0054
Chemical*time	S	0	S	7	Tukey	1.0000
Chemical*time	S	0	S	10	Tukey	0.8084
Chemical*time	S	0	S	14	Tukey	0.0827
Chemical*time	S	0	S	21	Tukey	<.0001
Chemical*time	S	0	S	35	Tukey	<.0001
Chemical*time	S	0	SS	0	Tukey	1.0000
Chemical*time	S	0	SS	4	Tukey	0.0008
Chemical*time	S	0	SS	7	Tukey	<.0001
Chemical*time	S	0	SS	10	Tukey	<.0001
Chemical*time	S	0	SS	14	Tukey	<.0001
Chemical*time	S	0	SS	21	Tukey	<.0001
Chemical*time	S	0	SS	35	Tukey	<.0001
Chemical*time	S	4	S	7	Tukey	0.0334
Chemical*time	S	4	S	10	Tukey	0.8506
Chemical*time	S	4	S	14	Tukey	1.0000
Chemical*time	S	4	S	21	Tukey	0.8084
Chemical*time	S	4	S	35	Tukey	<.0001
Chemical*time	S	4	SS	0	Tukey	0.0296
Chemical*time	S	4	SS	4	Tukey	1.0000
Chemical*time	S	4	SS	7	Tukey	0.9605
Chemical*time	S	4	SS	10	Tukey	0.8696
Chemical*time	S	4	SS	14	Tukey	0.6255
Chemical*time	S	4	SS	21	Tukey	<.0001
Chemical*time	S	4	SS	35	Tukey	<.0001
Chemical*time	S	7	S	10	Tukey	0.9842
Chemical*time	S	7	S	14	Tukey	0.2996
Chemical*time	S	7	S	21	Tukey	<.0001
Chemical*time	S	7	S	35	Tukey	<.0001
Chemical*time	S	7	SS	0	Tukey	1.0000
Chemical*time	S	7	SS	4	Tukey	0.0062
Chemical*time	S	7	SS	7	Tukey	<.0001

Chemical*time	S	7	SS	10	Tukey	<.0001
Chemical*time	S	7	SS	14	Tukey	<.0001
Chemical*time	S	7	SS	21	Tukey	<.0001
Chemical*time	S	7	SS	35	Tukey	<.0001
Chemical*time	S	10	S	14	Tukey	0.9994
Chemical*time	S	10	S	21	Tukey	0.0054
Chemical*time	S	10	S	35	Tukey	<.0001
Chemical*time	S	10	SS	0	Tukey	0.9797
Chemical*time	S	10	SS	4	Tukey	0.5100
Chemical*time	S	10	SS	7	Tukey	0.0205
Chemical*time	S	10	SS	10	Tukey	0.0082
Chemical*time	S	10	SS	14	Tukey	0.0020
Chemical*time	S	10	SS	21	Tukey	<.0001
Chemical*time	S	10	SS	35	Tukey	<.0001
Chemical*time	S	14	S	21	Tukey	0.2357
Chemical*time	S	14	S	35	Tukey	<.0001
Chemical*time	S	14	SS	0	Tukey	0.2772
Chemical*time	S	14	SS	4	Tukey	0.9987
Chemical*time	S	14	SS	7	Tukey	0.4815
Chemical*time	S	14	SS	10	Tukey	0.2996
Chemical*time	S	14	SS	14	Tukey	0.1246
Chemical*time	S	14	SS	21	Tukey	<.0001
Chemical*time	S	14	SS	35	Tukey	<.0001
Chemical*time	S	21	S	35	Tukey	<.0001
Chemical*time	S	21	SS	0	Tukey	<.0001
Chemical*time	S	21	SS	4	Tukey	0.9797
Chemical*time	S	21	SS	7	Tukey	1.0000
Chemical*time	S	21	SS	10	Tukey	1.0000
Chemical*time	S	21	SS	14	Tukey	1.0000
Chemical*time	S	21	SS	21	Tukey	<.0001
Chemical*time	S	21	SS	35	Tukey	<.0001
Chemical*time	S	35	SS	0	Tukey	<.0001
Chemical*time	S	35	SS	4	Tukey	<.0001
Chemical*time	S	35	SS	7	Tukey	<.0001
Chemical*time	S	35	SS	10	Tukey	<.0001
Chemical*time	S	35	SS	14	Tukey	<.0001
Chemical*time	S	35	SS	21	Tukey	0.0180
Chemical*time	S	35	SS	35	Tukey	<.0001
Chemical*time	SS	0	SS	4	Tukey	0.0054
Chemical*time	SS	0	SS	7	Tukey	<.0001
Chemical*time	SS	0	SS	10	Tukey	<.0001
Chemical*time	SS	0	SS	14	Tukey	<.0001
Chemical*time	SS	0	SS	21	Tukey	<.0001
Chemical*time	SS	0	SS	35	Tukey	<.0001
Chemical*time	SS	4	SS	7	Tukey	0.9991
Chemical*time	SS	4	SS	10	Tukey	0.9907

Chemical*time	SS	4	SS	14	Tukey	0.9174
Chemical*time	SS	4	SS	21	Tukey	<.0001
Chemical*time	SS	4	SS	35	Tukey	<.0001
Chemical*time	SS	7	SS	10	Tukey	1.0000
Chemical*time	SS	7	SS	14	Tukey	1.0000
Chemical*time	SS	7	SS	21	Tukey	<.0001
Chemical*time	SS	7	SS	35	Tukey	<.0001
Chemical*time	SS	10	SS	14	Tukey	1.0000
Chemical*time	SS	10	SS	21	Tukey	<.0001
Chemical*time	SS	10	SS	35	Tukey	<.0001
Chemical*time	SS	14	SS	21	Tukey	<.0001
Chemical*time	SS	14	SS	35	Tukey	<.0001
Chemical*time	SS	21	SS	35	Tukey	<.0001

C. *L. monocytogenes* on beef bologna during storage at 4 °C for 28 days

(Initially inoculated *L. monocytogenes* 10^3 CFU/g)

Effect	Num DF	Den DF	F Value	Pr > F
Chemical	2	42	2189.83	<.0001
time	6	42	101.19	<.0001
Chemical*time	12	42	281.67	<.0001

Effect	Chemical	time	Chemical	time	Adjustment	Adj P
Chemical	C		S		Tukey	<.0001
Chemical	C		SS		Tukey	<.0001
Chemical	S		SS		Tukey	<.0001
time		0		4	Tukey	0.9321
time		0		7	Tukey	0.9994
time		0		10	Tukey	1.0000
time		0		14	Tukey	0.0912
time		0		21	Tukey	<.0001
time		0		28	Tukey	<.0001
time		4		7	Tukey	0.7388
time		4		10	Tukey	0.8828
time		4		14	Tukey	0.0056
time		4		21	Tukey	<.0001
time		4		28	Tukey	<.0001
time		7		10	Tukey	0.9999
time		7		14	Tukey	0.2217
time		7		21	Tukey	<.0001
time		7		28	Tukey	<.0001
time		10		14	Tukey	0.1249

time	10		21		Tukey	<.0001
time	10		28		Tukey	<.0001
time	14		21		Tukey	<.0001
time	14		28		Tukey	<.0001
time	21		28		Tukey	<.0001
Chemical*time	C	0	C	4	Tukey	0.9966
Chemical*time	C	0	C	7	Tukey	<.0001
Chemical*time	C	0	C	10	Tukey	<.0001
Chemical*time	C	0	C	14	Tukey	<.0001
Chemical*time	C	0	C	21	Tukey	<.0001
Chemical*time	C	0	C	28	Tukey	<.0001
Chemical*time	C	0	S	0	Tukey	1.0000
Chemical*time	C	0	S	4	Tukey	0.9795
Chemical*time	C	0	S	7	Tukey	0.0863
Chemical*time	C	0	S	10	Tukey	0.1190
Chemical*time	C	0	S	14	Tukey	0.0023
Chemical*time	C	0	S	21	Tukey	<.0001
Chemical*time	C	0	S	28	Tukey	0.0002
Chemical*time	C	0	SS	0	Tukey	1.0000
Chemical*time	C	0	SS	4	Tukey	0.8747
Chemical*time	C	0	SS	7	Tukey	0.0004
Chemical*time	C	0	SS	10	Tukey	<.0001
Chemical*time	C	0	SS	14	Tukey	<.0001
Chemical*time	C	0	SS	21	Tukey	<.0001
Chemical*time	C	0	SS	28	Tukey	<.0001
Chemical*time	C	4	C	7	Tukey	<.0001
Chemical*time	C	4	C	10	Tukey	<.0001
Chemical*time	C	4	C	14	Tukey	<.0001
Chemical*time	C	4	C	21	Tukey	<.0001
Chemical*time	C	4	C	28	Tukey	<.0001
Chemical*time	C	4	S	0	Tukey	0.8747
Chemical*time	C	4	S	4	Tukey	0.2519
Chemical*time	C	4	S	7	Tukey	0.0017
Chemical*time	C	4	S	10	Tukey	0.0026
Chemical*time	C	4	S	14	Tukey	<.0001
Chemical*time	C	4	S	21	Tukey	<.0001
Chemical*time	C	4	S	28	Tukey	<.0001
Chemical*time	C	4	SS	0	Tukey	0.9995
Chemical*time	C	4	SS	4	Tukey	0.1118
Chemical*time	C	4	SS	7	Tukey	<.0001
Chemical*time	C	4	SS	10	Tukey	<.0001
Chemical*time	C	4	SS	14	Tukey	<.0001
Chemical*time	C	4	SS	21	Tukey	<.0001
Chemical*time	C	4	SS	28	Tukey	<.0001
Chemical*time	C	7	C	10	Tukey	1.0000
Chemical*time	C	7	C	14	Tukey	<.0001

Chemical*time	C	7	C	21	Tukey	<.0001
Chemical*time	C	7	C	28	Tukey	<.0001
Chemical*time	C	7	S	0	Tukey	<.0001
Chemical*time	C	7	S	4	Tukey	<.0001
Chemical*time	C	7	S	7	Tukey	<.0001
Chemical*time	C	7	S	10	Tukey	<.0001
Chemical*time	C	7	S	14	Tukey	<.0001
Chemical*time	C	7	S	21	Tukey	<.0001
Chemical*time	C	7	S	28	Tukey	<.0001
Chemical*time	C	7	SS	0	Tukey	<.0001
Chemical*time	C	7	SS	4	Tukey	<.0001
Chemical*time	C	7	SS	7	Tukey	<.0001
Chemical*time	C	7	SS	10	Tukey	<.0001
Chemical*time	C	7	SS	14	Tukey	<.0001
Chemical*time	C	7	SS	21	Tukey	<.0001
Chemical*time	C	7	SS	28	Tukey	<.0001
Chemical*time	C	10	C	14	Tukey	<.0001
Chemical*time	C	10	C	21	Tukey	<.0001
Chemical*time	C	10	C	28	Tukey	<.0001
Chemical*time	C	10	S	0	Tukey	<.0001
Chemical*time	C	10	S	4	Tukey	<.0001
Chemical*time	C	10	S	7	Tukey	<.0001
Chemical*time	C	10	S	10	Tukey	<.0001
Chemical*time	C	10	S	14	Tukey	<.0001
Chemical*time	C	10	S	21	Tukey	<.0001
Chemical*time	C	10	S	28	Tukey	<.0001
Chemical*time	C	10	SS	0	Tukey	<.0001
Chemical*time	C	10	SS	4	Tukey	<.0001
Chemical*time	C	10	SS	7	Tukey	<.0001
Chemical*time	C	10	SS	10	Tukey	<.0001
Chemical*time	C	10	SS	14	Tukey	<.0001
Chemical*time	C	10	SS	21	Tukey	<.0001
Chemical*time	C	10	SS	28	Tukey	<.0001
Chemical*time	C	14	C	21	Tukey	<.0001
Chemical*time	C	14	C	28	Tukey	<.0001
Chemical*time	C	14	S	0	Tukey	<.0001
Chemical*time	C	14	S	4	Tukey	<.0001
Chemical*time	C	14	S	7	Tukey	<.0001
Chemical*time	C	14	S	10	Tukey	<.0001
Chemical*time	C	14	S	14	Tukey	<.0001
Chemical*time	C	14	S	21	Tukey	<.0001
Chemical*time	C	14	S	28	Tukey	<.0001
Chemical*time	C	14	SS	0	Tukey	<.0001
Chemical*time	C	14	SS	4	Tukey	<.0001
Chemical*time	C	14	SS	7	Tukey	<.0001
Chemical*time	C	14	SS	10	Tukey	<.0001

Chemical*time	C	14	SS	14	Tukey	<.0001
Chemical*time	C	14	SS	21	Tukey	<.0001
Chemical*time	C	14	SS	28	Tukey	<.0001
Chemical*time	C	21	C	28	Tukey	<.0001
Chemical*time	C	21	S	0	Tukey	<.0001
Chemical*time	C	21	S	4	Tukey	<.0001
Chemical*time	C	21	S	7	Tukey	<.0001
Chemical*time	C	21	S	10	Tukey	<.0001
Chemical*time	C	21	S	14	Tukey	<.0001
Chemical*time	C	21	S	21	Tukey	<.0001
Chemical*time	C	21	S	28	Tukey	<.0001
Chemical*time	C	21	SS	0	Tukey	<.0001
Chemical*time	C	21	SS	4	Tukey	<.0001
Chemical*time	C	21	SS	7	Tukey	<.0001
Chemical*time	C	21	SS	10	Tukey	<.0001
Chemical*time	C	21	SS	14	Tukey	<.0001
Chemical*time	C	21	SS	21	Tukey	<.0001
Chemical*time	C	21	SS	28	Tukey	<.0001
Chemical*time	C	28	S	0	Tukey	<.0001
Chemical*time	C	28	S	4	Tukey	<.0001
Chemical*time	C	28	S	7	Tukey	<.0001
Chemical*time	C	28	S	10	Tukey	<.0001
Chemical*time	C	28	S	14	Tukey	<.0001
Chemical*time	C	28	S	21	Tukey	<.0001
Chemical*time	C	28	S	28	Tukey	<.0001
Chemical*time	C	28	SS	0	Tukey	<.0001
Chemical*time	C	28	SS	4	Tukey	<.0001
Chemical*time	C	28	SS	7	Tukey	<.0001
Chemical*time	C	28	SS	10	Tukey	<.0001
Chemical*time	C	28	SS	14	Tukey	<.0001
Chemical*time	C	28	SS	21	Tukey	<.0001
Chemical*time	C	28	SS	28	Tukey	<.0001
Chemical*time	S	0	S	4	Tukey	1.0000
Chemical*time	S	0	S	7	Tukey	0.3238
Chemical*time	S	0	S	10	Tukey	0.4064
Chemical*time	S	0	S	14	Tukey	0.0152
Chemical*time	S	0	S	21	Tukey	0.0001
Chemical*time	S	0	S	28	Tukey	0.0015
Chemical*time	S	0	SS	0	Tukey	1.0000
Chemical*time	S	0	SS	4	Tukey	0.9966
Chemical*time	S	0	SS	7	Tukey	0.0030
Chemical*time	S	0	SS	10	Tukey	0.0003
Chemical*time	S	0	SS	14	Tukey	<.0001
Chemical*time	S	0	SS	21	Tukey	<.0001
Chemical*time	S	0	SS	28	Tukey	<.0001
Chemical*time	S	4	S	7	Tukey	0.9267

Chemical*time	S	4	S	10	Tukey	0.9618
Chemical*time	S	4	S	14	Tukey	0.1918
Chemical*time	S	4	S	21	Tukey	0.0036
Chemical*time	S	4	S	28	Tukey	0.0302
Chemical*time	S	4	SS	0	Tukey	0.9427
Chemical*time	S	4	SS	4	Tukey	1.0000
Chemical*time	S	4	SS	7	Tukey	0.0537
Chemical*time	S	4	SS	10	Tukey	0.0064
Chemical*time	S	4	SS	14	Tukey	0.0020
Chemical*time	S	4	SS	21	Tukey	<.0001
Chemical*time	S	4	SS	28	Tukey	<.0001
Chemical*time	S	7	S	10	Tukey	1.0000
Chemical*time	S	7	S	14	Tukey	0.9988
Chemical*time	S	7	S	21	Tukey	0.3891
Chemical*time	S	7	S	28	Tukey	0.8623
Chemical*time	S	7	SS	0	Tukey	0.0537
Chemical*time	S	7	SS	4	Tukey	0.9919
Chemical*time	S	7	SS	7	Tukey	0.9427
Chemical*time	S	7	SS	10	Tukey	0.5162
Chemical*time	S	7	SS	14	Tukey	0.2793
Chemical*time	S	7	SS	21	Tukey	<.0001
Chemical*time	S	7	SS	28	Tukey	<.0001
Chemical*time	S	10	S	14	Tukey	0.9957
Chemical*time	S	10	S	21	Tukey	0.3085
Chemical*time	S	10	S	28	Tukey	0.7903
Chemical*time	S	10	SS	0	Tukey	0.0756
Chemical*time	S	10	SS	4	Tukey	0.9973
Chemical*time	S	10	SS	7	Tukey	0.8976
Chemical*time	S	10	SS	10	Tukey	0.4240
Chemical*time	S	10	SS	14	Tukey	0.2145
Chemical*time	S	10	SS	21	Tukey	<.0001
Chemical*time	S	10	SS	28	Tukey	<.0001
Chemical*time	S	14	S	21	Tukey	0.9919
Chemical*time	S	14	S	28	Tukey	1.0000
Chemical*time	S	14	SS	0	Tukey	0.0013
Chemical*time	S	14	SS	4	Tukey	0.3891
Chemical*time	S	14	SS	7	Tukey	1.0000
Chemical*time	S	14	SS	10	Tukey	0.9984
Chemical*time	S	14	SS	14	Tukey	0.9717
Chemical*time	S	14	SS	21	Tukey	0.0015
Chemical*time	S	14	SS	28	Tukey	<.0001
Chemical*time	S	21	S	28	Tukey	1.0000
Chemical*time	S	21	SS	0	Tukey	<.0001
Chemical*time	S	21	SS	4	Tukey	0.0111
Chemical*time	S	21	SS	7	Tukey	1.0000
Chemical*time	S	21	SS	10	Tukey	1.0000

Chemical*time	S	21	SS	14	Tukey	1.0000
Chemical*time	S	21	SS	21	Tukey	0.1049
Chemical*time	S	21	SS	28	Tukey	0.0014
Chemical*time	S	28	SS	0	Tukey	0.0001
Chemical*time	S	28	SS	4	Tukey	0.0808
Chemical*time	S	28	SS	7	Tukey	1.0000
Chemical*time	S	28	SS	10	Tukey	1.0000
Chemical*time	S	28	SS	14	Tukey	1.0000
Chemical*time	S	28	SS	21	Tukey	0.0152
Chemical*time	S	28	SS	28	Tukey	0.0001
Chemical*time	SS	0	SS	4	Tukey	0.7742
Chemical*time	SS	0	SS	7	Tukey	0.0002
Chemical*time	SS	0	SS	10	Tukey	<.0001
Chemical*time	SS	0	SS	14	Tukey	<.0001
Chemical*time	SS	0	SS	21	Tukey	<.0001
Chemical*time	SS	0	SS	28	Tukey	<.0001
Chemical*time	SS	4	SS	7	Tukey	0.1347
Chemical*time	SS	4	SS	10	Tukey	0.0192
Chemical*time	SS	4	SS	14	Tukey	0.0064
Chemical*time	SS	4	SS	21	Tukey	<.0001
Chemical*time	SS	4	SS	28	Tukey	<.0001
Chemical*time	SS	7	SS	10	Tukey	1.0000
Chemical*time	SS	7	SS	14	Tukey	0.9997
Chemical*time	SS	7	SS	21	Tukey	0.0081
Chemical*time	SS	7	SS	28	Tukey	<.0001
Chemical*time	SS	10	SS	14	Tukey	1.0000
Chemical*time	SS	10	SS	21	Tukey	0.0660
Chemical*time	SS	10	SS	28	Tukey	0.0008
Chemical*time	SS	14	SS	21	Tukey	0.1613
Chemical*time	SS	14	SS	28	Tukey	0.0026
Chemical*time	SS	21	SS	28	Tukey	0.9901

D. *L. monocytogenes* on beef bologna during storage at 4 °C for 28 days

(Initially inoculated *L. monocytogenes* 10^5 CFU/g)

Effect	Num DF	Den DF	F Value	Pr > F
Chemical	2	105	3234.61	<.0001
time	6	105	98.94	<.0001
Chemical*time	12	105	228.69	<.0001

Effect	Chemical	time	Chemical	time	Adjustment	Adj P
Chemical	C		S		Tukey	<.0001

Chemical	C		SS		Tukey	<.0001
Chemical	S		SS		Tukey	0.0001
time		0	4		Tukey	<.0001
time		0	7		Tukey	0.0002
time		0	10		Tukey	<.0001
time		0	14		Tukey	<.0001
time		0	21		Tukey	<.0001
time		0	28		Tukey	<.0001
time		4	7		Tukey	0.9565
time		4	10		Tukey	<.0001
time		4	14		Tukey	<.0001
time		4	21		Tukey	<.0001
time		4	28		Tukey	<.0001
time		7	10		Tukey	<.0001
time		7	14		Tukey	<.0001
time		7	21		Tukey	<.0001
time		7	28		Tukey	<.0001
time		10	14		Tukey	0.9015
time		10	21		Tukey	<.0001
time		10	28		Tukey	0.0002
time		14	21		Tukey	<.0001
time		14	28		Tukey	<.0001
time		21	28		Tukey	0.7887
Chemical*time	C	0	C	4	Tukey	<.0001
Chemical*time	C	0	C	7	Tukey	<.0001
Chemical*time	C	0	C	10	Tukey	<.0001
Chemical*time	C	0	C	14	Tukey	<.0001
Chemical*time	C	0	C	21	Tukey	<.0001
Chemical*time	C	0	C	28	Tukey	<.0001
Chemical*time	C	0	S	0	Tukey	1.0000
Chemical*time	C	0	S	4	Tukey	0.0880
Chemical*time	C	0	S	7	Tukey	0.1072
Chemical*time	C	0	S	10	Tukey	0.8776
Chemical*time	C	0	S	14	Tukey	0.4993
Chemical*time	C	0	S	21	Tukey	0.0010
Chemical*time	C	0	S	28	Tukey	<.0001
Chemical*time	C	0	SS	0	Tukey	1.0000
Chemical*time	C	0	SS	4	Tukey	0.3687
Chemical*time	C	0	SS	7	Tukey	0.0034
Chemical*time	C	0	SS	10	Tukey	0.5406
Chemical*time	C	0	SS	14	Tukey	0.0087
Chemical*time	C	0	SS	21	Tukey	0.0010
Chemical*time	C	0	SS	28	Tukey	<.0001
Chemical*time	C	4	C	7	Tukey	<.0001
Chemical*time	C	4	C	10	Tukey	<.0001
Chemical*time	C	4	C	14	Tukey	<.0001

Chemical*time	C	4	C	21	Tukey	<.0001
Chemical*time	C	4	C	28	Tukey	<.0001
Chemical*time	C	4	S	0	Tukey	<.0001
Chemical*time	C	4	S	4	Tukey	0.0001
Chemical*time	C	4	S	7	Tukey	<.0001
Chemical*time	C	4	S	10	Tukey	<.0001
Chemical*time	C	4	S	14	Tukey	<.0001
Chemical*time	C	4	S	21	Tukey	<.0001
Chemical*time	C	4	S	28	Tukey	<.0001
Chemical*time	C	4	SS	0	Tukey	<.0001
Chemical*time	C	4	SS	4	Tukey	<.0001
Chemical*time	C	4	SS	7	Tukey	<.0001
Chemical*time	C	4	SS	10	Tukey	<.0001
Chemical*time	C	4	SS	14	Tukey	<.0001
Chemical*time	C	4	SS	21	Tukey	<.0001
Chemical*time	C	4	SS	28	Tukey	<.0001
Chemical*time	C	7	C	10	Tukey	<.0001
Chemical*time	C	7	C	14	Tukey	<.0001
Chemical*time	C	7	C	21	Tukey	<.0001
Chemical*time	C	7	C	28	Tukey	<.0001
Chemical*time	C	7	S	0	Tukey	<.0001
Chemical*time	C	7	S	4	Tukey	<.0001
Chemical*time	C	7	S	7	Tukey	<.0001
Chemical*time	C	7	S	10	Tukey	<.0001
Chemical*time	C	7	S	14	Tukey	<.0001
Chemical*time	C	7	S	21	Tukey	<.0001
Chemical*time	C	7	S	28	Tukey	<.0001
Chemical*time	C	7	SS	0	Tukey	<.0001
Chemical*time	C	7	SS	4	Tukey	<.0001
Chemical*time	C	7	SS	7	Tukey	<.0001
Chemical*time	C	7	SS	10	Tukey	<.0001
Chemical*time	C	7	SS	14	Tukey	<.0001
Chemical*time	C	7	SS	21	Tukey	<.0001
Chemical*time	C	7	SS	28	Tukey	<.0001
Chemical*time	C	10	C	14	Tukey	1.0000
Chemical*time	C	10	C	21	Tukey	<.0001
Chemical*time	C	10	C	28	Tukey	<.0001
Chemical*time	C	10	S	0	Tukey	<.0001
Chemical*time	C	10	S	4	Tukey	<.0001
Chemical*time	C	10	S	7	Tukey	<.0001
Chemical*time	C	10	S	10	Tukey	<.0001
Chemical*time	C	10	S	14	Tukey	<.0001
Chemical*time	C	10	S	21	Tukey	<.0001
Chemical*time	C	10	S	28	Tukey	<.0001
Chemical*time	C	10	SS	0	Tukey	<.0001
Chemical*time	C	10	SS	4	Tukey	<.0001

Chemical*time	C	10	SS	7	Tukey	<.0001
Chemical*time	C	10	SS	10	Tukey	<.0001
Chemical*time	C	10	SS	14	Tukey	<.0001
Chemical*time	C	10	SS	21	Tukey	<.0001
Chemical*time	C	10	SS	28	Tukey	<.0001
Chemical*time	C	14	C	21	Tukey	<.0001
Chemical*time	C	14	C	28	Tukey	<.0001
Chemical*time	C	14	S	0	Tukey	<.0001
Chemical*time	C	14	S	4	Tukey	<.0001
Chemical*time	C	14	S	7	Tukey	<.0001
Chemical*time	C	14	S	10	Tukey	<.0001
Chemical*time	C	14	S	14	Tukey	<.0001
Chemical*time	C	14	S	21	Tukey	<.0001
Chemical*time	C	14	S	28	Tukey	<.0001
Chemical*time	C	14	SS	0	Tukey	<.0001
Chemical*time	C	14	SS	4	Tukey	<.0001
Chemical*time	C	14	SS	7	Tukey	<.0001
Chemical*time	C	14	SS	10	Tukey	<.0001
Chemical*time	C	14	SS	14	Tukey	<.0001
Chemical*time	C	14	SS	21	Tukey	<.0001
Chemical*time	C	14	SS	28	Tukey	<.0001
Chemical*time	C	21	C	28	Tukey	1.0000
Chemical*time	C	21	S	0	Tukey	<.0001
Chemical*time	C	21	S	4	Tukey	<.0001
Chemical*time	C	21	S	7	Tukey	<.0001
Chemical*time	C	21	S	10	Tukey	<.0001
Chemical*time	C	21	S	14	Tukey	<.0001
Chemical*time	C	21	S	21	Tukey	<.0001
Chemical*time	C	21	S	28	Tukey	<.0001
Chemical*time	C	21	SS	0	Tukey	<.0001
Chemical*time	C	21	SS	4	Tukey	<.0001
Chemical*time	C	21	SS	7	Tukey	<.0001
Chemical*time	C	21	SS	10	Tukey	<.0001
Chemical*time	C	21	SS	14	Tukey	<.0001
Chemical*time	C	21	SS	21	Tukey	<.0001
Chemical*time	C	21	SS	28	Tukey	<.0001
Chemical*time	C	28	S	0	Tukey	<.0001
Chemical*time	C	28	S	4	Tukey	<.0001
Chemical*time	C	28	S	7	Tukey	<.0001
Chemical*time	C	28	S	10	Tukey	<.0001
Chemical*time	C	28	S	14	Tukey	<.0001
Chemical*time	C	28	S	21	Tukey	<.0001
Chemical*time	C	28	S	28	Tukey	<.0001
Chemical*time	C	28	SS	0	Tukey	<.0001
Chemical*time	C	28	SS	4	Tukey	<.0001
Chemical*time	C	28	SS	7	Tukey	<.0001

Chemical*time	C	28	SS	10	Tukey	<.0001
Chemical*time	C	28	SS	14	Tukey	<.0001
Chemical*time	C	28	SS	21	Tukey	<.0001
Chemical*time	C	28	SS	28	Tukey	<.0001
Chemical*time	S	0	S	4	Tukey	0.1856
Chemical*time	S	0	S	7	Tukey	0.0468
Chemical*time	S	0	S	10	Tukey	0.7036
Chemical*time	S	0	S	14	Tukey	0.2991
Chemical*time	S	0	S	21	Tukey	0.0003
Chemical*time	S	0	S	28	Tukey	<.0001
Chemical*time	S	0	SS	0	Tukey	1.0000
Chemical*time	S	0	SS	4	Tukey	0.2020
Chemical*time	S	0	SS	7	Tukey	0.0011
Chemical*time	S	0	SS	10	Tukey	0.3329
Chemical*time	S	0	SS	14	Tukey	0.0030
Chemical*time	S	0	SS	21	Tukey	0.0003
Chemical*time	S	0	SS	28	Tukey	<.0001
Chemical*time	S	4	S	7	Tukey	<.0001
Chemical*time	S	4	S	10	Tukey	<.0001
Chemical*time	S	4	S	14	Tukey	<.0001
Chemical*time	S	4	S	21	Tukey	<.0001
Chemical*time	S	4	S	28	Tukey	<.0001
Chemical*time	S	4	SS	0	Tukey	0.0128
Chemical*time	S	4	SS	4	Tukey	<.0001
Chemical*time	S	4	SS	7	Tukey	<.0001
Chemical*time	S	4	SS	10	Tukey	<.0001
Chemical*time	S	4	SS	14	Tukey	<.0001
Chemical*time	S	4	SS	21	Tukey	<.0001
Chemical*time	S	4	SS	28	Tukey	<.0001
Chemical*time	S	7	S	10	Tukey	0.9991
Chemical*time	S	7	S	14	Tukey	1.0000
Chemical*time	S	7	S	21	Tukey	0.9982
Chemical*time	S	7	S	28	Tukey	0.5130
Chemical*time	S	7	SS	0	Tukey	0.4192
Chemical*time	S	7	SS	4	Tukey	1.0000
Chemical*time	S	7	SS	7	Tukey	1.0000
Chemical*time	S	7	SS	10	Tukey	1.0000
Chemical*time	S	7	SS	14	Tukey	1.0000
Chemical*time	S	7	SS	21	Tukey	0.9982
Chemical*time	S	7	SS	28	Tukey	0.0551
Chemical*time	S	10	S	14	Tukey	1.0000
Chemical*time	S	10	S	21	Tukey	0.4587
Chemical*time	S	10	S	28	Tukey	0.0209
Chemical*time	S	10	SS	0	Tukey	0.9971
Chemical*time	S	10	SS	4	Tukey	1.0000
Chemical*time	S	10	SS	7	Tukey	0.6906

Chemical*time	S	10	SS	10	Tukey	1.0000
Chemical*time	S	10	SS	14	Tukey	0.8509
Chemical*time	S	10	SS	21	Tukey	0.4587
Chemical*time	S	10	SS	28	Tukey	0.0005
Chemical*time	S	14	S	21	Tukey	0.8509
Chemical*time	S	14	S	28	Tukey	0.1125
Chemical*time	S	14	SS	0	Tukey	0.9012
Chemical*time	S	14	SS	4	Tukey	1.0000
Chemical*time	S	14	SS	7	Tukey	0.9616
Chemical*time	S	14	SS	10	Tukey	1.0000
Chemical*time	S	14	SS	14	Tukey	0.9927
Chemical*time	S	14	SS	21	Tukey	0.8509
Chemical*time	S	14	SS	28	Tukey	0.0048
Chemical*time	S	21	S	28	Tukey	0.9996
Chemical*time	S	21	SS	0	Tukey	0.0106
Chemical*time	S	21	SS	4	Tukey	0.9277
Chemical*time	S	21	SS	7	Tukey	1.0000
Chemical*time	S	21	SS	10	Tukey	0.8212
Chemical*time	S	21	SS	14	Tukey	1.0000
Chemical*time	S	21	SS	21	Tukey	1.0000
Chemical*time	S	21	SS	28	Tukey	0.7887
Chemical*time	S	28	SS	0	Tukey	<.0001
Chemical*time	S	28	SS	4	Tukey	0.1778
Chemical*time	S	28	SS	7	Tukey	0.9927
Chemical*time	S	28	SS	10	Tukey	0.0972
Chemical*time	S	28	SS	14	Tukey	0.9616
Chemical*time	S	28	SS	21	Tukey	0.9996
Chemical*time	S	28	SS	28	Tukey	1.0000
Chemical*time	SS	0	SS	4	Tukey	0.8107
Chemical*time	SS	0	SS	7	Tukey	0.0297
Chemical*time	SS	0	SS	10	Tukey	0.9216
Chemical*time	SS	0	SS	14	Tukey	0.0646
Chemical*time	SS	0	SS	21	Tukey	0.0106
Chemical*time	SS	0	SS	28	Tukey	<.0001
Chemical*time	SS	4	SS	7	Tukey	0.9875
Chemical*time	SS	4	SS	10	Tukey	1.0000
Chemical*time	SS	4	SS	14	Tukey	0.9985
Chemical*time	SS	4	SS	21	Tukey	0.9277
Chemical*time	SS	4	SS	28	Tukey	0.0093
Chemical*time	SS	7	SS	10	Tukey	0.9490
Chemical*time	SS	7	SS	14	Tukey	1.0000
Chemical*time	SS	7	SS	21	Tukey	1.0000
Chemical*time	SS	7	SS	28	Tukey	0.5683
Chemical*time	SS	10	SS	14	Tukey	0.9890
Chemical*time	SS	10	SS	21	Tukey	0.8212
Chemical*time	SS	10	SS	28	Tukey	0.0039

Chemical*time	SS	14	SS	21	Tukey	1.0000
Chemical*time	SS	14	SS	28	Tukey	0.3811
Chemical*time	SS	21	SS	28	Tukey	0.7887

E. Mesophilic bacteria on Cheddar cheese during storage at 4 °C for 35 days

(Initially inoculated *L. monocytogenes* 10^3 CFU/g)

Effect	Num DF	Den DF	F Value	Pr > F
Chemical	2	75	88.82	<.0001
time	4	75	1417.30	<.0001
Chemical*time	8	75	17.11	<.0001

Effect	Chemical	time	Chemical	time	Adjustment	Adj P
Chemical	C		S		Tukey	<.0001
Chemical	C		SS		Tukey	<.0001
Chemical	S		SS		Tukey	<.0001
time		0		7	Tukey	<.0001
time		0		14	Tukey	<.0001
time		0		21	Tukey	<.0001
time		0		35	Tukey	<.0001
time		7		14	Tukey	0.0084
time		7		21	Tukey	<.0001
time		7		35	Tukey	<.0001
time		14		21	Tukey	<.0001
time		14		35	Tukey	<.0001
time		21		35	Tukey	<.0001
Chemical*time	C	0	C	7	Tukey	<.0001
Chemical*time	C	0	C	14	Tukey	<.0001
Chemical*time	C	0	C	21	Tukey	<.0001
Chemical*time	C	0	C	35	Tukey	<.0001
Chemical*time	C	0	S	0	Tukey	1.0000
Chemical*time	C	0	S	7	Tukey	<.0001
Chemical*time	C	0	S	14	Tukey	<.0001
Chemical*time	C	0	S	21	Tukey	<.0001
Chemical*time	C	0	S	35	Tukey	<.0001
Chemical*time	C	0	SS	0	Tukey	1.0000
Chemical*time	C	0	SS	7	Tukey	<.0001
Chemical*time	C	0	SS	14	Tukey	<.0001
Chemical*time	C	0	SS	21	Tukey	<.0001
Chemical*time	C	0	SS	35	Tukey	<.0001
Chemical*time	C	7	C	14	Tukey	<.0001
Chemical*time	C	7	C	21	Tukey	<.0001

Chemical*time	C	7	C	35	Tukey	<.0001
Chemical*time	C	7	S	0	Tukey	<.0001
Chemical*time	C	7	S	7	Tukey	0.5798
Chemical*time	C	7	S	14	Tukey	0.6762
Chemical*time	C	7	S	21	Tukey	<.0001
Chemical*time	C	7	S	35	Tukey	<.0001
Chemical*time	C	7	SS	0	Tukey	<.0001
Chemical*time	C	7	SS	7	Tukey	0.6762
Chemical*time	C	7	SS	14	Tukey	0.3609
Chemical*time	C	7	SS	21	Tukey	<.0001
Chemical*time	C	7	SS	35	Tukey	0.7795
Chemical*time	C	14	C	21	Tukey	<.0001
Chemical*time	C	14	C	35	Tukey	0.9989
Chemical*time	C	14	S	0	Tukey	<.0001
Chemical*time	C	14	S	7	Tukey	<.0001
Chemical*time	C	14	S	14	Tukey	<.0001
Chemical*time	C	14	S	21	Tukey	<.0001
Chemical*time	C	14	S	35	Tukey	1.0000
Chemical*time	C	14	SS	0	Tukey	<.0001
Chemical*time	C	14	SS	7	Tukey	<.0001
Chemical*time	C	14	SS	14	Tukey	<.0001
Chemical*time	C	14	SS	21	Tukey	0.9989
Chemical*time	C	14	SS	35	Tukey	0.0050
Chemical*time	C	21	C	35	Tukey	<.0001
Chemical*time	C	21	S	0	Tukey	<.0001
Chemical*time	C	21	S	7	Tukey	<.0001
Chemical*time	C	21	S	14	Tukey	<.0001
Chemical*time	C	21	S	21	Tukey	0.0002
Chemical*time	C	21	S	35	Tukey	<.0001
Chemical*time	C	21	SS	0	Tukey	<.0001
Chemical*time	C	21	SS	7	Tukey	<.0001
Chemical*time	C	21	SS	14	Tukey	<.0001
Chemical*time	C	21	SS	21	Tukey	<.0001
Chemical*time	C	21	SS	35	Tukey	<.0001
Chemical*time	C	35	S	0	Tukey	<.0001
Chemical*time	C	35	S	7	Tukey	<.0001
Chemical*time	C	35	S	14	Tukey	<.0001
Chemical*time	C	35	S	21	Tukey	<.0001
Chemical*time	C	35	S	35	Tukey	1.0000
Chemical*time	C	35	SS	0	Tukey	<.0001
Chemical*time	C	35	SS	7	Tukey	<.0001
Chemical*time	C	35	SS	14	Tukey	<.0001
Chemical*time	C	35	SS	21	Tukey	1.0000
Chemical*time	C	35	SS	35	Tukey	<.0001
Chemical*time	S	0	S	7	Tukey	<.0001
Chemical*time	S	0	S	14	Tukey	<.0001

Chemical*time	S	0	S	21	Tukey	<.0001
Chemical*time	S	0	S	35	Tukey	<.0001
Chemical*time	S	0	SS	0	Tukey	1.0000
Chemical*time	S	0	SS	7	Tukey	<.0001
Chemical*time	S	0	SS	14	Tukey	<.0001
Chemical*time	S	0	SS	21	Tukey	<.0001
Chemical*time	S	0	SS	35	Tukey	<.0001
Chemical*time	S	7	S	14	Tukey	1.0000
Chemical*time	S	7	S	21	Tukey	<.0001
Chemical*time	S	7	S	35	Tukey	<.0001
Chemical*time	S	7	SS	0	Tukey	<.0001
Chemical*time	S	7	SS	7	Tukey	1.0000
Chemical*time	S	7	SS	14	Tukey	1.0000
Chemical*time	S	7	SS	21	Tukey	<.0001
Chemical*time	S	7	SS	35	Tukey	0.0037
Chemical*time	S	14	S	21	Tukey	<.0001
Chemical*time	S	14	S	35	Tukey	<.0001
Chemical*time	S	14	SS	0	Tukey	<.0001
Chemical*time	S	14	SS	7	Tukey	1.0000
Chemical*time	S	14	SS	14	Tukey	1.0000
Chemical*time	S	14	SS	21	Tukey	<.0001
Chemical*time	S	14	SS	35	Tukey	0.0059
Chemical*time	S	21	S	35	Tukey	<.0001
Chemical*time	S	21	SS	0	Tukey	<.0001
Chemical*time	S	21	SS	7	Tukey	<.0001
Chemical*time	S	21	SS	14	Tukey	<.0001
Chemical*time	S	21	SS	21	Tukey	<.0001
Chemical*time	S	21	SS	35	Tukey	<.0001
Chemical*time	S	35	SS	0	Tukey	<.0001
Chemical*time	S	35	SS	7	Tukey	<.0001
Chemical*time	S	35	SS	14	Tukey	<.0001
Chemical*time	S	35	SS	21	Tukey	1.0000
Chemical*time	S	35	SS	35	Tukey	0.0017
Chemical*time	SS	0	SS	7	Tukey	<.0001
Chemical*time	SS	0	SS	14	Tukey	<.0001
Chemical*time	SS	0	SS	21	Tukey	<.0001
Chemical*time	SS	0	SS	35	Tukey	<.0001
Chemical*time	SS	7	SS	14	Tukey	1.0000
Chemical*time	SS	7	SS	21	Tukey	<.0001
Chemical*time	SS	7	SS	35	Tukey	0.0059
Chemical*time	SS	14	SS	21	Tukey	<.0001
Chemical*time	SS	14	SS	35	Tukey	0.0012
Chemical*time	SS	21	SS	35	Tukey	<.0001

F. Mesophilic bacteria on Cheddar cheese during storage at 4 °C for 35 days

(Initially inoculated *L. monocytogenes* 10^5 CFU/g)

Effect	Num DF	Den DF	F Value	Pr > F
Chemical	2	105	34.99	<.0001
time	6	105	81.81	<.0001
Chemical*time	12	105	10.68	<.0001

Effect	Chemical	time	Chemical	time	Adjustment	Adj P
Chemical	C		S		Tukey	<.0001
Chemical	C		SS		Tukey	<.0001
Chemical	S		SS		Tukey	0.2751
time		0		4	Tukey	1.0000
time		0		7	Tukey	0.9999
time		0		10	Tukey	<.0001
time		0		14	Tukey	<.0001
time		0		21	Tukey	<.0001
time		0		35	Tukey	<.0001
time		4		7	Tukey	1.0000
time		4		10	Tukey	<.0001
time		4		14	Tukey	<.0001
time		4		21	Tukey	<.0001
time		4		35	Tukey	<.0001
time		7		10	Tukey	<.0001
time		7		14	Tukey	<.0001
time		7		21	Tukey	<.0001
time		7		35	Tukey	<.0001
time		10		14	Tukey	0.7970
time		10		21	Tukey	0.0232
time		10		35	Tukey	0.0208
time		14		21	Tukey	0.5041
time		14		35	Tukey	0.4796
time		21		35	Tukey	1.0000
Chemical*time	C	0	C	4	Tukey	1.0000
Chemical*time	C	0	C	7	Tukey	0.9099
Chemical*time	C	0	C	10	Tukey	<.0001
Chemical*time	C	0	C	14	Tukey	<.0001
Chemical*time	C	0	C	21	Tukey	<.0001
Chemical*time	C	0	C	35	Tukey	<.0001
Chemical*time	C	0	S	0	Tukey	1.0000
Chemical*time	C	0	S	4	Tukey	1.0000
Chemical*time	C	0	S	7	Tukey	0.9952

Chemical*time	C	0	S	10	Tukey	<.0001
Chemical*time	C	0	S	14	Tukey	0.0044
Chemical*time	C	0	S	21	Tukey	0.0361
Chemical*time	C	0	S	35	Tukey	0.0295
Chemical*time	C	0	SS	0	Tukey	1.0000
Chemical*time	C	0	SS	4	Tukey	1.0000
Chemical*time	C	0	SS	7	Tukey	1.0000
Chemical*time	C	0	SS	10	Tukey	<.0001
Chemical*time	C	0	SS	14	Tukey	<.0001
Chemical*time	C	0	SS	21	Tukey	0.1932
Chemical*time	C	0	SS	35	Tukey	0.2363
Chemical*time	C	4	C	7	Tukey	1.0000
Chemical*time	C	4	C	10	Tukey	<.0001
Chemical*time	C	4	C	14	Tukey	<.0001
Chemical*time	C	4	C	21	Tukey	<.0001
Chemical*time	C	4	C	35	Tukey	<.0001
Chemical*time	C	4	S	0	Tukey	0.9985
Chemical*time	C	4	S	4	Tukey	0.9985
Chemical*time	C	4	S	7	Tukey	0.5508
Chemical*time	C	4	S	10	Tukey	<.0001
Chemical*time	C	4	S	14	Tukey	<.0001
Chemical*time	C	4	S	21	Tukey	0.0008
Chemical*time	C	4	S	35	Tukey	0.0006
Chemical*time	C	4	SS	0	Tukey	1.0000
Chemical*time	C	4	SS	4	Tukey	1.0000
Chemical*time	C	4	SS	7	Tukey	1.0000
Chemical*time	C	4	SS	10	Tukey	<.0001
Chemical*time	C	4	SS	14	Tukey	<.0001
Chemical*time	C	4	SS	21	Tukey	0.0081
Chemical*time	C	4	SS	35	Tukey	0.0111
Chemical*time	C	7	C	10	Tukey	<.0001
Chemical*time	C	7	C	14	Tukey	<.0001
Chemical*time	C	7	C	21	Tukey	<.0001
Chemical*time	C	7	C	35	Tukey	<.0001
Chemical*time	C	7	S	0	Tukey	0.7553
Chemical*time	C	7	S	4	Tukey	0.7553
Chemical*time	C	7	S	7	Tukey	0.0797
Chemical*time	C	7	S	10	Tukey	<.0001
Chemical*time	C	7	S	14	Tukey	<.0001
Chemical*time	C	7	S	21	Tukey	<.0001
Chemical*time	C	7	S	35	Tukey	<.0001
Chemical*time	C	7	SS	0	Tukey	0.9999
Chemical*time	C	7	SS	4	Tukey	0.9668
Chemical*time	C	7	SS	7	Tukey	0.9978
Chemical*time	C	7	SS	10	Tukey	<.0001
Chemical*time	C	7	SS	14	Tukey	<.0001

Chemical*time	C	7	SS	21	Tukey	0.0002
Chemical*time	C	7	SS	35	Tukey	0.0003
Chemical*time	C	10	C	14	Tukey	0.9990
Chemical*time	C	10	C	21	Tukey	1.0000
Chemical*time	C	10	C	35	Tukey	1.0000
Chemical*time	C	10	S	0	Tukey	<.0001
Chemical*time	C	10	S	4	Tukey	<.0001
Chemical*time	C	10	S	7	Tukey	<.0001
Chemical*time	C	10	S	10	Tukey	0.5702
Chemical*time	C	10	S	14	Tukey	0.0005
Chemical*time	C	10	S	21	Tukey	<.0001
Chemical*time	C	10	S	35	Tukey	<.0001
Chemical*time	C	10	SS	0	Tukey	<.0001
Chemical*time	C	10	SS	4	Tukey	<.0001
Chemical*time	C	10	SS	7	Tukey	<.0001
Chemical*time	C	10	SS	10	Tukey	0.5799
Chemical*time	C	10	SS	14	Tukey	0.0240
Chemical*time	C	10	SS	21	Tukey	<.0001
Chemical*time	C	10	SS	35	Tukey	<.0001
Chemical*time	C	14	C	21	Tukey	1.0000
Chemical*time	C	14	C	35	Tukey	1.0000
Chemical*time	C	14	S	0	Tukey	<.0001
Chemical*time	C	14	S	4	Tukey	<.0001
Chemical*time	C	14	S	7	Tukey	<.0001
Chemical*time	C	14	S	10	Tukey	0.0250
Chemical*time	C	14	S	14	Tukey	<.0001
Chemical*time	C	14	S	21	Tukey	<.0001
Chemical*time	C	14	S	35	Tukey	<.0001
Chemical*time	C	14	SS	0	Tukey	<.0001
Chemical*time	C	14	SS	4	Tukey	<.0001
Chemical*time	C	14	SS	7	Tukey	<.0001
Chemical*time	C	14	SS	10	Tukey	0.0261
Chemical*time	C	14	SS	14	Tukey	0.0002
Chemical*time	C	14	SS	21	Tukey	<.0001
Chemical*time	C	14	SS	35	Tukey	<.0001
Chemical*time	C	21	C	35	Tukey	1.0000
Chemical*time	C	21	S	0	Tukey	<.0001
Chemical*time	C	21	S	4	Tukey	<.0001
Chemical*time	C	21	S	7	Tukey	<.0001
Chemical*time	C	21	S	10	Tukey	0.0641
Chemical*time	C	21	S	14	Tukey	<.0001
Chemical*time	C	21	S	21	Tukey	<.0001
Chemical*time	C	21	S	35	Tukey	<.0001
Chemical*time	C	21	SS	0	Tukey	<.0001
Chemical*time	C	21	SS	4	Tukey	<.0001
Chemical*time	C	21	SS	7	Tukey	<.0001

Chemical*time	C	21	SS	10	Tukey	0.0665
Chemical*time	C	21	SS	14	Tukey	0.0006
Chemical*time	C	21	SS	21	Tukey	<.0001
Chemical*time	C	21	SS	35	Tukey	<.0001
Chemical*time	C	35	S	0	Tukey	<.0001
Chemical*time	C	35	S	4	Tukey	<.0001
Chemical*time	C	35	S	7	Tukey	<.0001
Chemical*time	C	35	S	10	Tukey	0.0716
Chemical*time	C	35	S	14	Tukey	<.0001
Chemical*time	C	35	S	21	Tukey	<.0001
Chemical*time	C	35	S	35	Tukey	<.0001
Chemical*time	C	35	SS	0	Tukey	<.0001
Chemical*time	C	35	SS	4	Tukey	<.0001
Chemical*time	C	35	SS	7	Tukey	<.0001
Chemical*time	C	35	SS	10	Tukey	0.0742
Chemical*time	C	35	SS	14	Tukey	0.0007
Chemical*time	C	35	SS	21	Tukey	<.0001
Chemical*time	C	35	SS	35	Tukey	<.0001
Chemical*time	S	0	S	4	Tukey	1.0000
Chemical*time	S	0	S	7	Tukey	0.9998
Chemical*time	S	0	S	10	Tukey	<.0001
Chemical*time	S	0	S	14	Tukey	0.0126
Chemical*time	S	0	S	21	Tukey	0.0856
Chemical*time	S	0	S	35	Tukey	0.0716
Chemical*time	S	0	SS	0	Tukey	0.9998
Chemical*time	S	0	SS	4	Tukey	1.0000
Chemical*time	S	0	SS	7	Tukey	1.0000
Chemical*time	S	0	SS	10	Tukey	<.0001
Chemical*time	S	0	SS	14	Tukey	0.0002
Chemical*time	S	0	SS	21	Tukey	0.3571
Chemical*time	S	0	SS	35	Tukey	0.4184
Chemical*time	S	4	S	7	Tukey	0.9998
Chemical*time	S	4	S	10	Tukey	<.0001
Chemical*time	S	4	S	14	Tukey	0.0126
Chemical*time	S	4	S	21	Tukey	0.0856
Chemical*time	S	4	S	35	Tukey	0.0716
Chemical*time	S	4	SS	0	Tukey	0.9998
Chemical*time	S	4	SS	4	Tukey	1.0000
Chemical*time	S	4	SS	7	Tukey	1.0000
Chemical*time	S	4	SS	10	Tukey	<.0001
Chemical*time	S	4	SS	14	Tukey	0.0002
Chemical*time	S	4	SS	21	Tukey	0.3571
Chemical*time	S	4	SS	35	Tukey	0.4184
Chemical*time	S	7	S	10	Tukey	0.0001
Chemical*time	S	7	S	14	Tukey	0.3322
Chemical*time	S	7	S	21	Tukey	0.7719

Chemical*time	S	7	S	35	Tukey	0.7295
Chemical*time	S	7	SS	0	Tukey	0.7028
Chemical*time	S	7	SS	4	Tukey	0.9789
Chemical*time	S	7	SS	7	Tukey	0.8719
Chemical*time	S	7	SS	10	Tukey	0.0001
Chemical*time	S	7	SS	14	Tukey	0.0203
Chemical*time	S	7	SS	21	Tukey	0.9861
Chemical*time	S	7	SS	35	Tukey	0.9927
Chemical*time	S	10	S	14	Tukey	0.6753
Chemical*time	S	10	S	21	Tukey	0.2498
Chemical*time	S	10	S	35	Tukey	0.2855
Chemical*time	S	10	SS	0	Tukey	<.0001
Chemical*time	S	10	SS	4	Tukey	<.0001
Chemical*time	S	10	SS	7	Tukey	<.0001
Chemical*time	S	10	SS	10	Tukey	1.0000
Chemical*time	S	10	SS	14	Tukey	0.9988
Chemical*time	S	10	SS	21	Tukey	0.0512
Chemical*time	S	10	SS	35	Tukey	0.0390
Chemical*time	S	14	S	21	Tukey	1.0000
Chemical*time	S	14	S	35	Tukey	1.0000
Chemical*time	S	14	SS	0	Tukey	0.0001
Chemical*time	S	14	SS	4	Tukey	0.0020
Chemical*time	S	14	SS	7	Tukey	0.0005
Chemical*time	S	14	SS	10	Tukey	0.6659
Chemical*time	S	14	SS	14	Tukey	1.0000
Chemical*time	S	14	SS	21	Tukey	0.9996
Chemical*time	S	14	SS	35	Tukey	0.9990
Chemical*time	S	21	S	35	Tukey	1.0000
Chemical*time	S	21	SS	0	Tukey	0.0018
Chemical*time	S	21	SS	4	Tukey	0.0187
Chemical*time	S	21	SS	7	Tukey	0.0051
Chemical*time	S	21	SS	10	Tukey	0.2430
Chemical*time	S	21	SS	14	Tukey	0.9820
Chemical*time	S	21	SS	21	Tukey	1.0000
Chemical*time	S	21	SS	35	Tukey	1.0000
Chemical*time	S	35	SS	0	Tukey	0.0014
Chemical*time	S	35	SS	4	Tukey	0.0151
Chemical*time	S	35	SS	7	Tukey	0.0040
Chemical*time	S	35	SS	10	Tukey	0.2781
Chemical*time	S	35	SS	14	Tukey	0.9883
Chemical*time	S	35	SS	21	Tukey	1.0000
Chemical*time	S	35	SS	35	Tukey	1.0000
Chemical*time	SS	0	SS	4	Tukey	1.0000
Chemical*time	SS	0	SS	7	Tukey	1.0000
Chemical*time	SS	0	SS	10	Tukey	<.0001
Chemical*time	SS	0	SS	14	Tukey	<.0001

Chemical*time	SS	0	SS	21	Tukey	0.0164
Chemical*time	SS	0	SS	35	Tukey	0.0221
Chemical*time	SS	4	SS	7	Tukey	1.0000
Chemical*time	SS	4	SS	10	Tukey	<.0001
Chemical*time	SS	4	SS	14	Tukey	<.0001
Chemical*time	SS	4	SS	21	Tukey	0.1166
Chemical*time	SS	4	SS	35	Tukey	0.1465
Chemical*time	SS	7	SS	10	Tukey	<.0001
Chemical*time	SS	7	SS	14	Tukey	<.0001
Chemical*time	SS	7	SS	21	Tukey	0.0406
Chemical*time	SS	7	SS	35	Tukey	0.0532
Chemical*time	SS	10	SS	14	Tukey	0.9986
Chemical*time	SS	10	SS	21	Tukey	0.0493
Chemical*time	SS	10	SS	35	Tukey	0.0375
Chemical*time	SS	14	SS	21	Tukey	0.7468
Chemical*time	SS	14	SS	35	Tukey	0.6845
Chemical*time	SS	21	SS	35	Tukey	1.0000

G. Mesophilic bacteria on beef bologna during storage at 4 °C for 28 days

(Initially inoculated *L. monocytogenes* 10^3 CFU/g)

Effect	Num DF	Den DF	F Value	Pr > F
Chemical	2	42	1230.97	<.0001
time	6	42	51.86	<.0001
Chemical*time	12	42	128.55	<.0001

Effect	Chemical	time	Chemical	time	Adjustment	Adj P
Chemical	C		S		Tukey	<.0001
Chemical	C		SS		Tukey	<.0001
Chemical	S		SS		Tukey	0.0001
time		0		4	Tukey	0.0117
time		0		7	Tukey	1.0000
time		0		10	Tukey	0.9987
time		0		14	Tukey	0.0678
time		0		21	Tukey	<.0001
time		0		28	Tukey	<.0001
time		4		7	Tukey	0.0191
time		4		10	Tukey	0.0440
time		4		14	Tukey	0.9929
time		4		21	Tukey	<.0001
time		4		28	Tukey	<.0001
time		7		10	Tukey	0.9999

time		7		14	Tukey	0.1018
time		7		21	Tukey	<.0001
time		7		28	Tukey	<.0001
time		10		14	Tukey	0.1986
time		10		21	Tukey	<.0001
time		10		28	Tukey	<.0001
time		14		21	Tukey	<.0001
time		14		28	Tukey	<.0001
time		21		28	Tukey	0.0071
Chemical*time	C	0	C	4	Tukey	0.0004
Chemical*time	C	0	C	7	Tukey	<.0001
Chemical*time	C	0	C	10	Tukey	<.0001
Chemical*time	C	0	C	14	Tukey	<.0001
Chemical*time	C	0	C	21	Tukey	<.0001
Chemical*time	C	0	C	28	Tukey	<.0001
Chemical*time	C	0	S	0	Tukey	1.0000
Chemical*time	C	0	S	4	Tukey	1.0000
Chemical*time	C	0	S	7	Tukey	0.1329
Chemical*time	C	0	S	10	Tukey	0.5034
Chemical*time	C	0	S	14	Tukey	0.6497
Chemical*time	C	0	S	21	Tukey	0.3107
Chemical*time	C	0	S	28	Tukey	0.0145
Chemical*time	C	0	SS	0	Tukey	1.0000
Chemical*time	C	0	SS	4	Tukey	1.0000
Chemical*time	C	0	SS	7	Tukey	0.0852
Chemical*time	C	0	SS	10	Tukey	0.0008
Chemical*time	C	0	SS	14	Tukey	0.0012
Chemical*time	C	0	SS	21	Tukey	<.0001
Chemical*time	C	0	SS	28	Tukey	<.0001
Chemical*time	C	4	C	7	Tukey	0.9992
Chemical*time	C	4	C	10	Tukey	0.4515
Chemical*time	C	4	C	14	Tukey	<.0001
Chemical*time	C	4	C	21	Tukey	<.0001
Chemical*time	C	4	C	28	Tukey	<.0001
Chemical*time	C	4	S	0	Tukey	0.0001
Chemical*time	C	4	S	4	Tukey	0.0009
Chemical*time	C	4	S	7	Tukey	<.0001
Chemical*time	C	4	S	10	Tukey	<.0001
Chemical*time	C	4	S	14	Tukey	<.0001
Chemical*time	C	4	S	21	Tukey	<.0001
Chemical*time	C	4	S	28	Tukey	<.0001
Chemical*time	C	4	SS	0	Tukey	0.0003
Chemical*time	C	4	SS	4	Tukey	0.0005
Chemical*time	C	4	SS	7	Tukey	<.0001
Chemical*time	C	4	SS	10	Tukey	<.0001
Chemical*time	C	4	SS	14	Tukey	<.0001

Chemical*time	C	4	SS	21	Tukey	<.0001
Chemical*time	C	4	SS	28	Tukey	<.0001
Chemical*time	C	7	C	10	Tukey	0.9946
Chemical*time	C	7	C	14	Tukey	0.0005
Chemical*time	C	7	C	21	Tukey	<.0001
Chemical*time	C	7	C	28	Tukey	<.0001
Chemical*time	C	7	S	0	Tukey	<.0001
Chemical*time	C	7	S	4	Tukey	<.0001
Chemical*time	C	7	S	7	Tukey	<.0001
Chemical*time	C	7	S	10	Tukey	<.0001
Chemical*time	C	7	S	14	Tukey	<.0001
Chemical*time	C	7	S	21	Tukey	<.0001
Chemical*time	C	7	S	28	Tukey	<.0001
Chemical*time	C	7	SS	0	Tukey	<.0001
Chemical*time	C	7	SS	4	Tukey	<.0001
Chemical*time	C	7	SS	7	Tukey	<.0001
Chemical*time	C	7	SS	10	Tukey	<.0001
Chemical*time	C	7	SS	14	Tukey	<.0001
Chemical*time	C	7	SS	21	Tukey	<.0001
Chemical*time	C	7	SS	28	Tukey	<.0001
Chemical*time	C	10	C	14	Tukey	0.0374
Chemical*time	C	10	C	21	Tukey	<.0001
Chemical*time	C	10	C	28	Tukey	<.0001
Chemical*time	C	10	S	0	Tukey	<.0001
Chemical*time	C	10	S	4	Tukey	<.0001
Chemical*time	C	10	S	7	Tukey	<.0001
Chemical*time	C	10	S	10	Tukey	<.0001
Chemical*time	C	10	S	14	Tukey	<.0001
Chemical*time	C	10	S	21	Tukey	<.0001
Chemical*time	C	10	S	28	Tukey	<.0001
Chemical*time	C	10	SS	0	Tukey	<.0001
Chemical*time	C	10	SS	4	Tukey	<.0001
Chemical*time	C	10	SS	7	Tukey	<.0001
Chemical*time	C	10	SS	10	Tukey	<.0001
Chemical*time	C	10	SS	14	Tukey	<.0001
Chemical*time	C	10	SS	21	Tukey	<.0001
Chemical*time	C	10	SS	28	Tukey	<.0001
Chemical*time	C	14	C	21	Tukey	<.0001
Chemical*time	C	14	C	28	Tukey	<.0001
Chemical*time	C	14	S	0	Tukey	<.0001
Chemical*time	C	14	S	4	Tukey	<.0001
Chemical*time	C	14	S	7	Tukey	<.0001
Chemical*time	C	14	S	10	Tukey	<.0001
Chemical*time	C	14	S	14	Tukey	<.0001
Chemical*time	C	14	S	21	Tukey	<.0001
Chemical*time	C	14	S	28	Tukey	<.0001

Chemical*time	C	14	SS	0	Tukey	<.0001
Chemical*time	C	14	SS	4	Tukey	<.0001
Chemical*time	C	14	SS	7	Tukey	<.0001
Chemical*time	C	14	SS	10	Tukey	<.0001
Chemical*time	C	14	SS	14	Tukey	<.0001
Chemical*time	C	14	SS	21	Tukey	<.0001
Chemical*time	C	14	SS	28	Tukey	<.0001
Chemical*time	C	21	C	28	Tukey	<.0001
Chemical*time	C	21	S	0	Tukey	<.0001
Chemical*time	C	21	S	4	Tukey	<.0001
Chemical*time	C	21	S	7	Tukey	<.0001
Chemical*time	C	21	S	10	Tukey	<.0001
Chemical*time	C	21	S	14	Tukey	<.0001
Chemical*time	C	21	S	21	Tukey	<.0001
Chemical*time	C	21	S	28	Tukey	<.0001
Chemical*time	C	21	SS	0	Tukey	<.0001
Chemical*time	C	21	SS	4	Tukey	<.0001
Chemical*time	C	21	SS	7	Tukey	<.0001
Chemical*time	C	21	SS	10	Tukey	<.0001
Chemical*time	C	21	SS	14	Tukey	<.0001
Chemical*time	C	21	SS	21	Tukey	<.0001
Chemical*time	C	21	SS	28	Tukey	<.0001
Chemical*time	C	28	S	0	Tukey	<.0001
Chemical*time	C	28	S	4	Tukey	<.0001
Chemical*time	C	28	S	7	Tukey	<.0001
Chemical*time	C	28	S	10	Tukey	<.0001
Chemical*time	C	28	S	14	Tukey	<.0001
Chemical*time	C	28	S	21	Tukey	<.0001
Chemical*time	C	28	S	28	Tukey	<.0001
Chemical*time	C	28	SS	0	Tukey	<.0001
Chemical*time	C	28	SS	4	Tukey	<.0001
Chemical*time	C	28	SS	7	Tukey	<.0001
Chemical*time	C	28	SS	10	Tukey	<.0001
Chemical*time	C	28	SS	14	Tukey	<.0001
Chemical*time	C	28	SS	21	Tukey	<.0001
Chemical*time	C	28	SS	28	Tukey	<.0001
Chemical*time	S	0	S	4	Tukey	1.0000
Chemical*time	S	0	S	7	Tukey	0.3214
Chemical*time	S	0	S	10	Tukey	0.7964
Chemical*time	S	0	S	14	Tukey	0.8983
Chemical*time	S	0	S	21	Tukey	0.5966
Chemical*time	S	0	S	28	Tukey	0.0480
Chemical*time	S	0	SS	0	Tukey	1.0000
Chemical*time	S	0	SS	4	Tukey	1.0000
Chemical*time	S	0	SS	7	Tukey	0.2248
Chemical*time	S	0	SS	10	Tukey	0.0031

Chemical*time	S	0	SS	14	Tukey	0.0047
Chemical*time	S	0	SS	21	Tukey	<.0001
Chemical*time	S	0	SS	28	Tukey	<.0001
Chemical*time	S	4	S	7	Tukey	0.0777
Chemical*time	S	4	S	10	Tukey	0.3546
Chemical*time	S	4	S	14	Tukey	0.4902
Chemical*time	S	4	S	21	Tukey	0.2003
Chemical*time	S	4	S	28	Tukey	0.0075
Chemical*time	S	4	SS	0	Tukey	1.0000
Chemical*time	S	4	SS	4	Tukey	1.0000
Chemical*time	S	4	SS	7	Tukey	0.0480
Chemical*time	S	4	SS	10	Tukey	0.0004
Chemical*time	S	4	SS	14	Tukey	0.0006
Chemical*time	S	4	SS	21	Tukey	<.0001
Chemical*time	S	4	SS	28	Tukey	<.0001
Chemical*time	S	7	S	10	Tukey	1.0000
Chemical*time	S	7	S	14	Tukey	1.0000
Chemical*time	S	7	S	21	Tukey	1.0000
Chemical*time	S	7	S	28	Tukey	1.0000
Chemical*time	S	7	SS	0	Tukey	0.1851
Chemical*time	S	7	SS	4	Tukey	0.1167
Chemical*time	S	7	SS	7	Tukey	1.0000
Chemical*time	S	7	SS	10	Tukey	0.9473
Chemical*time	S	7	SS	14	Tukey	0.9740
Chemical*time	S	7	SS	21	Tukey	0.0584
Chemical*time	S	7	SS	28	Tukey	0.1329
Chemical*time	S	10	S	14	Tukey	1.0000
Chemical*time	S	10	S	21	Tukey	1.0000
Chemical*time	S	10	S	28	Tukey	0.9856
Chemical*time	S	10	SS	0	Tukey	0.6100
Chemical*time	S	10	SS	4	Tukey	0.4643
Chemical*time	S	10	SS	7	Tukey	1.0000
Chemical*time	S	10	SS	10	Tukey	0.5565
Chemical*time	S	10	SS	14	Tukey	0.6497
Chemical*time	S	10	SS	21	Tukey	0.0079
Chemical*time	S	10	SS	28	Tukey	0.0211
Chemical*time	S	14	S	21	Tukey	1.0000
Chemical*time	S	14	S	28	Tukey	0.9520
Chemical*time	S	14	SS	0	Tukey	0.7506
Chemical*time	S	14	SS	4	Tukey	0.6100
Chemical*time	S	14	SS	7	Tukey	0.9998
Chemical*time	S	14	SS	10	Tukey	0.4139
Chemical*time	S	14	SS	14	Tukey	0.5034
Chemical*time	S	14	SS	21	Tukey	0.0042
Chemical*time	S	14	SS	28	Tukey	0.0116
Chemical*time	S	21	S	28	Tukey	0.9989

Chemical*time	S	21	SS	0	Tukey	0.4017
Chemical*time	S	21	SS	4	Tukey	0.2800
Chemical*time	S	21	SS	7	Tukey	1.0000
Chemical*time	S	21	SS	10	Tukey	0.7624
Chemical*time	S	21	SS	14	Tukey	0.8378
Chemical*time	S	21	SS	21	Tukey	0.0190
Chemical*time	S	21	SS	28	Tukey	0.0480
Chemical*time	S	28	SS	0	Tukey	0.0223
Chemical*time	S	28	SS	4	Tukey	0.0123
Chemical*time	S	28	SS	7	Tukey	1.0000
Chemical*time	S	28	SS	10	Tukey	1.0000
Chemical*time	S	28	SS	14	Tukey	1.0000
Chemical*time	S	28	SS	21	Tukey	0.3661
Chemical*time	S	28	SS	28	Tukey	0.5966
Chemical*time	SS	0	SS	4	Tukey	1.0000
Chemical*time	SS	0	SS	7	Tukey	0.1219
Chemical*time	SS	0	SS	10	Tukey	0.0013
Chemical*time	SS	0	SS	14	Tukey	0.0020
Chemical*time	SS	0	SS	21	Tukey	<.0001
Chemical*time	SS	0	SS	28	Tukey	<.0001
Chemical*time	SS	4	SS	7	Tukey	0.0741
Chemical*time	SS	4	SS	10	Tukey	0.0007
Chemical*time	SS	4	SS	14	Tukey	0.0010
Chemical*time	SS	4	SS	21	Tukey	<.0001
Chemical*time	SS	4	SS	28	Tukey	<.0001
Chemical*time	SS	7	SS	10	Tukey	0.9816
Chemical*time	SS	7	SS	14	Tukey	0.9927
Chemical*time	SS	7	SS	21	Tukey	0.0934
Chemical*time	SS	7	SS	28	Tukey	0.2003
Chemical*time	SS	10	SS	14	Tukey	1.0000
Chemical*time	SS	10	SS	21	Tukey	0.9314
Chemical*time	SS	10	SS	28	Tukey	0.9903
Chemical*time	SS	14	SS	21	Tukey	0.8827
Chemical*time	SS	14	SS	28	Tukey	0.9767
Chemical*time	SS	21	SS	28	Tukey	1.0000

H. Mesophilic bacteria on beef bologna during storage at 4 °C for 28 days

(Initially inoculated *L. monocytogenes* 10^5 CFU/g)

Effect	Num DF	Den DF	F Value	Pr > F
Chemical	2	105	1727.65	<.0001
time	6	105	71.35	<.0001
Chemical*time	12	105	106.42	<.0001

Effect	Chemical	time	Chemical	time	Adjustment	Adj P
Chemical		C		S	Tukey	<.0001
Chemical		C		SS	Tukey	<.0001
Chemical		S		SS	Tukey	0.0010
time		0		4	Tukey	0.0072
time		0		7	Tukey	0.0185
time		0		10	Tukey	<.0001
time		0		14	Tukey	<.0001
time		0		21	Tukey	<.0001
time		0		28	Tukey	<.0001
time		4		7	Tukey	<.0001
time		4		10	Tukey	<.0001
time		4		14	Tukey	<.0001
time		4		21	Tukey	<.0001
time		4		28	Tukey	<.0001
time		7		10	Tukey	<.0001
time		7		14	Tukey	<.0001
time		7		21	Tukey	<.0001
time		7		28	Tukey	<.0001
time		10		14	Tukey	0.3245
time		10		21	Tukey	0.0121
time		10		28	Tukey	0.1895
time		14		21	Tukey	0.8363
time		14		28	Tukey	1.0000
time		21		28	Tukey	0.9421
Chemical*time	C	0	C	4	Tukey	0.0012
Chemical*time	C	0	C	7	Tukey	<.0001
Chemical*time	C	0	C	10	Tukey	<.0001
Chemical*time	C	0	C	14	Tukey	<.0001
Chemical*time	C	0	C	21	Tukey	<.0001
Chemical*time	C	0	C	28	Tukey	<.0001
Chemical*time	C	0	S	0	Tukey	1.0000
Chemical*time	C	0	S	4	Tukey	0.9995
Chemical*time	C	0	S	7	Tukey	0.9997
Chemical*time	C	0	S	10	Tukey	0.7574
Chemical*time	C	0	S	14	Tukey	0.0182
Chemical*time	C	0	S	21	Tukey	0.0092
Chemical*time	C	0	S	28	Tukey	0.0015
Chemical*time	C	0	SS	0	Tukey	1.0000
Chemical*time	C	0	SS	4	Tukey	<.0001
Chemical*time	C	0	SS	7	Tukey	0.2343
Chemical*time	C	0	SS	10	Tukey	0.9883
Chemical*time	C	0	SS	14	Tukey	0.1137
Chemical*time	C	0	SS	21	Tukey	0.0489

Chemical*time	C	0	SS	28	Tukey	0.0003
Chemical*time	C	4	C	7	Tukey	0.0010
Chemical*time	C	4	C	10	Tukey	<.0001
Chemical*time	C	4	C	14	Tukey	<.0001
Chemical*time	C	4	C	21	Tukey	<.0001
Chemical*time	C	4	C	28	Tukey	<.0001
Chemical*time	C	4	S	0	Tukey	0.0006
Chemical*time	C	4	S	4	Tukey	<.0001
Chemical*time	C	4	S	7	Tukey	<.0001
Chemical*time	C	4	S	10	Tukey	<.0001
Chemical*time	C	4	S	14	Tukey	<.0001
Chemical*time	C	4	S	21	Tukey	<.0001
Chemical*time	C	4	S	28	Tukey	<.0001
Chemical*time	C	4	SS	0	Tukey	0.0002
Chemical*time	C	4	SS	4	Tukey	<.0001
Chemical*time	C	4	SS	7	Tukey	<.0001
Chemical*time	C	4	SS	10	Tukey	<.0001
Chemical*time	C	4	SS	14	Tukey	<.0001
Chemical*time	C	4	SS	21	Tukey	<.0001
Chemical*time	C	4	SS	28	Tukey	<.0001
Chemical*time	C	7	C	10	Tukey	<.0001
Chemical*time	C	7	C	14	Tukey	<.0001
Chemical*time	C	7	C	21	Tukey	<.0001
Chemical*time	C	7	C	28	Tukey	<.0001
Chemical*time	C	7	S	0	Tukey	<.0001
Chemical*time	C	7	S	4	Tukey	<.0001
Chemical*time	C	7	S	7	Tukey	<.0001
Chemical*time	C	7	S	10	Tukey	<.0001
Chemical*time	C	7	S	14	Tukey	<.0001
Chemical*time	C	7	S	21	Tukey	<.0001
Chemical*time	C	7	S	28	Tukey	<.0001
Chemical*time	C	7	SS	0	Tukey	<.0001
Chemical*time	C	7	SS	4	Tukey	<.0001
Chemical*time	C	7	SS	7	Tukey	<.0001
Chemical*time	C	7	SS	10	Tukey	<.0001
Chemical*time	C	7	SS	14	Tukey	<.0001
Chemical*time	C	7	SS	21	Tukey	<.0001
Chemical*time	C	7	SS	28	Tukey	<.0001
Chemical*time	C	10	C	14	Tukey	<.0001
Chemical*time	C	10	C	21	Tukey	<.0001
Chemical*time	C	10	C	28	Tukey	<.0001
Chemical*time	C	10	S	0	Tukey	<.0001
Chemical*time	C	10	S	4	Tukey	<.0001
Chemical*time	C	10	S	7	Tukey	<.0001
Chemical*time	C	10	S	10	Tukey	<.0001
Chemical*time	C	10	S	14	Tukey	<.0001

Chemical*time	C	10	S	21	Tukey	<.0001
Chemical*time	C	10	S	28	Tukey	<.0001
Chemical*time	C	10	SS	0	Tukey	<.0001
Chemical*time	C	10	SS	4	Tukey	<.0001
Chemical*time	C	10	SS	7	Tukey	<.0001
Chemical*time	C	10	SS	10	Tukey	<.0001
Chemical*time	C	10	SS	14	Tukey	<.0001
Chemical*time	C	10	SS	21	Tukey	<.0001
Chemical*time	C	10	SS	28	Tukey	<.0001
Chemical*time	C	14	C	21	Tukey	0.3847
Chemical*time	C	14	C	28	Tukey	0.3194
Chemical*time	C	14	S	0	Tukey	<.0001
Chemical*time	C	14	S	4	Tukey	<.0001
Chemical*time	C	14	S	7	Tukey	<.0001
Chemical*time	C	14	S	10	Tukey	<.0001
Chemical*time	C	14	S	14	Tukey	<.0001
Chemical*time	C	14	S	21	Tukey	<.0001
Chemical*time	C	14	S	28	Tukey	<.0001
Chemical*time	C	14	SS	0	Tukey	<.0001
Chemical*time	C	14	SS	4	Tukey	<.0001
Chemical*time	C	14	SS	7	Tukey	<.0001
Chemical*time	C	14	SS	10	Tukey	<.0001
Chemical*time	C	14	SS	14	Tukey	<.0001
Chemical*time	C	14	SS	21	Tukey	<.0001
Chemical*time	C	14	SS	28	Tukey	<.0001
Chemical*time	C	21	C	28	Tukey	1.0000
Chemical*time	C	21	S	0	Tukey	<.0001
Chemical*time	C	21	S	4	Tukey	<.0001
Chemical*time	C	21	S	7	Tukey	<.0001
Chemical*time	C	21	S	10	Tukey	<.0001
Chemical*time	C	21	S	14	Tukey	<.0001
Chemical*time	C	21	S	21	Tukey	<.0001
Chemical*time	C	21	S	28	Tukey	<.0001
Chemical*time	C	21	SS	0	Tukey	<.0001
Chemical*time	C	21	SS	4	Tukey	<.0001
Chemical*time	C	21	SS	7	Tukey	<.0001
Chemical*time	C	21	SS	10	Tukey	<.0001
Chemical*time	C	21	SS	14	Tukey	<.0001
Chemical*time	C	21	SS	21	Tukey	<.0001
Chemical*time	C	21	SS	28	Tukey	<.0001
Chemical*time	C	28	S	0	Tukey	<.0001
Chemical*time	C	28	S	4	Tukey	<.0001
Chemical*time	C	28	S	7	Tukey	<.0001
Chemical*time	C	28	S	10	Tukey	<.0001
Chemical*time	C	28	S	14	Tukey	<.0001
Chemical*time	C	28	S	21	Tukey	<.0001

Chemical*time	C	28	S	28	Tukey	<.0001
Chemical*time	C	28	SS	0	Tukey	<.0001
Chemical*time	C	28	SS	4	Tukey	<.0001
Chemical*time	C	28	SS	7	Tukey	<.0001
Chemical*time	C	28	SS	10	Tukey	<.0001
Chemical*time	C	28	SS	14	Tukey	<.0001
Chemical*time	C	28	SS	21	Tukey	<.0001
Chemical*time	C	28	SS	28	Tukey	<.0001
Chemical*time	S	0	S	4	Tukey	0.9999
Chemical*time	S	0	S	7	Tukey	1.0000
Chemical*time	S	0	S	10	Tukey	0.8527
Chemical*time	S	0	S	14	Tukey	0.0308
Chemical*time	S	0	S	21	Tukey	0.0160
Chemical*time	S	0	S	28	Tukey	0.0028
Chemical*time	S	0	SS	0	Tukey	1.0000
Chemical*time	S	0	SS	4	Tukey	<.0001
Chemical*time	S	0	SS	7	Tukey	0.3272
Chemical*time	S	0	SS	10	Tukey	0.9968
Chemical*time	S	0	SS	14	Tukey	0.1710
Chemical*time	S	0	SS	21	Tukey	0.0783
Chemical*time	S	0	SS	28	Tukey	0.0006
Chemical*time	S	4	S	7	Tukey	1.0000
Chemical*time	S	4	S	10	Tukey	1.0000
Chemical*time	S	4	S	14	Tukey	0.4464
Chemical*time	S	4	S	21	Tukey	0.3117
Chemical*time	S	4	S	28	Tukey	0.0996
Chemical*time	S	4	SS	0	Tukey	1.0000
Chemical*time	S	4	SS	4	Tukey	<.0001
Chemical*time	S	4	SS	7	Tukey	0.9651
Chemical*time	S	4	SS	10	Tukey	1.0000
Chemical*time	S	4	SS	14	Tukey	0.8652
Chemical*time	S	4	SS	21	Tukey	0.6795
Chemical*time	S	4	SS	28	Tukey	0.0308
Chemical*time	S	7	S	10	Tukey	1.0000
Chemical*time	S	7	S	14	Tukey	0.4195
Chemical*time	S	7	S	21	Tukey	0.2892
Chemical*time	S	7	S	28	Tukey	0.0900
Chemical*time	S	7	SS	0	Tukey	1.0000
Chemical*time	S	7	SS	4	Tukey	<.0001
Chemical*time	S	7	SS	7	Tukey	0.9573
Chemical*time	S	7	SS	10	Tukey	1.0000
Chemical*time	S	7	SS	14	Tukey	0.8463
Chemical*time	S	7	SS	21	Tukey	0.6521
Chemical*time	S	7	SS	28	Tukey	0.0274
Chemical*time	S	10	S	14	Tukey	0.9807
Chemical*time	S	10	S	21	Tukey	0.9416

Chemical*time	S	10	S	28	Tukey	0.6885
Chemical*time	S	10	SS	0	Tukey	0.9483
Chemical*time	S	10	SS	4	Tukey	<.0001
Chemical*time	S	10	SS	7	Tukey	1.0000
Chemical*time	S	10	SS	10	Tukey	1.0000
Chemical*time	S	10	SS	14	Tukey	1.0000
Chemical*time	S	10	SS	21	Tukey	0.9984
Chemical*time	S	10	SS	28	Tukey	0.3933
Chemical*time	S	14	S	21	Tukey	1.0000
Chemical*time	S	14	S	28	Tukey	1.0000
Chemical*time	S	14	SS	0	Tukey	0.0656
Chemical*time	S	14	SS	4	Tukey	<.0001
Chemical*time	S	14	SS	7	Tukey	1.0000
Chemical*time	S	14	SS	10	Tukey	0.7063
Chemical*time	S	14	SS	14	Tukey	1.0000
Chemical*time	S	14	SS	21	Tukey	1.0000
Chemical*time	S	14	SS	28	Tukey	0.9999
Chemical*time	S	21	S	28	Tukey	1.0000
Chemical*time	S	21	SS	0	Tukey	0.0361
Chemical*time	S	21	SS	4	Tukey	<.0001
Chemical*time	S	21	SS	7	Tukey	0.9999
Chemical*time	S	21	SS	10	Tukey	0.5583
Chemical*time	S	21	SS	14	Tukey	1.0000
Chemical*time	S	21	SS	21	Tukey	1.0000
Chemical*time	S	21	SS	28	Tukey	1.0000
Chemical*time	S	28	SS	0	Tukey	0.0070
Chemical*time	S	28	SS	4	Tukey	<.0001
Chemical*time	S	28	SS	7	Tukey	0.9883
Chemical*time	S	28	SS	10	Tukey	0.2343
Chemical*time	S	28	SS	14	Tukey	0.9991
Chemical*time	S	28	SS	21	Tukey	1.0000
Chemical*time	S	28	SS	28	Tukey	1.0000
Chemical*time	SS	0	SS	4	Tukey	<.0001
Chemical*time	SS	0	SS	7	Tukey	0.5017
Chemical*time	SS	0	SS	10	Tukey	0.9998
Chemical*time	SS	0	SS	14	Tukey	0.2966
Chemical*time	SS	0	SS	21	Tukey	0.1514
Chemical*time	SS	0	SS	28	Tukey	0.0016
Chemical*time	SS	4	SS	7	Tukey	<.0001
Chemical*time	SS	4	SS	10	Tukey	<.0001
Chemical*time	SS	4	SS	14	Tukey	<.0001
Chemical*time	SS	4	SS	21	Tukey	<.0001
Chemical*time	SS	4	SS	28	Tukey	0.0001
Chemical*time	SS	7	SS	10	Tukey	0.9971
Chemical*time	SS	7	SS	14	Tukey	1.0000
Chemical*time	SS	7	SS	21	Tukey	1.0000

Chemical*time	SS	7	SS	28	Tukey	0.8987
Chemical*time	SS	10	SS	14	Tukey	0.9738
Chemical*time	SS	10	SS	21	Tukey	0.8881
Chemical*time	SS	10	SS	28	Tukey	0.0869
Chemical*time	SS	14	SS	21	Tukey	1.0000
Chemical*time	SS	14	SS	28	Tukey	0.9775
Chemical*time	SS	21	SS	28	Tukey	0.9977

I. Lactic acid bacteria on Cheddar cheese during storage at 4 °C for 35 days

(Initially inoculated *L. monocytogenes* 10^3 CFU/g)

Effect	Num DF	Den DF	F Value	Pr > F
Chemical	2	75	81.29	<.0001
time	4	75	69.92	<.0001
Chemical*time	8	75	13.45	<.0001

Effect	Chemical	time	Chemical	time	Adjustment	Adj P
Chemical	C		S		Tukey	0.0360
Chemical	C		SS		Tukey	<.0001
Chemical	S		SS		Tukey	<.0001
time		0		7	Tukey	<.0001
time		0		14	Tukey	<.0001
time		0		21	Tukey	0.7649
time		0		35	Tukey	0.9372
time		7		14	Tukey	0.6787
time		7		21	Tukey	<.0001
time		7		35	Tukey	<.0001
time		14		21	Tukey	<.0001
time		14		35	Tukey	<.0001
time		21		35	Tukey	0.3004
Chemical*time	C	0	C	7	Tukey	<.0001
Chemical*time	C	0	C	14	Tukey	<.0001
Chemical*time	C	0	C	21	Tukey	0.1535
Chemical*time	C	0	C	35	Tukey	0.0232
Chemical*time	C	0	S	0	Tukey	1.0000
Chemical*time	C	0	S	7	Tukey	<.0001
Chemical*time	C	0	S	14	Tukey	<.0001
Chemical*time	C	0	S	21	Tukey	0.3665
Chemical*time	C	0	S	35	Tukey	0.4186
Chemical*time	C	0	SS	0	Tukey	1.0000
Chemical*time	C	0	SS	7	Tukey	<.0001
Chemical*time	C	0	SS	14	Tukey	0.3007

Chemical*time	C	0	SS	21	Tukey	0.0155
Chemical*time	C	0	SS	35	Tukey	<.0001
Chemical*time	C	7	C	14	Tukey	0.8927
Chemical*time	C	7	C	21	Tukey	0.0055
Chemical*time	C	7	C	35	Tukey	0.0483
Chemical*time	C	7	S	0	Tukey	<.0001
Chemical*time	C	7	S	7	Tukey	1.0000
Chemical*time	C	7	S	14	Tukey	0.9043
Chemical*time	C	7	S	21	Tukey	0.0012
Chemical*time	C	7	S	35	Tukey	0.0009
Chemical*time	C	7	SS	0	Tukey	<.0001
Chemical*time	C	7	SS	7	Tukey	0.8621
Chemical*time	C	7	SS	14	Tukey	0.0018
Chemical*time	C	7	SS	21	Tukey	<.0001
Chemical*time	C	7	SS	35	Tukey	<.0001
Chemical*time	C	14	C	21	Tukey	<.0001
Chemical*time	C	14	C	35	Tukey	0.0001
Chemical*time	C	14	S	0	Tukey	<.0001
Chemical*time	C	14	S	7	Tukey	0.6923
Chemical*time	C	14	S	14	Tukey	0.0431
Chemical*time	C	14	S	21	Tukey	<.0001
Chemical*time	C	14	S	35	Tukey	<.0001
Chemical*time	C	14	SS	0	Tukey	<.0001
Chemical*time	C	14	SS	7	Tukey	0.0323
Chemical*time	C	14	SS	14	Tukey	<.0001
Chemical*time	C	14	SS	21	Tukey	<.0001
Chemical*time	C	14	SS	35	Tukey	<.0001
Chemical*time	C	21	C	35	Tukey	1.0000
Chemical*time	C	21	S	0	Tukey	0.1302
Chemical*time	C	21	S	7	Tukey	0.0176
Chemical*time	C	21	S	14	Tukey	0.4803
Chemical*time	C	21	S	21	Tukey	1.0000
Chemical*time	C	21	S	35	Tukey	1.0000
Chemical*time	C	21	SS	0	Tukey	0.1019
Chemical*time	C	21	SS	7	Tukey	0.5513
Chemical*time	C	21	SS	14	Tukey	1.0000
Chemical*time	C	21	SS	21	Tukey	<.0001
Chemical*time	C	21	SS	35	Tukey	<.0001
Chemical*time	C	35	S	0	Tukey	0.0187
Chemical*time	C	35	S	7	Tukey	0.1241
Chemical*time	C	35	S	14	Tukey	0.9081
Chemical*time	C	35	S	21	Tukey	0.9980
Chemical*time	C	35	S	35	Tukey	0.9959
Chemical*time	C	35	SS	0	Tukey	0.0137
Chemical*time	C	35	SS	7	Tukey	0.9400
Chemical*time	C	35	SS	14	Tukey	0.9993

Chemical*time	C	35	SS	21	Tukey	<.0001
Chemical*time	C	35	SS	35	Tukey	<.0001
Chemical*time	S	0	S	7	Tukey	<.0001
Chemical*time	S	0	S	14	Tukey	<.0001
Chemical*time	S	0	S	21	Tukey	0.3238
Chemical*time	S	0	S	35	Tukey	0.3729
Chemical*time	S	0	SS	0	Tukey	1.0000
Chemical*time	S	0	SS	7	Tukey	<.0001
Chemical*time	S	0	SS	14	Tukey	0.2627
Chemical*time	S	0	SS	21	Tukey	0.0193
Chemical*time	S	0	SS	35	Tukey	<.0001
Chemical*time	S	7	S	14	Tukey	0.9847
Chemical*time	S	7	S	21	Tukey	0.0043
Chemical*time	S	7	S	35	Tukey	0.0033
Chemical*time	S	7	SS	0	Tukey	<.0001
Chemical*time	S	7	SS	7	Tukey	0.9721
Chemical*time	S	7	SS	14	Tukey	0.0062
Chemical*time	S	7	SS	21	Tukey	<.0001
Chemical*time	S	7	SS	35	Tukey	<.0001
Chemical*time	S	14	S	21	Tukey	0.2232
Chemical*time	S	14	S	35	Tukey	0.1880
Chemical*time	S	14	SS	0	Tukey	<.0001
Chemical*time	S	14	SS	7	Tukey	1.0000
Chemical*time	S	14	SS	14	Tukey	0.2786
Chemical*time	S	14	SS	21	Tukey	<.0001
Chemical*time	S	14	SS	35	Tukey	<.0001
Chemical*time	S	21	S	35	Tukey	1.0000
Chemical*time	S	21	SS	0	Tukey	0.2679
Chemical*time	S	21	SS	7	Tukey	0.2732
Chemical*time	S	21	SS	14	Tukey	1.0000
Chemical*time	S	21	SS	21	Tukey	<.0001
Chemical*time	S	21	SS	35	Tukey	<.0001
Chemical*time	S	35	SS	0	Tukey	0.3121
Chemical*time	S	35	SS	7	Tukey	0.2327
Chemical*time	S	35	SS	14	Tukey	1.0000
Chemical*time	S	35	SS	21	Tukey	<.0001
Chemical*time	S	35	SS	35	Tukey	<.0001
Chemical*time	SS	0	SS	7	Tukey	<.0001
Chemical*time	SS	0	SS	14	Tukey	0.2140
Chemical*time	SS	0	SS	21	Tukey	0.0262
Chemical*time	SS	0	SS	35	Tukey	<.0001
Chemical*time	SS	7	SS	14	Tukey	0.3357
Chemical*time	SS	7	SS	21	Tukey	<.0001
Chemical*time	SS	7	SS	35	Tukey	<.0001
Chemical*time	SS	14	SS	21	Tukey	<.0001
Chemical*time	SS	14	SS	35	Tukey	<.0001

Chemical*time	SS	21	SS	35	Tukey	0.0113

J. Lactic acid bacteria on Cheddar cheese during storage at 4 °C for 35 days

(Initially inoculated *L. monocytogenes* 10^5 CFU/g)

Effect	Num DF	Den DF	F Value	Pr > F
Chemical	2	105	26.56	<.0001
time	6	105	470.37	<.0001
Chemical*time	12	105	12.38	<.0001

Effect	Chemical	time	Chemical	time	Adjustment	Adj P
Chemical	C		S		Tukey	<.0001
Chemical	C		SS		Tukey	0.0009
Chemical	S		SS		Tukey	0.0016
time		0		4	Tukey	0.0010
time		0		7	Tukey	<.0001
time		0		10	Tukey	<.0001
time		0		14	Tukey	<.0001
time		0		21	Tukey	<.0001
time		0		35	Tukey	<.0001
time		4		7	Tukey	<.0001
time		4		10	Tukey	<.0001
time		4		14	Tukey	<.0001
time		4		21	Tukey	<.0001
time		4		35	Tukey	<.0001
time		7		10	Tukey	<.0001
time		7		14	Tukey	<.0001
time		7		21	Tukey	<.0001
time		7		35	Tukey	<.0001
time		10		14	Tukey	0.9987
time		10		21	Tukey	0.2492
time		10		35	Tukey	<.0001
time		14		21	Tukey	0.0821
time		14		35	Tukey	<.0001
time		21		35	Tukey	<.0001
Chemical*time	C	0	C	4	Tukey	0.4697
Chemical*time	C	0	C	7	Tukey	<.0001
Chemical*time	C	0	C	10	Tukey	<.0001
Chemical*time	C	0	C	14	Tukey	<.0001
Chemical*time	C	0	C	21	Tukey	<.0001
Chemical*time	C	0	C	35	Tukey	<.0001
Chemical*time	C	0	S	0	Tukey	0.3633

Chemical*time	C	0	S	4	Tukey	1.0000
Chemical*time	C	0	S	7	Tukey	<.0001
Chemical*time	C	0	S	10	Tukey	<.0001
Chemical*time	C	0	S	14	Tukey	<.0001
Chemical*time	C	0	S	21	Tukey	<.0001
Chemical*time	C	0	S	35	Tukey	<.0001
Chemical*time	C	0	SS	0	Tukey	1.0000
Chemical*time	C	0	SS	4	Tukey	1.0000
Chemical*time	C	0	SS	7	Tukey	<.0001
Chemical*time	C	0	SS	10	Tukey	<.0001
Chemical*time	C	0	SS	14	Tukey	<.0001
Chemical*time	C	0	SS	21	Tukey	<.0001
Chemical*time	C	0	SS	35	Tukey	<.0001
Chemical*time	C	4	C	7	Tukey	0.0083
Chemical*time	C	4	C	10	Tukey	<.0001
Chemical*time	C	4	C	14	Tukey	<.0001
Chemical*time	C	4	C	21	Tukey	<.0001
Chemical*time	C	4	C	35	Tukey	<.0001
Chemical*time	C	4	S	0	Tukey	<.0001
Chemical*time	C	4	S	4	Tukey	0.8556
Chemical*time	C	4	S	7	Tukey	<.0001
Chemical*time	C	4	S	10	Tukey	<.0001
Chemical*time	C	4	S	14	Tukey	<.0001
Chemical*time	C	4	S	21	Tukey	<.0001
Chemical*time	C	4	S	35	Tukey	<.0001
Chemical*time	C	4	SS	0	Tukey	0.1015
Chemical*time	C	4	SS	4	Tukey	0.8084
Chemical*time	C	4	SS	7	Tukey	<.0001
Chemical*time	C	4	SS	10	Tukey	<.0001
Chemical*time	C	4	SS	14	Tukey	<.0001
Chemical*time	C	4	SS	21	Tukey	<.0001
Chemical*time	C	4	SS	35	Tukey	<.0001
Chemical*time	C	7	C	10	Tukey	<.0001
Chemical*time	C	7	C	14	Tukey	<.0001
Chemical*time	C	7	C	21	Tukey	<.0001
Chemical*time	C	7	C	35	Tukey	<.0001
Chemical*time	C	7	S	0	Tukey	<.0001
Chemical*time	C	7	S	4	Tukey	<.0001
Chemical*time	C	7	S	7	Tukey	0.1075
Chemical*time	C	7	S	10	Tukey	<.0001
Chemical*time	C	7	S	14	Tukey	0.9958
Chemical*time	C	7	S	21	Tukey	0.9867
Chemical*time	C	7	S	35	Tukey	<.0001
Chemical*time	C	7	SS	0	Tukey	<.0001
Chemical*time	C	7	SS	4	Tukey	<.0001
Chemical*time	C	7	SS	7	Tukey	0.7253

Chemical*time	C	7	SS	10	Tukey	<.0001
Chemical*time	C	7	SS	14	Tukey	<.0001
Chemical*time	C	7	SS	21	Tukey	<.0001
Chemical*time	C	7	SS	35	Tukey	<.0001
Chemical*time	C	10	C	14	Tukey	0.6158
Chemical*time	C	10	C	21	Tukey	1.0000
Chemical*time	C	10	C	35	Tukey	<.0001
Chemical*time	C	10	S	0	Tukey	<.0001
Chemical*time	C	10	S	4	Tukey	<.0001
Chemical*time	C	10	S	7	Tukey	0.0427
Chemical*time	C	10	S	10	Tukey	1.0000
Chemical*time	C	10	S	14	Tukey	<.0001
Chemical*time	C	10	S	21	Tukey	<.0001
Chemical*time	C	10	S	35	Tukey	<.0001
Chemical*time	C	10	SS	0	Tukey	<.0001
Chemical*time	C	10	SS	4	Tukey	<.0001
Chemical*time	C	10	SS	7	Tukey	0.0011
Chemical*time	C	10	SS	10	Tukey	0.9916
Chemical*time	C	10	SS	14	Tukey	0.5343
Chemical*time	C	10	SS	21	Tukey	1.0000
Chemical*time	C	10	SS	35	Tukey	<.0001
Chemical*time	C	14	C	21	Tukey	0.9822
Chemical*time	C	14	C	35	Tukey	<.0001
Chemical*time	C	14	S	0	Tukey	<.0001
Chemical*time	C	14	S	4	Tukey	<.0001
Chemical*time	C	14	S	7	Tukey	<.0001
Chemical*time	C	14	S	10	Tukey	0.7400
Chemical*time	C	14	S	14	Tukey	<.0001
Chemical*time	C	14	S	21	Tukey	<.0001
Chemical*time	C	14	S	35	Tukey	<.0001
Chemical*time	C	14	SS	0	Tukey	<.0001
Chemical*time	C	14	SS	4	Tukey	<.0001
Chemical*time	C	14	SS	7	Tukey	<.0001
Chemical*time	C	14	SS	10	Tukey	0.0140
Chemical*time	C	14	SS	14	Tukey	1.0000
Chemical*time	C	14	SS	21	Tukey	0.1579
Chemical*time	C	14	SS	35	Tukey	<.0001
Chemical*time	C	21	C	35	Tukey	<.0001
Chemical*time	C	21	S	0	Tukey	<.0001
Chemical*time	C	21	S	4	Tukey	<.0001
Chemical*time	C	21	S	7	Tukey	0.0027
Chemical*time	C	21	S	10	Tukey	1.0000
Chemical*time	C	21	S	14	Tukey	<.0001
Chemical*time	C	21	S	21	Tukey	<.0001
Chemical*time	C	21	S	35	Tukey	<.0001
Chemical*time	C	21	SS	0	Tukey	<.0001

Chemical*time	C	21	SS	4	Tukey	<.0001
Chemical*time	C	21	SS	7	Tukey	<.0001
Chemical*time	C	21	SS	10	Tukey	0.6951
Chemical*time	C	21	SS	14	Tukey	0.9660
Chemical*time	C	21	SS	21	Tukey	0.9929
Chemical*time	C	21	SS	35	Tukey	<.0001
Chemical*time	C	35	S	0	Tukey	0.1939
Chemical*time	C	35	S	4	Tukey	<.0001
Chemical*time	C	35	S	7	Tukey	<.0001
Chemical*time	C	35	S	10	Tukey	<.0001
Chemical*time	C	35	S	14	Tukey	<.0001
Chemical*time	C	35	S	21	Tukey	<.0001
Chemical*time	C	35	S	35	Tukey	0.0801
Chemical*time	C	35	SS	0	Tukey	0.0001
Chemical*time	C	35	SS	4	Tukey	<.0001
Chemical*time	C	35	SS	7	Tukey	<.0001
Chemical*time	C	35	SS	10	Tukey	<.0001
Chemical*time	C	35	SS	14	Tukey	<.0001
Chemical*time	C	35	SS	21	Tukey	<.0001
Chemical*time	C	35	SS	35	Tukey	<.0001
Chemical*time	S	0	S	4	Tukey	0.1015
Chemical*time	S	0	S	7	Tukey	<.0001
Chemical*time	S	0	S	10	Tukey	<.0001
Chemical*time	S	0	S	14	Tukey	<.0001
Chemical*time	S	0	S	21	Tukey	<.0001
Chemical*time	S	0	S	35	Tukey	<.0001
Chemical*time	S	0	SS	0	Tukey	0.8556
Chemical*time	S	0	SS	4	Tukey	0.1272
Chemical*time	S	0	SS	7	Tukey	<.0001
Chemical*time	S	0	SS	10	Tukey	<.0001
Chemical*time	S	0	SS	14	Tukey	<.0001
Chemical*time	S	0	SS	21	Tukey	<.0001
Chemical*time	S	0	SS	35	Tukey	<.0001
Chemical*time	S	4	S	7	Tukey	<.0001
Chemical*time	S	4	S	10	Tukey	<.0001
Chemical*time	S	4	S	14	Tukey	<.0001
Chemical*time	S	4	S	21	Tukey	<.0001
Chemical*time	S	4	S	35	Tukey	<.0001
Chemical*time	S	4	SS	0	Tukey	0.9993
Chemical*time	S	4	SS	4	Tukey	1.0000
Chemical*time	S	4	SS	7	Tukey	<.0001
Chemical*time	S	4	SS	10	Tukey	<.0001
Chemical*time	S	4	SS	14	Tukey	<.0001
Chemical*time	S	4	SS	21	Tukey	<.0001
Chemical*time	S	4	SS	35	Tukey	<.0001
Chemical*time	S	7	S	10	Tukey	0.0248

Chemical*time	S	7	S	14	Tukey	0.9406
Chemical*time	S	7	S	21	Tukey	0.9735
Chemical*time	S	7	S	35	Tukey	<.0001
Chemical*time	S	7	SS	0	Tukey	<.0001
Chemical*time	S	7	SS	4	Tukey	<.0001
Chemical*time	S	7	SS	7	Tukey	1.0000
Chemical*time	S	7	SS	10	Tukey	0.8445
Chemical*time	S	7	SS	14	Tukey	<.0001
Chemical*time	S	7	SS	21	Tukey	0.2826
Chemical*time	S	7	SS	35	Tukey	<.0001
Chemical*time	S	10	S	14	Tukey	<.0001
Chemical*time	S	10	S	21	Tukey	<.0001
Chemical*time	S	10	S	35	Tukey	<.0001
Chemical*time	S	10	SS	0	Tukey	<.0001
Chemical*time	S	10	SS	4	Tukey	<.0001
Chemical*time	S	10	SS	7	Tukey	0.0005
Chemical*time	S	10	SS	10	Tukey	0.9735
Chemical*time	S	10	SS	14	Tukey	0.6639
Chemical*time	S	10	SS	21	Tukey	1.0000
Chemical*time	S	10	SS	35	Tukey	<.0001
Chemical*time	S	14	S	21	Tukey	1.0000
Chemical*time	S	14	S	35	Tukey	<.0001
Chemical*time	S	14	SS	0	Tukey	<.0001
Chemical*time	S	14	SS	4	Tukey	<.0001
Chemical*time	S	14	SS	7	Tukey	1.0000
Chemical*time	S	14	SS	10	Tukey	0.0150
Chemical*time	S	14	SS	14	Tukey	<.0001
Chemical*time	S	14	SS	21	Tukey	0.0007
Chemical*time	S	14	SS	35	Tukey	<.0001
Chemical*time	S	21	S	35	Tukey	<.0001
Chemical*time	S	21	SS	0	Tukey	<.0001
Chemical*time	S	21	SS	4	Tukey	<.0001
Chemical*time	S	21	SS	7	Tukey	1.0000
Chemical*time	S	21	SS	10	Tukey	0.0248
Chemical*time	S	21	SS	14	Tukey	<.0001
Chemical*time	S	21	SS	21	Tukey	0.0013
Chemical*time	S	21	SS	35	Tukey	<.0001
Chemical*time	S	35	SS	0	Tukey	<.0001
Chemical*time	S	35	SS	4	Tukey	<.0001
Chemical*time	S	35	SS	7	Tukey	<.0001
Chemical*time	S	35	SS	10	Tukey	<.0001
Chemical*time	S	35	SS	14	Tukey	<.0001
Chemical*time	S	35	SS	21	Tukey	<.0001
Chemical*time	S	35	SS	35	Tukey	0.4228
Chemical*time	SS	0	SS	4	Tukey	0.9998
Chemical*time	SS	0	SS	7	Tukey	<.0001

Chemical*time	SS	0	SS	10	Tukey	<.0001
Chemical*time	SS	0	SS	14	Tukey	<.0001
Chemical*time	SS	0	SS	21	Tukey	<.0001
Chemical*time	SS	0	SS	35	Tukey	<.0001
Chemical*time	SS	4	SS	7	Tukey	<.0001
Chemical*time	SS	4	SS	10	Tukey	<.0001
Chemical*time	SS	4	SS	14	Tukey	<.0001
Chemical*time	SS	4	SS	21	Tukey	<.0001
Chemical*time	SS	4	SS	35	Tukey	<.0001
Chemical*time	SS	7	SS	10	Tukey	0.1752
Chemical*time	SS	7	SS	14	Tukey	<.0001
Chemical*time	SS	7	SS	21	Tukey	0.0162
Chemical*time	SS	7	SS	35	Tukey	<.0001
Chemical*time	SS	10	SS	14	Tukey	0.0096
Chemical*time	SS	10	SS	21	Tukey	1.0000
Chemical*time	SS	10	SS	35	Tukey	<.0001
Chemical*time	SS	14	SS	21	Tukey	0.1204
Chemical*time	SS	14	SS	35	Tukey	<.0001
Chemical*time	SS	21	SS	35	Tukey	<.0001

K. Lactic acid bacteria on beef bologna during storage at 4 °C for 28 days

(Initially inoculated *L. monocytogenes* 10^3 CFU/g)

Effect	Num DF	Den DF	F Value	Pr > F
Chemical	2	42	1000.97	<.0001
time	6	42	465.06	<.0001
Chemical*time	12	42	98.51	<.0001

Effect	Chemical	time	Chemical	time	Adjustment	Adj P
Chemical	C		S		Tukey	<.0001
Chemical	C		SS		Tukey	<.0001
Chemical	S		SS		Tukey	0.0585
time		0		4	Tukey	1.0000
time		0		7	Tukey	<.0001
time		0		10	Tukey	<.0001
time		0		14	Tukey	<.0001
time		0		21	Tukey	<.0001
time		0		28	Tukey	<.0001
time		4		7	Tukey	<.0001
time		4		10	Tukey	<.0001
time		4		14	Tukey	<.0001
time		4		21	Tukey	<.0001

time		4		28	Tukey	<.0001
time		7		10	Tukey	<.0001
time		7		14	Tukey	<.0001
time		7		21	Tukey	<.0001
time		7		28	Tukey	<.0001
time		10		14	Tukey	0.0040
time		10		21	Tukey	<.0001
time		10		28	Tukey	<.0001
time		14		21	Tukey	0.0397
time		14		28	Tukey	<.0001
time		21		28	Tukey	0.0003
Chemical*time	C	0	C	4	Tukey	1.0000
Chemical*time	C	0	C	7	Tukey	<.0001
Chemical*time	C	0	C	10	Tukey	<.0001
Chemical*time	C	0	C	14	Tukey	<.0001
Chemical*time	C	0	C	21	Tukey	<.0001
Chemical*time	C	0	C	28	Tukey	<.0001
Chemical*time	C	0	S	0	Tukey	1.0000
Chemical*time	C	0	S	4	Tukey	1.0000
Chemical*time	C	0	S	7	Tukey	1.0000
Chemical*time	C	0	S	10	Tukey	<.0001
Chemical*time	C	0	S	14	Tukey	<.0001
Chemical*time	C	0	S	21	Tukey	<.0001
Chemical*time	C	0	S	28	Tukey	<.0001
Chemical*time	C	0	SS	0	Tukey	1.0000
Chemical*time	C	0	SS	4	Tukey	1.0000
Chemical*time	C	0	SS	7	Tukey	1.0000
Chemical*time	C	0	SS	10	Tukey	<.0001
Chemical*time	C	0	SS	14	Tukey	<.0001
Chemical*time	C	0	SS	21	Tukey	<.0001
Chemical*time	C	0	SS	28	Tukey	<.0001
Chemical*time	C	4	C	7	Tukey	<.0001
Chemical*time	C	4	C	10	Tukey	<.0001
Chemical*time	C	4	C	14	Tukey	<.0001
Chemical*time	C	4	C	21	Tukey	<.0001
Chemical*time	C	4	C	28	Tukey	<.0001
Chemical*time	C	4	S	0	Tukey	1.0000
Chemical*time	C	4	S	4	Tukey	1.0000
Chemical*time	C	4	S	7	Tukey	1.0000
Chemical*time	C	4	S	10	Tukey	<.0001
Chemical*time	C	4	S	14	Tukey	<.0001
Chemical*time	C	4	S	21	Tukey	<.0001
Chemical*time	C	4	S	28	Tukey	<.0001
Chemical*time	C	4	SS	0	Tukey	1.0000
Chemical*time	C	4	SS	4	Tukey	1.0000
Chemical*time	C	4	SS	7	Tukey	1.0000

Chemical*time	C	4	SS	10	Tukey	<.0001
Chemical*time	C	4	SS	14	Tukey	<.0001
Chemical*time	C	4	SS	21	Tukey	<.0001
Chemical*time	C	4	SS	28	Tukey	<.0001
Chemical*time	C	7	C	10	Tukey	0.6518
Chemical*time	C	7	C	14	Tukey	<.0001
Chemical*time	C	7	C	21	Tukey	<.0001
Chemical*time	C	7	C	28	Tukey	<.0001
Chemical*time	C	7	S	0	Tukey	<.0001
Chemical*time	C	7	S	4	Tukey	<.0001
Chemical*time	C	7	S	7	Tukey	<.0001
Chemical*time	C	7	S	10	Tukey	<.0001
Chemical*time	C	7	S	14	Tukey	<.0001
Chemical*time	C	7	S	21	Tukey	<.0001
Chemical*time	C	7	S	28	Tukey	<.0001
Chemical*time	C	7	SS	0	Tukey	<.0001
Chemical*time	C	7	SS	4	Tukey	<.0001
Chemical*time	C	7	SS	7	Tukey	<.0001
Chemical*time	C	7	SS	10	Tukey	<.0001
Chemical*time	C	7	SS	14	Tukey	<.0001
Chemical*time	C	7	SS	21	Tukey	<.0001
Chemical*time	C	7	SS	28	Tukey	<.0001
Chemical*time	C	10	C	14	Tukey	<.0001
Chemical*time	C	10	C	21	Tukey	<.0001
Chemical*time	C	10	C	28	Tukey	<.0001
Chemical*time	C	10	S	0	Tukey	<.0001
Chemical*time	C	10	S	4	Tukey	<.0001
Chemical*time	C	10	S	7	Tukey	<.0001
Chemical*time	C	10	S	10	Tukey	<.0001
Chemical*time	C	10	S	14	Tukey	<.0001
Chemical*time	C	10	S	21	Tukey	<.0001
Chemical*time	C	10	S	28	Tukey	<.0001
Chemical*time	C	10	SS	0	Tukey	<.0001
Chemical*time	C	10	SS	4	Tukey	<.0001
Chemical*time	C	10	SS	7	Tukey	<.0001
Chemical*time	C	10	SS	10	Tukey	<.0001
Chemical*time	C	10	SS	14	Tukey	<.0001
Chemical*time	C	10	SS	21	Tukey	<.0001
Chemical*time	C	10	SS	28	Tukey	<.0001
Chemical*time	C	14	C	21	Tukey	<.0001
Chemical*time	C	14	C	28	Tukey	<.0001
Chemical*time	C	14	S	0	Tukey	<.0001
Chemical*time	C	14	S	4	Tukey	<.0001
Chemical*time	C	14	S	7	Tukey	<.0001
Chemical*time	C	14	S	10	Tukey	<.0001
Chemical*time	C	14	S	14	Tukey	<.0001

Chemical*time	C	14	S	21	Tukey	<.0001
Chemical*time	C	14	S	28	Tukey	<.0001
Chemical*time	C	14	SS	0	Tukey	<.0001
Chemical*time	C	14	SS	4	Tukey	<.0001
Chemical*time	C	14	SS	7	Tukey	<.0001
Chemical*time	C	14	SS	10	Tukey	<.0001
Chemical*time	C	14	SS	14	Tukey	<.0001
Chemical*time	C	14	SS	21	Tukey	<.0001
Chemical*time	C	14	SS	28	Tukey	<.0001
Chemical*time	C	21	C	28	Tukey	0.0465
Chemical*time	C	21	S	0	Tukey	<.0001
Chemical*time	C	21	S	4	Tukey	<.0001
Chemical*time	C	21	S	7	Tukey	<.0001
Chemical*time	C	21	S	10	Tukey	<.0001
Chemical*time	C	21	S	14	Tukey	<.0001
Chemical*time	C	21	S	21	Tukey	<.0001
Chemical*time	C	21	S	28	Tukey	<.0001
Chemical*time	C	21	SS	0	Tukey	<.0001
Chemical*time	C	21	SS	4	Tukey	<.0001
Chemical*time	C	21	SS	7	Tukey	<.0001
Chemical*time	C	21	SS	10	Tukey	<.0001
Chemical*time	C	21	SS	14	Tukey	<.0001
Chemical*time	C	21	SS	21	Tukey	<.0001
Chemical*time	C	21	SS	28	Tukey	<.0001
Chemical*time	C	28	S	0	Tukey	<.0001
Chemical*time	C	28	S	4	Tukey	<.0001
Chemical*time	C	28	S	7	Tukey	<.0001
Chemical*time	C	28	S	10	Tukey	<.0001
Chemical*time	C	28	S	14	Tukey	<.0001
Chemical*time	C	28	S	21	Tukey	<.0001
Chemical*time	C	28	S	28	Tukey	<.0001
Chemical*time	C	28	SS	0	Tukey	<.0001
Chemical*time	C	28	SS	4	Tukey	<.0001
Chemical*time	C	28	SS	7	Tukey	<.0001
Chemical*time	C	28	SS	10	Tukey	<.0001
Chemical*time	C	28	SS	14	Tukey	<.0001
Chemical*time	C	28	SS	21	Tukey	<.0001
Chemical*time	C	28	SS	28	Tukey	<.0001
Chemical*time	S	0	S	4	Tukey	1.0000
Chemical*time	S	0	S	7	Tukey	1.0000
Chemical*time	S	0	S	10	Tukey	<.0001
Chemical*time	S	0	S	14	Tukey	<.0001
Chemical*time	S	0	S	21	Tukey	<.0001
Chemical*time	S	0	S	28	Tukey	<.0001
Chemical*time	S	0	SS	0	Tukey	1.0000
Chemical*time	S	0	SS	4	Tukey	1.0000

Chemical*time	S	0	SS	7	Tukey	1.0000
Chemical*time	S	0	SS	10	Tukey	<.0001
Chemical*time	S	0	SS	14	Tukey	<.0001
Chemical*time	S	0	SS	21	Tukey	<.0001
Chemical*time	S	0	SS	28	Tukey	<.0001
Chemical*time	S	4	S	7	Tukey	1.0000
Chemical*time	S	4	S	10	Tukey	<.0001
Chemical*time	S	4	S	14	Tukey	<.0001
Chemical*time	S	4	S	21	Tukey	<.0001
Chemical*time	S	4	S	28	Tukey	<.0001
Chemical*time	S	4	SS	0	Tukey	1.0000
Chemical*time	S	4	SS	4	Tukey	1.0000
Chemical*time	S	4	SS	7	Tukey	1.0000
Chemical*time	S	4	SS	10	Tukey	<.0001
Chemical*time	S	4	SS	14	Tukey	<.0001
Chemical*time	S	4	SS	21	Tukey	<.0001
Chemical*time	S	4	SS	28	Tukey	<.0001
Chemical*time	S	7	S	10	Tukey	<.0001
Chemical*time	S	7	S	14	Tukey	<.0001
Chemical*time	S	7	S	21	Tukey	<.0001
Chemical*time	S	7	S	28	Tukey	<.0001
Chemical*time	S	7	SS	0	Tukey	1.0000
Chemical*time	S	7	SS	4	Tukey	1.0000
Chemical*time	S	7	SS	7	Tukey	1.0000
Chemical*time	S	7	SS	10	Tukey	<.0001
Chemical*time	S	7	SS	14	Tukey	<.0001
Chemical*time	S	7	SS	21	Tukey	<.0001
Chemical*time	S	7	SS	28	Tukey	<.0001
Chemical*time	S	10	S	14	Tukey	0.9999
Chemical*time	S	10	S	21	Tukey	0.9992
Chemical*time	S	10	S	28	Tukey	1.0000
Chemical*time	S	10	SS	0	Tukey	<.0001
Chemical*time	S	10	SS	4	Tukey	<.0001
Chemical*time	S	10	SS	7	Tukey	<.0001
Chemical*time	S	10	SS	10	Tukey	0.8771
Chemical*time	S	10	SS	14	Tukey	1.0000
Chemical*time	S	10	SS	21	Tukey	0.0061
Chemical*time	S	10	SS	28	Tukey	0.9964
Chemical*time	S	14	S	21	Tukey	1.0000
Chemical*time	S	14	S	28	Tukey	0.9992
Chemical*time	S	14	SS	0	Tukey	<.0001
Chemical*time	S	14	SS	4	Tukey	<.0001
Chemical*time	S	14	SS	7	Tukey	<.0001
Chemical*time	S	14	SS	10	Tukey	1.0000
Chemical*time	S	14	SS	14	Tukey	1.0000
Chemical*time	S	14	SS	21	Tukey	0.1074

Chemical*time	S	14	SS	28	Tukey	1.0000
Chemical*time	S	21	S	28	Tukey	0.9953
Chemical*time	S	21	SS	0	Tukey	<.0001
Chemical*time	S	21	SS	4	Tukey	<.0001
Chemical*time	S	21	SS	7	Tukey	<.0001
Chemical*time	S	21	SS	10	Tukey	1.0000
Chemical*time	S	21	SS	14	Tukey	1.0000
Chemical*time	S	21	SS	21	Tukey	0.1625
Chemical*time	S	21	SS	28	Tukey	1.0000
Chemical*time	S	28	SS	0	Tukey	<.0001
Chemical*time	S	28	SS	4	Tukey	<.0001
Chemical*time	S	28	SS	7	Tukey	<.0001
Chemical*time	S	28	SS	10	Tukey	0.7813
Chemical*time	S	28	SS	14	Tukey	1.0000
Chemical*time	S	28	SS	21	Tukey	0.0035
Chemical*time	S	28	SS	28	Tukey	0.9854
Chemical*time	SS	0	SS	4	Tukey	1.0000
Chemical*time	SS	0	SS	7	Tukey	1.0000
Chemical*time	SS	0	SS	10	Tukey	<.0001
Chemical*time	SS	0	SS	14	Tukey	<.0001
Chemical*time	SS	0	SS	21	Tukey	<.0001
Chemical*time	SS	0	SS	28	Tukey	<.0001
Chemical*time	SS	4	SS	7	Tukey	1.0000
Chemical*time	SS	4	SS	10	Tukey	<.0001
Chemical*time	SS	4	SS	14	Tukey	<.0001
Chemical*time	SS	4	SS	21	Tukey	<.0001
Chemical*time	SS	4	SS	28	Tukey	<.0001
Chemical*time	SS	7	SS	10	Tukey	<.0001
Chemical*time	SS	7	SS	14	Tukey	<.0001
Chemical*time	SS	7	SS	21	Tukey	<.0001
Chemical*time	SS	7	SS	28	Tukey	<.0001
Chemical*time	SS	10	SS	14	Tukey	0.9764
Chemical*time	SS	10	SS	21	Tukey	0.5983
Chemical*time	SS	10	SS	28	Tukey	1.0000
Chemical*time	SS	14	SS	21	Tukey	0.0171
Chemical*time	SS	14	SS	28	Tukey	0.9999
Chemical*time	SS	21	SS	28	Tukey	0.2235

L. Lactic acid bacteria on beef bologna during storage at 4 °C for 28 days

(Initially inoculated *L. monocytogenes* 10^5 CFU/g)

Effect	Num DF	Den DF	F Value	Pr > F
Chemical	2	105	419.31	<.0001

time	6	105	705.04	<.0001
Chemical*time	12	105	34.34	<.0001

Effect	Chemical	time	Chemical	time	Adjustment	Adj P
Chemical	C		S		Tukey	<.0001
Chemical	C		SS		Tukey	<.0001
Chemical	S		SS		Tukey	0.0074
time		0		4	Tukey	<.0001
time		0		7	Tukey	<.0001
time		0		10	Tukey	<.0001
time		0		14	Tukey	<.0001
time		0		21	Tukey	<.0001
time		0		28	Tukey	<.0001
time		4		7	Tukey	<.0001
time		4		10	Tukey	<.0001
time		4		14	Tukey	<.0001
time		4		21	Tukey	<.0001
time		4		28	Tukey	<.0001
time		7		10	Tukey	0.5696
time		7		14	Tukey	0.4601
time		7		21	Tukey	0.6629
time		7		28	Tukey	<.0001
time		10		14	Tukey	1.0000
time		10		21	Tukey	1.0000
time		10		28	Tukey	0.0185
time		14		21	Tukey	0.9999
time		14		28	Tukey	0.0302
time		21		28	Tukey	0.0120
Chemical*time	C	0	C	4	Tukey	<.0001
Chemical*time	C	0	C	7	Tukey	<.0001
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APPENDIX II

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