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EVALUATION OF THE EFFECT OF CHLORINE
DIOXIDE AND ALLYL-ISOTHIOCYANATE ON THE
GROWTH OF *SALMONELLA* TYPHIMURIUM AND
LISTERIA MONOCYTOGENES ON FRESH CHICKEN
BREAST AND EFFECT OF CHLORINE DIOXIDE
EXPOSURE ON THE PHYSICAL PROPERTIES OF
PLASTIC FILMS

presented by

Joongmin Shin

has been accepted towards fulfillment
of the requirements for the

Ph.D degree in school of packaging

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By

Joongmin Shin

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ABSTRACT

EVALUATION OF THE EFFECT OF CHLORINE DIOXIDE AND ALLYL-ISOTHIOCYANATE ON THE GROWTH OF *SALMONELLA* TYPHIMURIUM AND *LISTERIA MONOCYTOGENES* ON FRESH CHICKEN BREAST AND EFFECT OF CHLORINE DIOXIDE EXPOSURE ON THE PHYSICAL PROPERTIES OF PLASTIC FILMS

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Listeria monocytogenes and *Salmonella Typhimurium* are major pathogenic bacteria associated with poultry products. The consumption of pathogen contaminated food can cause illness or even death. Controlled, constant release of antimicrobial agents (antimicrobial packaging) on the surface of poultry products has potential to inhibit the growth of pathogens and thus enhance food safety.

In this study, two gas type antimicrobial agents, chlorine dioxide (ClO_2) and allyl-isothiocyanate (AITC), were used with conventional modified atmosphere packaging (MAP) to determine their effectiveness against *Listeria monocytogenes* and *Salmonella Typhimurium* on fresh chicken breast at 4°C. Several different constant release rate ClO_2 sachets and AITC canisters were developed and placed into conventional MA packaging. Chicken breast was also inoculated with the pathogenic bacteria, and gas flushed with 30% CO_2 /70% N_2 . The effects of ClO_2 and AITC on microbial growth, headspace gas composition, color, and pH changes were evaluated periodically over 21 days.

Overall test results indicate that controlled, continuous release of ClO_2 or AITC with MA packaging retarded the growth of *Listeria monocytogenes* and *Salmonella Typhimurium*, and thus would enhance the safety of fresh chicken during storage. Some discoloration (L, a, b) was observed on the chicken breast at high ClO_2 or AITC release rate.

The effect of chlorine dioxide (ClO_2) gas exposure on physical properties of selected plastic films was investigated. LDPE, PP, and PS were treated with various concentrations of ClO_2 gas over selected treatment times. The control

and treated films were evaluated for their mechanical, barrier, and optical properties. In the concentration range of 250 to 2000 ppm, the ClO₂ treatment was shown to significantly affect the mechanical properties, oxygen transmission rate, and appearance of PS. However, LDPE and PP did not show any significant physical property changes associated with the treatment.

This dissertation is dedicated to my dearest family, Suwan Lee and Yewon Shin

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INTRODUCTION

Microbial contamination of food products is a major problem that can reduce the shelf life of food. Microbial contamination of food usually occurs in post processing during packaging and distribution. Natural or chemical preservatives have been developed and can be added directly to food to extend its shelf life. However, food additives are becoming less attractive and consumers are demanding minimally processed and preservative free products (Collins-Thompson and Hwang, 2000). Moreover, substances that are directly added to the food may be neutralized on contact or may rapidly diffuse so that their effectiveness is limited. Also, the addition of additives can cause an undesirable taste in the food at high concentrations (Ahvenainen, 2003).

Antimicrobial packaging is an active packaging concept to control microbial growth and enhance food safety. In this concept, the preservative agent is released from the packaging instead of adding it directly to the product. The application can be more effective than direct addition of the preservative, because the packaging system can maintain the required minimum inhibition level through constant release with lesser amounts of the active agents.

Chlorine dioxide (ClO_2) and allyl isothiocyanate (AITC) are rapidly gaining attention as antimicrobial agents because of their excellent microbial control performance even at very low treatment levels (Brody, 2005).

Chlorine dioxide (ClO_2) is a highly oxidizing greenish yellow gas. It reacts with the proteins of cell membranes in microorganisms, and destroys them through cell wall oxidation. Microorganisms are unable to mutate to resistant

forms due to the oxidation and destruction of the proteins within the cell (Du et al., 2002). ClO₂ can be used to kill large populations of fungi, bacteria, viruses, and algae at low concentrations (Clordisys, 2003), and is widely used in multiple industries such as water purification, vegetable/fruit processing plants, and a variety of other facilities. Its application as a food antimicrobial agent has not been studied a lot but several studies have shown its potential. Du et al. (2002) tested the bactericidal effects of chlorine dioxide against *Listeria monocytogenes* on apple surfaces. At a dosage level of 4.0 mg/l, *Listeria monocytogenes* was decreased by 3.9~6.6 log cfu. Han (2000) achieved 3 log and 6.45 log reduction of *Listeria monocytogenes* on green peppers with 0.62 mg/liter of ClO₂ and 1.25 mg/l of ClO₂, respectively. Gas type ClO₂ treatment has proved more effective than aqueous ClO₂ treatment. Han et al. (2001) found that ClO₂ gas treatment (0.3 and 3.0 mg/liter) was more effective than aqueous ClO₂ on green peppers inoculated with *Listeria monocytogenes*. Singh et al. (2002) found that ClO₂ gas was more effective than washing with aqueous ClO₂ for lettuce and baby carrots inoculated with E. coli O157.

Allyl isothiocyanate (AITC) is a pungent volatile component in all plants belonging to the family Cruciferae, such as Wasabi, black mustard, etc. For a long time, it has been used as a food flavor fortifier, and its powerful antimicrobial effect has been shown in many studies. Isshiki et al. (1992) tested fresh beef and sliced raw tuna using AITC as an antimicrobial, and showed a 5 log reduction in mold (*Aspergillus niger* and so on) . Farber (1991) applied AITC to sandwiches and pizza, alone and in combination with MAP packaging. The shelf

life of the foods was increased by 30 days and 17 days, respectively. Kim et al. (2002) found that AITC extract in combination with acetic acid completely inhibited aerobic microbial growth in cooked rice.

Gas phase AITC requires less dosage than an aqueous solution to obtain the same result. Sekiyama et al. (1996) showed that AITC vapor application required 1/500-1/1000 of the minimum inhibitory concentration when the liquid was mixed with agar. Isshiki et al. (1992) suppressed the growth of bacteria and yeast with a very small amount of AITC gas (16 to 110 ng/ml).

Fresh poultry cuts like boneless, skinless breast have become very popular and represent one of the fastest growing markets because of their moderate cost, healthy nutrients and convenience (Greene, 1998). Consumption of poultry meat in the US has an annual growth rate of 7% and the market segment has reached \$17.5 billion. Like other fresh or minimally processed food products, microbial contamination is one of the biggest problems of the poultry industry, in terms of shelf life and food safety. The conventional chill pack of chicken has an approximate shelf life of 4-5 days (Sams, 2001). Microbial contamination of the product may occur in post processing during handling, packaging and distribution. *Salmonella spp.* and *Listeria monocytogenes* are common pathogenic organisms associated with raw meat and poultry products (Mead, 2004).

Salmonella Typhimurium is one species of salmonella isolated from chicken which causes gastrointestinal illness, including nausea, vomiting, abdominal cramps, diarrhea, and headaches (FDA, 1992). *Salmonella*

Typhimurium has the highest rate of hospitalization for any of the *Salmonella* species (Fisker et al., 2003).

Listeria monocytogenes is a major threat to the food industry as a post-processing contaminant. It is able to multiply on chill-stored poultry meat (Hart et al., 1991; Bajard et al., 1996), and is well adapted to food and food processing environments where the growth of other pathogens may be restricted. The infection commonly results in fever, muscle aches, and sometimes gastrointestinal symptoms such as nausea or diarrhea. Infections during pregnancy can cause serious problems such as miscarriage, premature delivery, or infection of the newborn (CDC, 2005).

Modified Atmosphere Packaging (MAP) is an effective way to suppress normal aerobic spoilage microorganisms. Low level oxygen packaging combined with a carbon dioxide and nitrogen flush inhibit Gram-negative bacteria. Jimenez et al. (1997) showed that aerobic bacteria were suppressed by modified atmosphere packaging. A combination of the antimicrobials (ClO_2 and AITC) with MAP may increase antimicrobial activity through synergetic mechanisms. Thus, the research hypothesis of this study is that gas phase ClO_2 or AITC plus MAP can be successfully used to enhance microbial safety of fresh chicken.

In addition, ClO_2 is a highly oxidizing agent (Lenntech, 2006). Degradation of packaging polymer properties by polymer oxidation has been reported in several studies (Hara, 1970; Ozen et al., 2002). Use of ClO_2 in food packages may involve higher concentrations than those used for package sterilization alone (Ozen et al., 2002). At high ClO_2 concentration, the treatment could affect

physical properties of the packaging materials. In order to prove the hypothesis, the effect of ClO₂ gas exposure at several different concentrations on common packaging films (LDPE, PVC, and PS) was investigated. Thus, objectives of this study are;

- (1) To investigate antimicrobial activity and minimum concentration of ClO₂ and AITC against *Salmonella* Typhimurium and *Listeria monocytogenes*.
- (2) To evaluate overall performance of ClO₂ or AITC plus MAP combinations for *Salmonella* Typhimurium and *Listeria monocytogenes* in inoculated fresh chicken.
- (3) To evaluate the effect of ClO₂ on the mechanical properties of the packaging films.

CHAPTER I LITERATURE REVIEW

1.1. Principle of antimicrobial packaging

Active packaging is a system which possesses attributes beyond basic barrier properties, and which is achieved by adding active ingredients into the packaging system (Ahvenainen, 2003). Antimicrobial packaging is a type of active packaging which could have a significant impact on product shelf life extension and food safety. Traditional methods used for microbial control are canning, aseptic processing, and MAP. Canned food cannot be marketed as fresh. Aseptic processing can be expensive and is limited to specific types of foods. MAP can promote the growth of pathogenic anaerobic bacteria (Farber, 1991). Directly adding antimicrobial agents to the food has limited effectiveness because the active substance can be neutralized on contact or absorbed into the food surface to affect its original taste (Quintavalla and Vicini, 2002).

If packaging materials have controlled release ability, the packaging system can be used to delay the lag phase or reduce the growth rate of microorganisms by controlled release of small amounts of antimicrobial agents. Han (Ahvenainen, 2003) explained the basic principle of antimicrobial packaging as part of a hurdle concept (Figure 1).

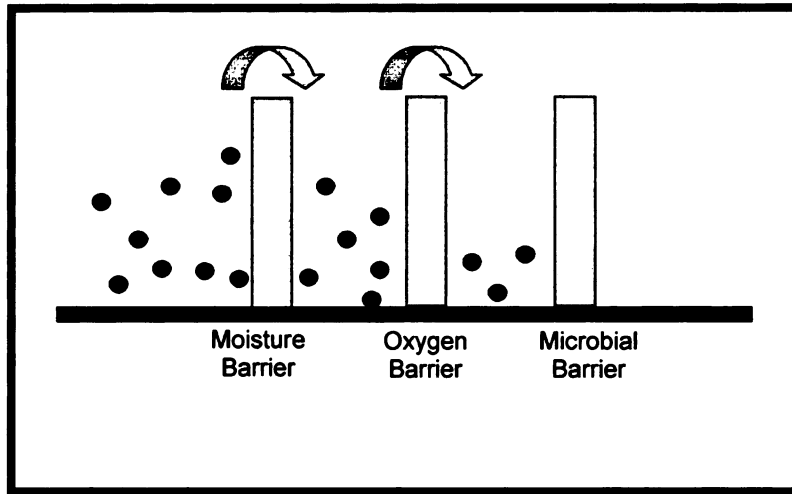


Figure 1. An antimicrobial packaging system as part of a Hurdle technology

(Source: Ahvenainen, 2003)

The antimicrobial function of the packaging system is another hurdle designed to help prevent product spoilage while the conventional layers satisfy the barrier requirement and provide physical protection. Embedding the antimicrobial agent into the packaging material provides active protection against microorganisms which is not achievable by conventional barrier packaging.

“Antimicrobial agents can be categorized into two types; those antimicrobial agents which intentionally migrate to the surface of the food, and those that are effective against surface growth without intentional migration of the active agent to the food (immobilization)” (Suppakul et al., 2003). The first type includes antimicrobial agents impregnated into packaging materials or coated onto their surface. The purpose is to migrate partially into or completely surround the food, thus extending the lag phase of the target microorganisms, or possibly inactivating the microorganisms. The antimicrobial agents can be in gas or solute

form. Solute agents have to be in direct contact with the food, while gaseous agents can fill the package headspace and thus contact more of the food product, whereas the solute agents can only control the direct contact area. Recently, a new antimicrobial migration system was developed. Thijssen patented a biologically triggered release system called the “BioSwitch” concept (Jong et al., 2005). This system releases its agents only if bacterial growth occurs. The advantage of this system is the lower active substance requirement because the impregnated antimicrobial agents are released only when there is microbial growth.

Immobilization systems do not release antimicrobial agents but inhibit microorganisms in the direct contact areas. Some antimicrobial packaging systems utilize covalently immobilized antimicrobial substances that suppress microbial growth when the target microorganism comes into contact with the antimicrobial surface (Kourai et al., 1994; Suppakul et al., 2003). This type of antimicrobial packaging can be more effective with liquid food products than with solids (Ahvenainen, 2003).

1.2 Types of antimicrobial packaging

1.2.1 Incorporation of antimicrobial agents into films

The antimicrobial packaging system is usually prepared by either incorporating the antimicrobial agent into the packaging material or by coating the active compound on the surface of the packaging film (Vermeiren et al., 2002). The incorporation of an antimicrobial substance into a packaging material

can take several approaches. One approach is to add the active agents to the resin mix in the extruder, and the film is then produced. However, this approach is often limited because of the heat liability of the component during extrusion, or incompatibility of the component in the packaging material (Quintavalla and Vicini, 2002). The process has poor cost effectiveness because the added active agents that typically migrate less to the surface of food than the original input. To limit heat loss, a master batch can be produced at low temperature. The antimicrobial agents can be mixed, extruded, and pelletized to create the master batch at low temperature (Brody, 2001). Han and Floros (1997) produced an LDPE master batch containing 1% potassium sorbate to prevent heat decomposition. The film extruded using the master batch inhibited the growth of *Saccharomyces spp.* on agar plates. However, Weng and Hotchkiss (1992) failed to suppress mold growth when they incorporated 1% sorbic acid into an LDPE film. Sorbic acid was insufficiently released from the film because of different polarity between the agent and polymer. While PE has been widely used as a heat seal layer in packaging, the copolymer polyethylene-co-methacrylic acid (PEMA) has been found to be preferable in some cases for incorporation of antimicrobials. Weng et al. (1999) modified films with NaOH, and then incorporated benzoic acid and sorbic acid. Tests then showed the antimicrobial films modified with NaOH exhibited more antimicrobial effectiveness due to a high release from the film.

Some antimicrobial packaging systems are covalently immobilized with active agents to suppress microbial growth. Appendini and Hotchkiss (1997) produced cellulose triacetate (CTA) film containing lysozyme. The presence of

the *Micrococcus lysodeikticus* spp. in tryptic soy broth (TSB) was effectively reduced by immobilized lysozyme containing CTA films (0.01 cm² / ml TSB).

1.2.2. Edible films and coatings for antimicrobial packaging

Edible films with various antimicrobial compounds also have been recently investigated for food packaging. Edible coatings and films are produced from polysaccharides, proteins and lipids. Edible coatings have advantages such as biodegradability, edibility, and good oxygen barrier properties. When using edible films and coatings as carriers for antimicrobial agents, the safety and edibility of the coating system is essential.

Rodrigues and Han (2000) investigated the effectiveness of whey protein isolate (WPI) films containing nisin, lysozyme and ethylenediamine tetracetic acid (EDTA). Both nisin and lysozyme containing films effectively inhibited *Brochothrix thermosphacta* but were not effective against *Listeria monocytogenes*. Research was also done by Ouattara et al. (2000) who prepared antimicrobial films with various organic acids and essential oils in a chitosan matrix. They found that the growth of *Enterobacteriaceae* and *Serratia liquefaciens* was reduced during 21 days of storage. Another study was done by Cagri et al. (2002). They produced whey protein isolate films containing 0.5 to 1.0% p-aminobenzoic acid (PABA) and/or sorbic acid (SA). The PABA and SA containing films were effective in decreasing *L. monocytogenes*, *E. coli*, and *S. Typhimurium* populations on inoculated slices of bologna and summer sausage.

1.2.3. Photon excited polymer

Ultraviolet irradiation treatment can increase surface amine concentrations which are surface active antimicrobial sites. Hagelstein treated nylon film with UV irradiation in order to increase the concentration of surface amine, and improved the film's antimicrobial effectiveness (Brody 2001). The author reported a 3 log reduction of *Staphylococcus aureus* by contact with the treated film. Another antimicrobial film was developed using a UV excimer laser. The polyamide film (nylon 6,6) was irradiated in air using a laser at 193 nm, and the amide groups on the surface of the nylon films were converted to amines (Brody 2001). Irradiation at 248 nm did not convert the chemical functional group from an amide to an amine. (Ozdemir et al., 1999)

1.2.4. Gas or volatile generating sachets

For volatile antimicrobial agents, a sachet type application is possible in an antimicrobial packaging system. Many volatile food grade antimicrobials can be encapsulated in a sachet. The release rate is then influenced by the permeability of the sachet. Ethanol, carbon dioxide, chlorine dioxide, and allyl isothiocyanate releasing sachets have been developed and commercialized for food applications (Kruijf et al., 2002; Suppakul et al., 2003).

1.3. Commercially used antimicrobial agents for foods

1.3.1. Chlorine dioxide (ClO₂)

Chlorine dioxide (ClO₂) is a synthetic, yellowish - green gas which possesses a similar odor to that of chlorine. It has been used for many years as a powerful biocide which exhibits rapid kill over a wide range of organisms. It works through oxidation, and penetrates bacterial cell walls and reacts with vital amino acids in the cytoplasm of the cell to kill the organism (Clordisys, 2003). Since its antimicrobial mechanism is oxidation (rather than chlorinating organic material like chlorine), it does not form undesirable pollutants such as trihalomethanes (THMs) or dioxins. Thus, the use of chlorine dioxide has the advantage that it produces less harmful byproducts than chlorine (Lenntech, 2006).

The first ClO₂ was produced in 1814 by Sir Humphrey Davy. He found that ClO₂ gas can be generated by reaction between sulfurous acid (H₂SO₃) and potassium chlorate (KClO₃). Nowadays, ClO₂ is generated by reaction of hydrochloric acid (HCl) and sodium chlorate (NaClO₃), which can be used to produce large quantities inexpensively (Clordisys, 2003).



Chlorine dioxide is currently used on food and medical equipment, counter surfaces, and processing facilities. The compound is unstable, and easily oxidized by sunlight. It is reduced to Cl⁻, ClO₂⁻, and ClO₃⁻ as end products. Since ClO₂ is an explosion hazard above 10% in air, it is difficult to store or transport as a gas (EPA, 1999). Therefore, it is often produced and used at the same location

(Lenntech, 2006). Thus, equipment for on-site chemical or electrochemical generation is often put in place. However, the significant capital equipment and operating costs have limited the use of chlorine dioxide to large-scale applications only. The use of ClO_2 is also governed by strict hazardous material handling regulations (Lenntech, 2006). Recently, ClO_2 sachets and films have been developed to offer cost - effective alternatives to bulk ClO_2 . For example, ICA Trinova and Engelhard manufacture ClO_2 release sachets. These utilize sodium chlorite and zeolites with acidic minerals to generate small, precise quantities of ClO_2 (Speronello, 2005). Bernard Technologies Inc. (Chicago, Illinois) patented ClO_2 generating LDPE films (Microatmosphere™) which consist of hydrophobic materials containing an acid releasing agent and hydrophilic materials containing anions that are able to react with hydronium ions to generate a gas (US Patent, 1994). Since it is an irritating oxidizer, high concentrations of ClO_2 gas may cause irritation of the eyes, skin, and lungs. (OSHA, 2006) The use of chlorine dioxide for antimicrobial food packaging has been recently permitted with “no objection” notification from FDA (2001). To minimize any potential adverse effects associated with ClO_2 , it is important to determine the minimum level of ClO_2 gas necessary to inhibit the target organisms.

Several studies have examined the effect of ClO_2 gas treatment for food packaging applications. Wellinghoff (1995) used ClO_2 to inhibit *Escherichia coli* in a ground beef meat product, and attained a 2-6 log reduction, depending on the dosage level. Knight (2001) reported a 90% microbial reduction using a high

concentration of ClO_2 (18 wt% of ClO_2) on raw beef. On the down side, an undesirable quality change was observed with the raw beef. The color of the beef changed from red to dark green when treated with ClO_2 . Ellis et al. (2005) used a combination of modified atmosphere packaging and ClO_2 (sachet) with fresh chicken breast, and reduced *Salmonella* Typhimurium by 1 log. There was no off-odor detected following ClO_2 treatment.

1.3.2. Allyl isothiocyanate (AITC)

Allyl isothiocyanate (AITC) is a major volatile pungent component of mustard, horseradish and wasabi. AITC is present as a precursor in the form of “sinigrin” in these natural plants. The sinigrin is hydrolyzed by the endogenous enzyme myrosinase when the plant tissues were suffered by physical injury, and yield ally isothiocyanate (Muthukumarasamy et al., 2003).

In the early 20th century, Hofmann discovered the effectiveness of AITC as a preservative in mustard oil (Winther and Nielsen, 2006). Since then, the strong antimicrobial effectiveness of both AITC liquid and vapor has been demonstrated in many studies (Lin et al., 2000). AITC has properties similar to those of other alkyl and aryl isothiocyanates which are used as a strong lachrymator or skin vesicant. AITC can be used as a flavor fortifier for foods (ex. mustard and horseradish) if used in the proper amount (Radomir et al., 1997).

The pungent AITC flavor is extremely reactive with nucleophiles such as water and SH- and OH- groups commonly found in food (Drobnaica et al., 1977). High concentrations of AITC are not typically useful for many food applications

because of its extremely pungent odor. Delaquis and Mazza (1995) mentioned that the amount of isothiocyanate (AITC) that can be successfully be used for food preservation is limited because of the possible negative sensory response it can cause. Kim et al. (2002) also found that too much AITC (2000 µg) was not acceptable to consumers due to its strong odor. Dunnick et al. (1982) observed transitional cell papilloma in the urinary bladder (in 4 of 49 rats) at a high dosage level (25 mg/kg/day). This indicates a possible safety concern at high concentration.

AITC can be applied both as a liquid and gas type. Gas type AITCs have been shown to be more effective as antimicrobial agents. Isshiki et al. (1992) presented data obtained from the Institute for Fermentation, Osaka (IFO, Osaka), the Japan Collection of Microorganisms (JCM, Saitama), and the American Type Culture Collection (ATCC, Rockville, MD). The growth of bacteria and yeast were suppressed at AITC concentrations of 31 to 420 µg/petri dish. These doses were equivalent to 16 to 110 ng/ml. They suggested that AITC could be used as a vapor for MAP. Nielsen and Rios (2000) used 3.5 µg/ml AITC in the gas phase for hot dogs and rye bread. All fungi (*Penicillium commune*, *P.roqueforti*, *Aspergillus flavus*, *Endomyces fibuliger*, etc) tested were eliminated, and it was anticipated that the minimum inhibition dosage could be decreased when used with MAP. Suhr and Nielsen (2003) reported that the minimum inhibitory concentration was 250 times lower in the gas type than in liquid type.

The use of naturally extracted AITC in certain food packaging systems has been allowed in Japan as a preservative (Isshiki et al., 1992). WasaOuro

(Mitsubishi-Kagaku Foods Corporation, Japan) is an example of a commercialized AITC preparation for food applications in Japan. The company offers antimicrobial labels, and sheets to prevent mold or bacteria growth on bakery, ready-to-eat foods, and vegetables. Recently, the use of AITC for food packaging was granted a no objection status from FDA as GRAS (FDA, 2006).

1.3.3. Silver ions

The antimicrobial activity of metals is due to the ions formed by the metals. Silver and copper ion, quaternary ammonium salts, and natural compounds such as Hinokitiol are generally considered safe antimicrobial agents (Suppakul et al., 2003). Silver is the most effective metallic antimicrobial at very low concentrations (0.02 – 0.05 ppm). Silver does not release the ion easily, compared to other metals such as copper. Thus, 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 silver zeolite. Silver substituted zeolite (Ag-zeolite) is the most common metallic antimicrobial agent. It retards a range of metabolic enzymes and has a uniquely broad microbial spectrum (Brody, 2001). Its antimicrobial effectiveness has been demonstrated in many studies against a wide range of bacteria, yeast, fungi and molds. Galeano et al. (2003) used stainless steel surfaces coated with silver zeolite (Aglon) to inhibit vegetative cells and *Bacillus* spores, and the silver zeolite coating inactivated approximately 3 log of vegetative cells within a 5 to 24 h period.

AglON™ (Aglon Technology, Inc., Wakefield, MA) produces a commercialized Ag-zeolite product. It is available in many food packaging applications such as bulk food storage containers, paperboard cartons, plastic and paper food wraps, and so on.

1.3.4. Ethanol

Ethanol (ethyl alcohol) is a flammable, colorless chemical compound with a distinctive perfume-like odor. It is used conventionally in medical and pharmaceutical packaging applications (Suppakul et al., 2003). Ethanol generating films and sachets have been developed and marketed to prevent mold growth of intermediate moisture foods, cheeses, and bakery products (Smith et al., 1987; Suppakul et al., 2003). Labuza et al. (1989) introduced an adhesive-backed film that could be taped on the inside of a package to provide antimicrobial activity.

In Japan, several ethanol vapor - generating sachets have been developed and commercialized, such as Ethicap (Freund Industrial Co. Ltd, Japan) and Ageless type SE (Mitsubishi Gas Chemical Co., Japan). Smith et al. (1987) used a commercialized ethanol release sachet (Ethicap) to extend the shelf life of apple pie, by reducing fungal growth. Franke et al. (2002) also used the sachet with pre-baked buns. Mold growth was delayed for 13 days in the presence of the ethanol emitting sachet while that in the control package normally occurred within 4-6 days. On the down side, though, the studies showed

that high ethanol release caused strong undesirable chemical odor in most food products tested with the sachets (Franke et al., 2002; Smith et al, 1987).

1.3.5. Nisin

Nisin is a bacteriocin which is included in a group of microbial synthesized proteinaceous antimicrobial agents. It is produced by the lactic acid bacteria, *Lactococcus lactis*, particularly during the exponential phase of bacterial growth (Buchman et al., 1988) and is effective against a broad range of Gram-positive bacteria, including *Listeria*, *Bacillus*, *Clostridium* and other lactic acid bacteria (Ahvenainen, 2003; Cleveland et al., 2001).

Nisin was the first bacteriocin to attain GRAS (General Recognized As Safe) status. Siragusa et al. (1999) reported the potential of incorporating Nisin directly into LDPE film, to enhance product safety. These peptide type compounds can only inhibit bacteria on food closely in contact with the microorganism. Nisaplin® (Danisco, Copenhagen, Denmark) is a commercialized Nisin-based antimicrobial agent. It can be applied directly onto or to foods or be incorporated into a packaging film. Cabo et al. (2001) applied Nisaplin® to fresh pizza as a preservative, in combination with MAP. The results showed significant increases in shelf life. Viskase Corp. (IL, USA) uses Nisaplin® in the casing of frankfurters and other deli meat products (Charest, 2004).

1.3.6. Natural extract

Antimicrobial extracts from plants are desirable for food packaging applications because of their apparent safety and the consumer's perception of natural materials. Many plant extracts from grapefruit seeds, cinnamon, and horseradish have been investigated as antimicrobials. Citrex™ (Citrex inc., Coconut Grove, FL) is a commercialized grapefruit seed extract (GFSE), that can be incorporated at 1% w/w into LDPE film (Lee et al., 1998). Lee et al. observed aerobic bacteria and yeast reduction on curled lettuce treated with GFSE. Ha et al. (2001) extruded GFSE incorporated multilayer films, and found coated film with 1% GFSE to have the highest effectiveness against E.coli, S.aureus, and Bacillus subtilis.

1.3.7. Carbon dioxide (CO₂)

Carbon dioxide (CO₂) is a well known antimicrobial gas which can suppress Gram-negative psychrotrophic bacteria (Daniels et al., 1985). The use of carbon dioxide to extend the lag phase and decrease the growth rate of microorganisms on foods is well known (Kruijf et al., 2002). Therefore, this antimicrobial has been commercially applied to many refrigerated products via modified atmosphere packaging.

There are many commercialized CO₂ generating sachets, such as FreshPax (Multisorb Tech Inc., USA), Ageless (Mitsubishi Gas Chemical Co., Japan), and Freshilizer (Toppan Printing Co., Japan). These sachets can also

be combined with an oxygen scavenging function. To enhance the antimicrobial effectiveness and prevent package collapse as a result of oxygen absorption (Smith et al., 1995), many commercialized CO₂ generating sachets are combined with oxygen scavengers (Smith et al. 1995, Suppakul et al., 2003).

High CO₂ concentrations, however, can cause changes in product flavor and the development of undesirable anaerobic glycolysis in fruits and vegetables. For these reasons, CO₂ generators are more useful in applications such as fresh meat, poultry, fish and cheese packaging (Suppakul et al., 2003).

1.4. Regulatory issues for antimicrobials in food packaging

Although antimicrobials have a promising potential, the current use of antimicrobial agents in food packaging is limited, with one reason being the regulations concerning their use. The use of antimicrobials in processed food and food packaging must follow the guidelines and regulations of the FDA¹ under section 409 of the FFDCA² (FDA/CSFAN, 1999). Agents used in antimicrobial packaging are considered food additives. Antimicrobial packaging can only contain agents which are approved by the authorization agency (FDA) or allowed for use within the concentration limits for food safety enhancement or preservation (Ahvenainen, 2003). Some organic acids, bacteriocins and volatile compounds derived from plants have been approved by the FDA. Table 1 shows the list of permitted antimicrobial agents which can be used for food packaging materials.

¹ FDA: Food and Drug Administration

² FFDCA: Federal Food, Drug and Cosmetic Act

Table 1. List of antimicrobial agents that are approved for food additive (food packaging) materials in USA

AM agents	Authority (US)	AM agents	Authority (US)
Acetic acid	GRAS	Lysozyme	FA
Allyl isothiocyanate	GRAS	Malic acid	GRAS
Adipic acid	GRAS	Natamycin	FA
Benzoic acid	GRAS	Nisin	GRAS
Calcium acetate	GRAS	Parabens	FA
Carvarcol	GRAS	Phosphoric acid	GRAS
Citral	FA	Polyphosphate	GRAS
Citric acid	GRAS	Potassium sorbate	GRAS
p-Cresol	GRAS	Propionic acid	GRAS
Chlorine dioxide	GRAS	Propyl paraben	GRAS
Ethanol	FA	Sodium acetate	GRAS
EDTA	FA	Sodium bicarbonate	GRAS
Geraniol	GRAS	Sodium benzoate	GRAS
Glucose oxidase	GRAS	Sorbic acid	GRAS
Lactic acid	GRAS	Succinic acid	GRAS
Lauric acid	GRAS		

1. Source: Suppakul et al. (2003), Davidson et al. (2005)

2. Classification in accordance with Food and Drug Administration (FDA) Title 21 of the Code of Federal Regulation (21 CFR) wherein substances intended for use in the manufacture of food stuffs for human consumption are classified into 3 categories: food additives (FA), pre-sanctioned food ingredients and substances generally recognized as safe (GRAS)

FDA safety reviews are sometimes in concert with other government agencies such as EPA¹. EPA also regulates pesticide chemicals (antimicrobials) with a tolerance or exemption from tolerance under section 408 of FIFRA² (Misko and Rothschild, 2001).

Historically, even if the antimicrobial is regulated as a food additive by the FDA, an antimicrobial intended to have any ongoing effect on a permanent or semi-permanent food contact article needs to have its safety reissued as an antimicrobial pesticide residue in or on the food packaging under section 408 of FIFRA. Therefore, confusion can exist when antimicrobials are incorporated into food packaging materials.

In 1998, the Antimicrobial Regulation Technical Corrections Act (ARTCA) (21 CFR Part 173) was enacted in part to correct the regulatory authority confusion between FDA and EPA. With this amendment, FDA now regulates all antimicrobials incorporated in, or applied to, food packaging materials regardless of whether the substance is intended to have an ongoing effect on any portion of the packaging. However, the use of ethylene oxide and propylene oxide on processed food is under the jurisdiction of EPA under section 408 of FIFRA (Misko and Rothschild, 2001). EPA also regulates antimicrobials that are incorporated in, or applied to, objects that have a semi-permanent or permanent food-contact surface, other than food packaging, in order to provide a sanitizing effect on surfaces such as a cutting board or a conveyor belt.

¹ EPA: Environmental Protection Agency

² FIFRA: Federal Insecticide, Fungicide, and Rodenticide Act

In Europe, no specific legislation has yet been approved about active packaging (including antimicrobial packaging). Antimicrobial compounds released from packaging are still regarded as migration agents so that the application has to follow the “Legislation for migration of components from the packaging material to the product” (Quintavalla and Vicini, 2002). Van Beest mentioned that the overall migration limit from packaging materials into a food is set too small (60mg/kg) to be compatible with the aim of antimicrobial release packaging (Suppakul et al., 2003). A new approach in food packaging antimicrobial regulation is needed.

The use of natural plant extracts as antimicrobial agents in food packaging goes through an easier regulating process when compared to chemical antimicrobial agents (Ahvenainen, 2003).

1.5. Common pathogenic organisms in meat products

1.5.1. *Salmonella* Typhimurium

Salmonella spp. is Gram-negative, facultatively anaerobic bacteria of the family Enterobacteriaceae, made up of nonspore-forming rods, usually motile with peritrichous flagella. It is one of the major foodborne pathogens commonly associated with raw poultry and poultry products (Mead, 2004). The usual symptoms of Salmonellosis include diarrhea, abdominal pain, cramps and fever 12 to 72 hours after infection and may last for up to 7 days (FDA, 1992).

Salmonella causes an estimated 1.4 million illnesses in the USA annually. The most common serotype was Typhimurium (CDC-b, 2006). *Salmonella*

Typhimurium DT 104 frequently causes serious complications within Salmonellosis, and caused the highest rate of hospitalization within the *Salmonella* species (Fisker et al., 2003). According to an epidemiological study done in 1993, 34 of 83 *Salmonella* Typhimurium DT 104 gastroenteritis cases required hospitalization and 10 people died (Bionewsonline, 2005). Another study done in 2006 reported that 27 of 58 *Salmonella* Typhimurium DT 104 cases resulted in hospitalization (CDC-b, 2006).

In July 1969, an outbreak of gastroenteritis due to *Salmonella* Typhimurium occurred in the greater Spokane area of Atlanta. The outbreak was associated with “ready to eat” barbecued chicken from a Spokane supermarket. 107 were known to have been ill, 29 were hospitalized, and two died (Werner et al., 1969). The Public Health Unit of the Dodge County Human Services and Health Department (DCHSHD) in Wisconsin reported 107 confirmed and 51 probable cases of *Salmonella* serotype Typhimurium gastrointestinal illness in 1995, associated with eating contaminated raw ground beef during the winter holiday season (CDC, 1995). One of the largest outbreaks of salmonella gastroenteritis was reported in Spain. As of 8 August 2005, a total of 2,138 cases were linked to processed chicken. The reported cases were linked to a single brand of pre-cooked, vacuum packed roast chicken which was distributed throughout Spain. All implicated chicken products were recalled from commercial outlets (Nathnac, 2005). As of November, 2006, Centers for Disease Control and Prevention reported a multistate outbreak of *Salmonella* Typhimurium linked to contaminated tomato consumption at restaurant chains. The outbreak caused

183 cases in 21 states, most patients had fever and diarrhea, and 22 (12%) were hospitalized; there have been no deaths reported (CDC, 2006c).

1.5.2. *Listeria monocytogenes*

Listeria monocytogenes is a psychrotrophic, Gram-positive, rod-shaped bacterium that can grow under refrigeration, in anaerobic conditions and in low oxygen atmosphere (Bonilauri et al., 2004). *Listeria monocytogenes* can persist in cool, damp areas of raw meat or processed meat plants. Drains and refrigeration freezing equipment can also harbor *Listeria monocytogenes* (Cox et al., 1989). This is a common contaminant of raw poultry and up to 60% of processed chicken carcasses may harbor low numbers of the organism (Cox et al., 1999). Live birds are rarely found to be positive and contamination occurs mainly during processing (Cox et al., 1989). *Listeria monocytogenes* is a major concern to the food industry. Approximately 2,500 illnesses and 500 deaths were caused by the pathogen, annually in the U.S (CDC, 2005b).

Consumption of *Listeria monocytogenes* contaminated foods causes the human disease called listeriosis. *Listeria monocytogenes* is a particularly lethal pathogen to the immunocompromised such as pregnant women and their fetuses, AIDS patients, alcoholics, and the elderly. Dairy products can be particularly susceptible to contamination by *Listeria monocytogenes* 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 (Donnelle, 1990). During August 1998 to February 1999, one listeriosis outbreak from hot

dogs resulted in 101 cases of listeriosis in 22 states, and created great concern among ready-to-eat food manufacturers (CDC, 1999). From May to November, 2000, a multistate outbreak was caused by a *Listeria monocytogenes* contamination with 29 illnesses, which were linked to eating deli turkey meat (CDC, 2000). From July to September, 2002, another multistate outbreak was reported of *Listeria monocytogenes* infections with 46 culture-confirmed cases, seven deaths, and three stillbirths or miscarriages in eight states. The outbreak was also linked to sliceable turkey deli meat in Northeastern United States. The meat was contaminated from a poultry processing plant (CDC, 2002).

1.5.3. Other foodborne pathogens

Thermophilic campylobacters, (especially *Campylobacter jejuni*), are recognized as a major, worldwide cause of human enteritis (Mead, 2004). The infection source is primarily poultry meat, contaminated water supplies, raw milk and pets. *Campylobacter* human infections are often associated with consumption of undercooked meat or handling of raw product since it is easily transferred to kitchen tools such as cutting boards and plates. Most cases are sporadic.

Eschericia coli include hundreds of bacterial strains which are commonly found in the intestines of healthy cattle, deer, goats, and sheep. Although most strains are harmless, one strain produces a powerful toxin that can cause severe illness. *E.coli* O-157:H7 is the most important serotype and has been the cause of various food associated outbreaks (Mead, 2004). *E. coli* O157:H7 was first

recognized as a cause of illness in 1982 during an outbreak of severe bloody diarrhea. People can become infected with *E.coli* O157:H7 in a variety of ways, and most illness has been associated with eating undercooked, contaminated ground beef (CDC, 2006a).

1.6. Chicken shelf life

Poultry meat is a highly perishable product because it provides an excellent medium for microorganisms. Thus, the primary cause of spoilage in fresh poultry is microbial growth, and shelf life can be extended by controlling growth of the usual spoilage organisms. Mead (2004) said that “the meaning of meat spoilage is that meat has become unfit for human consumption, due largely to the growth and metabolic activities of particular microorganisms.” Thus, there may be negative changes in the odor, flavor or appearance of meat making it unacceptable. In the case of chilled stored chicken portions, Ayres et al (1950) reported an ester-like odor and observed numerous small colonies on the surface of the chicken, which developed eventually into a layer of slime. At this stage, the odor becomes ammonium like and microbial populations exceed 1.0×10^8 cfu/cm². Elliott and Michener (1961) found that off odors were associated with 1.6×10^5 to 1.0×10^8 cfu/cm² organisms, and slime appeared between 3.2×10^7 to 1.0×10^9 cfu/cm². At spoilage, the predominant organisms were *pseudomonas* spp. (Cox et al, 1975). Most of the odor, color, and textural changes were attributable to microbial metabolism. The main spoilage organisms of chilled meat, in aerobic conditions, preferentially utilize glucose. During the logarithmic

phase of growth, glucose metabolism results in complex mixtures of short-chain fatty acids, ketones and alcohols (Dainty, 1996). At microbial populations above 10^7 cfu/cm², the source of glucose is soon depleted. Then, lactate and amino acids begin to be utilized and malodorous compounds are formed, especially from the sulphur - containing amino acids, cysteine, cystine and methionine. These contribute to the putrid, sulphury off odors of spoilage (McMeekin and Thomas, 1980). Once the pool of amino acids has been depleted and cells enter the stationary growth phase, having reached maximum numbers, there may be microbial proteolysis and lipolytic activity. The oxygen concentration in a meat package is greatly diminished and the concentration of carbon dioxide increased, resulting in a markedly reduced growth of *Pseudomonas spp.* This is mainly due to the inhibitory effect of the high level of carbon dioxide. The slower growing lactic acid bacteria tolerant to carbon dioxide then begin to dominate and ferment glucose to products that include lactic, iso-butanoic, and acetic acids, which give the meat a characteristic sour/cheesy odor (Mead, 2004).

1.7. Packaging of fresh meats

The oldest method of packaging and distributing fresh poultry meat is in a “wet shipper”. The wet shipper is a wax coated corrugated box in which whole birds are placed with ice (Sams, 2001). Currently, poultry meat packaging is including more small, consumer portions (known as “case ready”) processed and packaged at central processing locations (Mead, 2004). Still, almost 90% of these packaged chicken parts are packaged using highly oxygen permeable

polystyrene foam trays with a highly oxygen permeable PVC or other clear stretch film overwrap. Whole carcasses are packaged in polymer bags, either sealed or with clip closures (Sams, 2001).

Raw poultry meat is highly perishable even when stored in chilled conditions because the growth of psychrotrophic bacteria will cause spoilage. Normally, the shelf life of the conventional meat package is less than 5 days (Ellis et al., 2005). Packaging is important to maintain the safety of perishable products such as poultry and red meats. Modified atmosphere packaging (MAP) is a useful method that can be used to extend the shelf life of fresh meat. MAP is typically accomplished by flushing the package headspace with a desired gas mixture to replace ambient air. Carbon dioxide (CO₂) and nitrogen (N₂) have been used for this purpose. CO₂ particularly delays the lag phase of the microbial growth and lengthens the generation times of the organisms. Ogilvy and Ayres (1951) found that the shelf life of poultry meat can be expressed as a linear function of CO₂ concentration in the air. However, too high CO₂ treatment concentration can affect the color and texture of the meat product. For retail packaging, the CO₂ concentration should be limited to 35% to minimize package collapse and excessive purge (Sams, 2001). Many studies have been carried out in order to study the effectiveness of different gas compositions on the preservation of fresh meat. A minimum concentration of CO₂ is considered to be at least 20% to obtain significant improvement in shelf life (Greengrass, 1993). Jimenez et al. (1997) performed a study to determine the influence of gas composition on the shelf life of chicken breasts at 4 °C for 21 days. They found

that a 30%CO₂/70%N₂ blend extended shelf life up to 14 days and 70%CO₂/30%N₂ extended it to 21 days compared to 5 days for air-packaged samples. Lawlis and Fuller (1990) reported that refrigerated meat shelf life is typically 14 days with MAP, and the shelf life could be slightly longer if accompanied by deep chilling. N₂ may be used as a filler gas to minimize purge in the absence of oxygen. CO₂ with low levels of oxygen will inhibit many aerobic spoilage organisms such as pseudomonas (the main spoilage organism in chilled meat) (Mead, 2004). However, Sander and Soo (1978) reported that lactic acid bacteria ultimately predominate in the presence of CO₂.

1.8. Poultry meat market segment & packaging

According to a “US poultry market research trend analysis”(Global Information Inc., 2005), the total market growth from 1999 to 2004 has been 7%, and reached \$17.5 billion in 2004.

USDA's foreign agricultural service reported that meat production including beef, pork, and poultry has increased about 3% annually during the last decade. These gains have been led by poultry. Poultry meat production has expanded more than 5% each year on average, offsetting little or no growth in beef production since 1988 (Greene, 1998).

There are several factors contributing to this growth. One of the main factors is poultry's good nutritional profile, versatility and low cost. For example, poultry cuts like boneless, skinless breast have become popular because of their healthy nutrients (low fat, low carbohydrate, and high protein content) and

convenience. Semi-prepared and pre-marinated varieties of poultry products are also convenient and have been well-received. Cost is another reason; poultry meat is a cheaper source of protein than beef, and it has been extremely well-received in China, Russia, and Mexico (Greene, 1998). As a result of the strong, growing demand for poultry meat, global exports advanced at a double digit pace in the 1990s. The US supplies about 53% of global poultry imports, and promotion of poultry products in the fast food industry has also contributed to this demand.

CHAPTER II. MATERIALS AND METHODS

2.1. Culture preparation

Three strains each of *Listeria monocytogenes* (1002, 1176, 1304) and *Salmonella* Typhimurium (G10127, G10601, G10931) were obtained from Dr. E.T. Ryser, Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI. The cultures were stored at -70°C in trypticase soy broth (TSB) (Difco Laboratories, Detroit, MI) containing 10% (v/v) glycerol (J.T. Baker, Phillipsburg, NJ), and then subcultured twice in TSB containing 0.6% (w/v) yeast extract (Difco) (TSBYE) at 35°C /18~24 hours (10^9CFU/ml). The three strains of *Listeria monocytogenes* and *Salmonella* Typhimurium culture were then combined in equal volumes and agitated to obtain two three-strain cocktails. Finally, the two cocktails were serially diluted in 9 ml of 0.1% peptone water to produce different microbial densities (10^1 - 10^6). Figure 2 shows the *Listeria monocytogenes* and *Salmonella* Typhimurium culture preparation procedure.

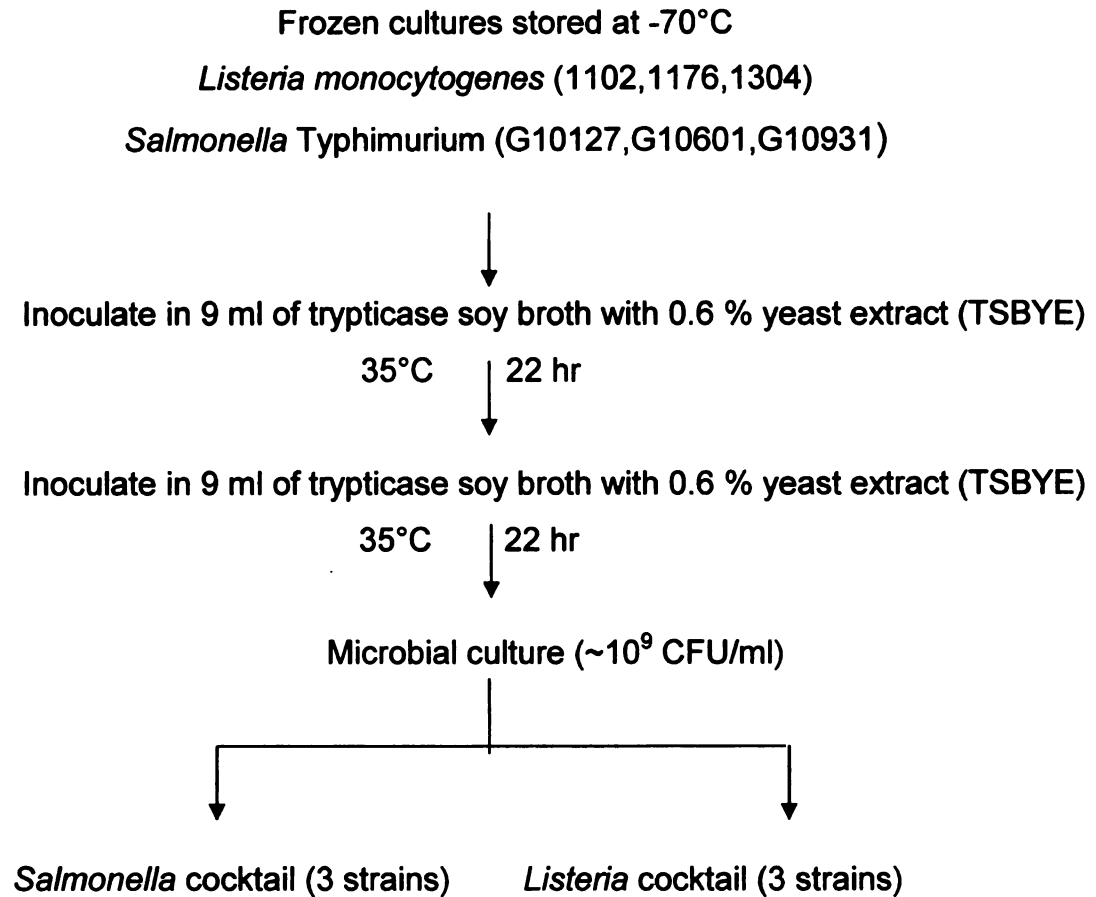


Figure 2. *Listeria* / *Salmonella* culture preparation used for inoculation of the chicken

2.2. Determine headspace concentration of chlorine dioxide (ClO₂) and its antimicrobial effectiveness

In order to investigate the antimicrobial effectiveness of ClO₂ against *L. monocytogene* and *S. Typhimurium*, each culture was inoculated on a different selective growth medium (agar), and incubated in the presence of different ClO₂ concentrations. To perform this study, 1 quart (946 ml) Mason glass canning jars were purchased from a local store (Meijer, Lansing). The metal lid of the glass jar was modified by cutting with holes (0.25 in) into the lid and attaching, and flexible Tygon tubes to the lid with 3/8 in diameter brass fittings. The tubes were closed using laboratory pinch clamps. Figure 3 provides a schematic of the jar used for ClO₂ analysis. A known amount of ClO₂ gas was injected into the jar headspace to yield a range of headspace concentrations in the glass jar.

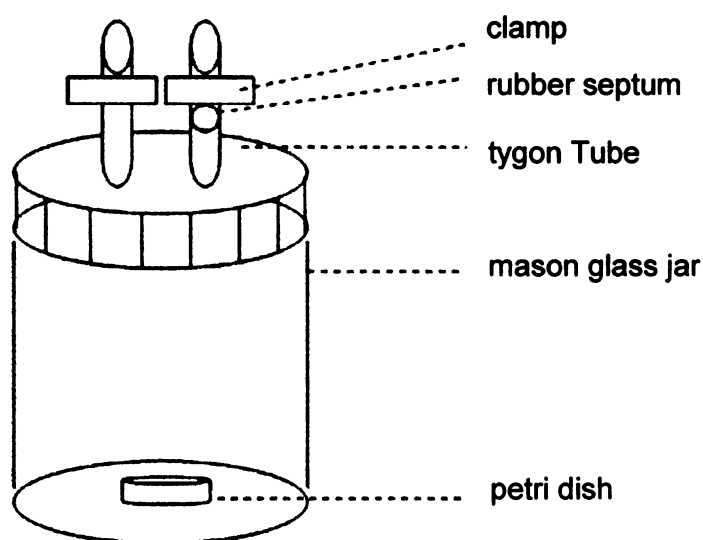


Figure 3. Schematic of canning jar used for ClO₂ concentration study

A ClO₂ sachet which is able to release ClO₂ gas in water was provided from ICA Trinova. The sachet contains 2g of the company's special formula, and release of ClO₂ gas was begun by soaking the sachet in distilled water. One sachet in 1 liter water provides approximately 40,000 ppm (107 mg/l) of dissolved ClO₂ gas. The ClO₂ in the aqueous mixture was stable in the dark and maintained a constant ClO₂ headspace concentration according to the vapor-liquid equilibrium relationship of Henry's law. (Montgomery, 1985)

$$X_g = HP_g$$

where,

X_g = equilibrium mole of dissolved gas

H = Henry's law constant (H of ClO₂ was 54 at 20°C, atm)

P_g = partial pressure of gas, atm

Figure 4 shows the glass container system used in the test. The ClO₂ aqueous mixture was diluted with distilled water to produce 1000 ppm (2.76 mg/l) of ClO₂ according to Henry's law. The headspace concentration was then measured using the ICA vapor equilibrated titration method (Appendix I). In order to create desirable ClO₂ concentration in the canning jar (Figure 3), headspace gas from the equilibrium ClO₂ generating container (Figure 4) was injected into the canning jar. Injection volumes varied depending on desired headspace concentration. For example, 1 ml of headspace gas containing 27.6 µg ClO₂ gas was injected to produce approximately 27.6 µg ClO₂/l (10 ppm) in the glass jar. In the same way, 0.5 ml of headspace gas was removed from the equilibrium ClO₂ gas generating container to produce 5 ppm.

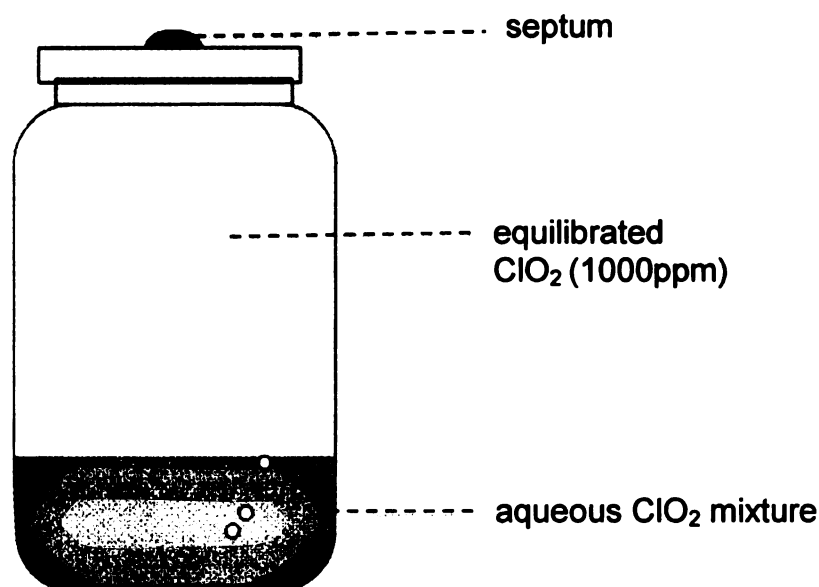


Figure 4. Equilibrium ClO_2 gas generating system

To evaluate antimicrobial activity as a function of ClO_2 treatment, the glass jar and lids were washed in hot soapy water, rinsed, and wiped with a 70% ethanol solution to sterilize. After drying in a hood, an inoculated agar plate was placed into the jar. 0.1 ml of each prepared cocktail (inoculum densities ranging from 10^0 CFU/ml to 10^6 CFU/ml) was inoculated onto the agar plates (60 mm diameter x 15 mm height). Modified Oxford agar (MOX) and Xylose Lysine Deoxycholate (XLD) agar were used for *Salmonella* Typhimurium and *Listeria monocytogenes*, respectively. After the metal lid was tightly closed, the prepared ClO_2 gas was injected through the septum attached to the Tygon tubing (Figure 3). The closed jar was then placed in an incubator (37°C), and stored for 48 hr. After the incubation period, the glass jars were removed, and evaluated to determine the antimicrobial effectiveness with the amounts of injected ClO_2 . Lack of growth on the plates was considered to be due to the inhibition of the inoculum densities by ClO_2 . The amount of remaining ClO_2 in the jar was also monitored

during the incubation time. Headspace ClO_2 concentrations in the canning jar were determined by using a chemical detector tube and Kwik-draw pump (MSA, Pittsburgh, PA). The detector tube marks the concentration of ClO_2 by color change (oxidation of aromatic amine by ClO_2) after absorbing 120 cc of headspace gas in the jar. To prevent development of a partial vacuum when the headspace gas was pulled out of the jar, nitrogen gas was flowed into the jar through another septum at an equal rate (approximately 150 cc/min).

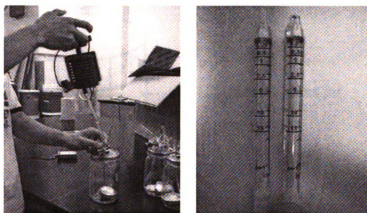


Figure 5. Photo of the detector tube and pump used to determine ClO_2 concentration

2.3. Headspace concentration of Allyl isothiocyanate (AITC) and its antimicrobial effectiveness

1 quart (946 ml) glass canning jars (Mason, Lansing, MI) were purchased from a local store (Menard, Lansing, MI). Their metal lid was equipped with a 3/8 in diameter septum adaptor. An orifice in the middle, sealed by silicon rubber, served as a septum for sampling the headspace gas in the glass jar. Figure 5 shows the schematic of the jar.

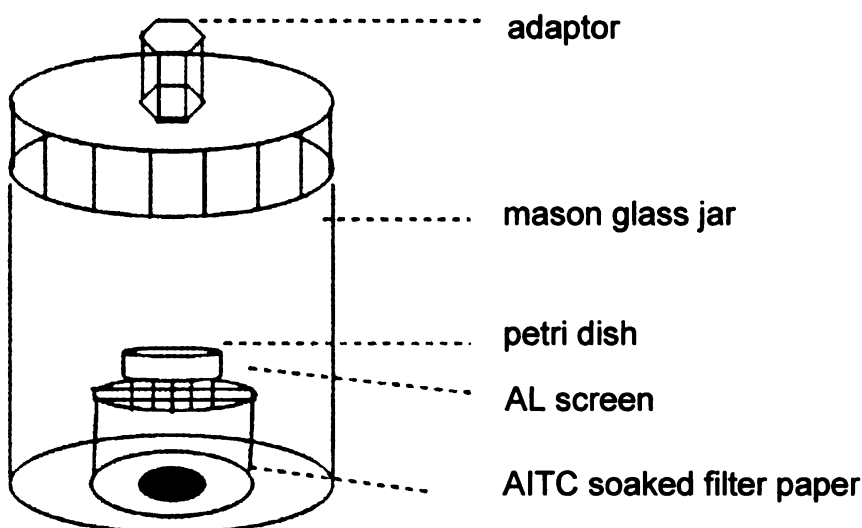


Figure 6. Schematic of canning jar used for AITC testing

AIT gas concentration was determined using a HP 6890 gas chromatograph (Hewlett-Packard, Wilmington, DE), equipped with a crosslinked 5% PHME siloxane column (30 m x 0.32 mm x 0.25 μ m film thickness). 1 ml of headspace gas was withdrawn with a gas tight syringe from the glass jar, and injected into the gas chromatograph. The flow rates of nitrogen carrier, hydrogen and air to the FID detector were 30, 30, and 240 ml/min, respectively. The temperature of

the oven was programmed to hold at 45°C for 4.5 min and increase at 60°C/min to 230°C, and hold for 5 min. The temperatures of the injection port and detector were set at 250°C and 290°C, respectively. To develop the standard curves, pure AITC (95% Sigma-Aldrich, St Louis, MO) was mixed and diluted with hexane, and a series of standard solution of known AITC amounts were injected into the GC to prepare a standard calibration curve. The standard curve is shown in Figure 7, and a copy of the actual chromatogram is shown in Figure 8.

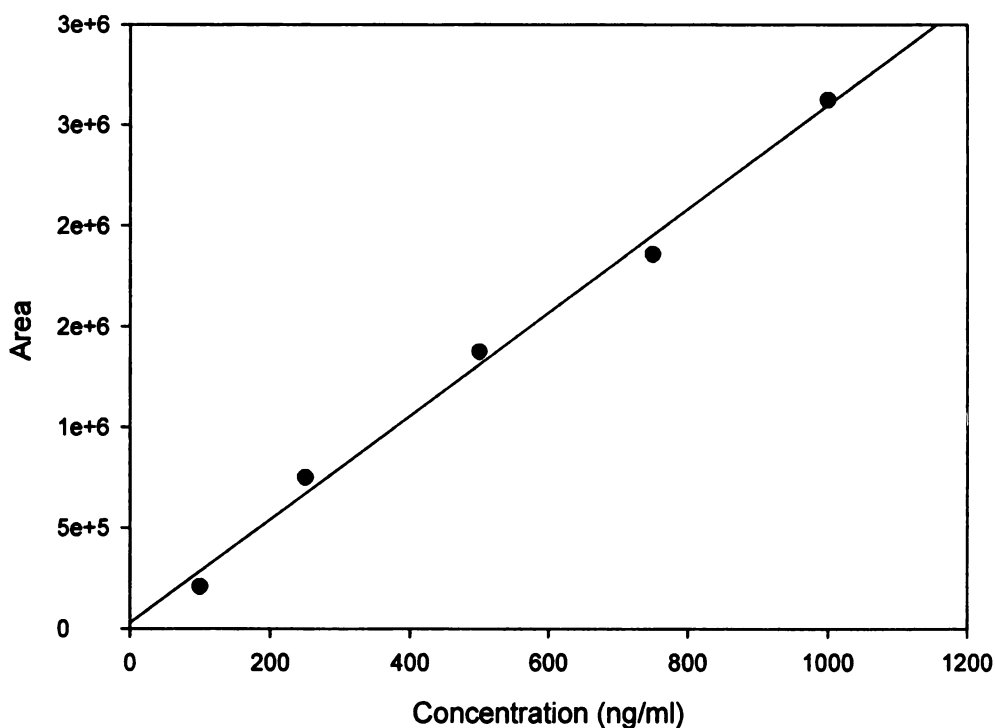


Figure 7. Standard curve of AITC concentration vs. area in a hexane solution

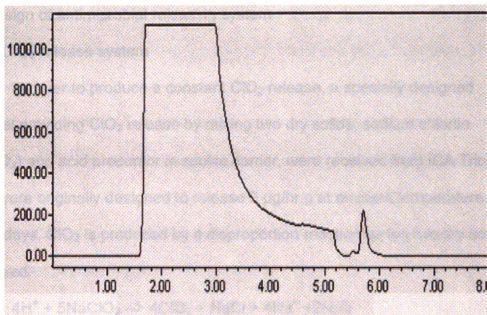


Figure 8. Chromatogram of AITC in a Hexane- AITC solution

In order to determine the antimicrobial activity of AITC, pure allyl isothiocyanate (95%, Sigma Chemicals, St Louis, MO) was mixed in warmed (30°C) corn oil, and AITC-oil mixtures of 0.5, 1, 2.5, and 5 ul/ml were added to filter paper (4.24 cm diameter, Whatman Inc., New Jersey, USA).

Listeria monocytogene and *Salmonella Typhimurium* inoculated agar plates were prepared as previously determined (section 2.2). To prevent direct contact of the AITC soaked filter paper and the agar plate, the AITC treated filter paper was positioned on an aluminum screen stand as is shown in Figure 5. After 48 hr of incubation, the glass jars were opened and the agar plates removed. Growth of the above microorganisms on the agar plate was then determined. Lack of growth on each of the different densities of plates ($10^1 - 10^6$ CFU/ml) was considered to be due to the inhibition of the inoculum densities by AITC.

2.4 Design of antimicrobial releasing system

2.4.1. ClO₂ release system

In order to produce a constant ClO₂ release, a specially designed material providing ClO₂ release by mixing two dry solids, sodium chlorite (NaClO₂) and acid precursor in zeolite carrier, were received from ICA Trinova. They were originally designed to release 3 µg/hr.g at ambient temperature (23°C) for 31 days. ClO₂ is produced by a disproportion reaction as the two dry solids are mixed:



2.4.2. AITC release system

2.4.2.1 AITC vapor pressure control

Since pure AITC solution is very volatile, it can quickly vaporize into the atmosphere. In order to control AITC vapor pressure, Sekiyama et al. (1994) and Lim and Tung (1997) used a triglyceride (ODO, oleic-capric-oleic) such as vegetable oil to lower the vapor pressure of pure AITC. In this experiment, the equilibrium AITC vapor pressures for various mole fractions in triglyceride (equilibrium AITC vapor pressure in the liquid-liquid mixture) were investigated at different storage temperatures.

5 ml of AITC liquid was introduced into a 10 ml vial with an orificed metal cap. A rubber septum was inserted into the metal cap, and then the cap was tightly closed using a metal clamp. The AITC containing vials were stored at three temperatures (4, 22, and 37°C) for more than 30 min to produce a

saturated vapor in the vial. The equilibrium point was determined when the headspace concentration did not increase during three serial injections. Then, the saturated headspace gas was withdrawn and analyzed using gas chromatography (GC). The AITC vapors were assumed to behave like an ideal gas ($pV = nRT$). Partial vapor pressures were determined from the measured concentrations, using the ideal gas equation. The experimentally determined AITC vapor pressures for the triglyceride mixtures were then compared to the results determined according to Raoult's law

$$P_1 = P_1^0 \times X_1$$

where

P_1 = vapor pressure of the solvent with added solute

X_1 = mole fraction of solvent

P_1^0 = vapor pressure of the pure solvent

The relationship between vapor pressure and AITC mole fraction at different temperatures was used to control the vapor pressure in the AITC canister.

2.4.2.2. AITC permeability in PE film

Figure 9 shows the schematic of the AITC canister used in this study. To design a constant releasing canister system, the relationship between partial vapor pressure and permeability was used to control the AITC release rate.

The AITC vapor transmission rate through PE film was determined in order to predict the organic vapor released from the canister. The Hatzidimitriu et al. (1987) permeation cell method was used to determine the permeability

through the PE film. The cell consists of two stainless steel chambers tightened around PE film. AITC liquid was placed in the lower part of the chamber, and the permeated AITC vapor in the top cell was detected. The top part of the cell (total volume 50 cc) contained an injection port with a rubber septum from which the sample gas was extracted. An o-ring was used and vacuum sealing grease was applied to assure a good seal between the film and surroundings. The concentrations of the permeating vapors were monitored using gas chromatography by removing 1 ml of headspace gas with a gas tight syringe. A plot of the concentration versus sampling time was used to determine when steady state was reached. Figure 10 shows a schematic of the stainless steel permeability cell used in the test. Two cells were tightened together with a bolt and nut, and a sample was withdrawn every 15 min until the permeant flux reached steady state.

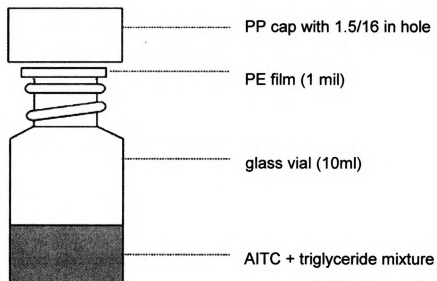


Figure 9. Schematic of the AITC release canister

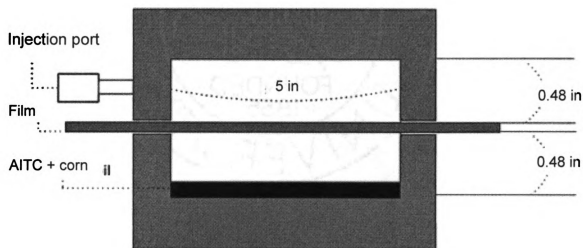


Figure 10. Quasi-isotatic cell used for measuring AITC permeability of the film sample

2.5. Determination of the ClO_2 and AITC release rates needed to inhibit microbial growth on fresh chicken

Both AITC and ClO_2 release systems were applied to fresh chicken breasts and the microbial inhibition effectiveness of the two antimicrobial agents investigated over a period of 8 days. Fresh boneless skinless chicken breasts were obtained from a local grocery store (Goodridge ShopRite, East Lansing, MI), transported to the microbiology laboratory (Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI), and stored for no longer than 1 day at 4°C before use. Each chicken breast was evenly cut with a sterilized knife to get a standardized surface area. The average surface area and weight were 286 cm² and 200 g, respectively. Figure 11 shows how the chicken samples were evenly cut before packaging.



Figure 11. Photo of chicken breast cut to provide a standardized surface area

The chicken samples were packaged in multilayer barrier trays, and heat sealed with lid stock (CS 907, Cryovac lid 1050), which is typical packaging used

for fresh, modified atmosphere meat packaging. The total volume was 1010 ml (7.5 in x 5.5 in x 1.5 in). A Multivac T-200 Traysealer (Multivac Inc, Kansas, MO) was used to seal the MAP product in the package. After the chicken was placed into the tray, it was inoculated evenly on the top surface of the chicken, and each antimicrobial release agent (ClO_2 sachet or AITC canister) was inserted into the package before the lid was sealed by the tray sealer. To prevent direct contact between the chicken and antimicrobial, the antimicrobials were attached to the walls of the package using pressure sensitive tape. Figure 12 shows a photo of the tray after the antimicrobial sachets were applied.



Figure 12. Inoculated chicken sample with ClO_2 and AITC sachets

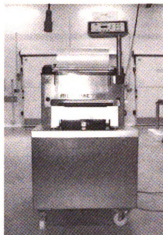


Figure 13. T-200 packaging machine used to pack the chicken breasts into the trays

Each culture cocktail of *Listeria monocytogenes* and *Salmonella*.

Typhimurium (see 2.1) was inoculated onto separate chicken pieces to avoid any interaction between them. 3, 4, 6, and 8 $\mu\text{g/hr}$ ClO_2 releasing sachets were prepared for the ClO_2 treatments, and 5 ml AITC-triglyceride mixtures able to release 0.3, 0.6, 1.2, and 1.4 $\mu\text{g/hr}$ of AITC continuously from the canister were prepared for the AITC treatments. Each antimicrobial system was attached to the inner wall of the tray, and sealed under a gas flush. A 70% N_2 /30% CO_2 gas mixture was used for antimicrobial plus modified atmosphere packaging. An ambient air flushed tray was also used to imitate conventional overwrapped chicken packaging. All filled trays were stored at 7°C for 12 days.

Every two days, 10 packages (4 for ClO_2 , 4 for AITC, 1 for MAP, and 1 for ambient air) containing chicken were opened and the contents placed into a stomacher filter bag. 200 ml of 0.1% peptone water was added to the stomacher filter bag and homogenized for 2 minute. 1 ml of the homogenized sample was diluted serially with 9 ml of 0.1% peptone water. The samples were plated on the

different selective growth media to quantify the number of *Listeria monocytogenes* and *Salmonella* Typhimurium.

Modified Oxford agar (MOX) and Xylose Lysine Deoxycholate (XLD) agar were used for each organism respectively. In order to recover the injured pathogen organisms, the MOX and XLD were overlaid with Tryptic Soy agar with 0.6% yeast extract (TSAYE) as shown in Figure 14, and the sample was inoculated directly on the nonselective thin agar layer. Colony counts were expressed as $\log_{10}$ colony forming units (cfu/g). Products from each packaging treatment were sampled three times (n=3).

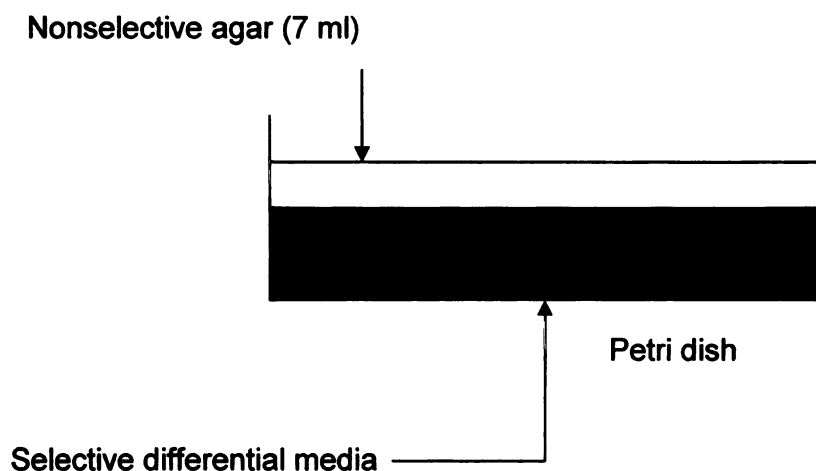


Figure 14. Schematic illustration of the thin agar overlay method

2.6. Evaluation of the synergic effect of antimicrobials (ClO₂ and AITC) and MAP combination.

The chicken samples were prepared and packaged as mentioned previously (section 2.5). In order to confirm the MAP and antimicrobial synergic effectiveness, half of the packages were packaged with air, and the other half were packaged using a 70% N₂/30% CO₂ gas flush. All packaged trays were stored in an environmental chamber for 8 days with storage temperature of 4°C. Then, the growth of *Salmonella* Typhimurium, and *Listeria monocytogenes*, and total aerobic bacteria were enumerated on growth media (section 2.5).

2.7. Fresh chicken packaged with antimicrobial and stored for 21 day

2.7.1 Sample and packaging

To determine the effective minimum ClO₂ and AITC release necessary to attain an antimicrobial impact, packages were prepared and packed with fresh chicken breast and antimicrobials. Chicken was obtained from a local store (fresh, boneless, skinless chicken breasts), cut, and packaged with ClO₂ or AITC after gas flushing (30% CO₂/70% N₂). The ClO₂ amounts used were 8 and 16 g sachets, with release rates of 4 µg/hr and 8 µg/hr, respectively at 4°C. For AITC treatment, 1.0 and 1.5 ml of AITC were mixed in a 5 ml AITC-triglyceride mixture. All of the antimicrobial packages were packed with a 30% CO₂/70% N₂ gas mixture. Samples were also packed without antimicrobial treatment as control samples, to compare the microbial inhibition effectiveness. Half of the controls were packaged in ambient atmosphere, and the other half were packaged with a

30% CO₂/70% N₂ gas mixture. All sample trays were stored in an environmental chamber for 21 days at 4°C.

2.7.2. Microbial analysis

Every three days, three trays from each treatment were removed and the amount of microbial growth (0, 3, 6, 9, 12, 15, 18, and 21 day) determined. The microbial analysis was discussed in a previous section (2.5).

2.7.3. Headspace gas analysis

In order to determine the internal atmosphere of the chicken packages, gas headspace analysis was carried out throughout the storage period. Gas composition of CO₂ and O₂ within the package was monitored using a headspace gas analyzer M-6600 (Illinois Instruments Inc, IL, US) at 0, 6, 12, 18, and 21 days. Headspace samples from the tray packages were withdrawn at a rate of 40 ml/min using the internal pump of the instrument, and passed by CO₂ and O₂ sensors.

2.7.4. pH measurement

During the storage period, pH was measured on the samples from storage every 3 days. To determine pH, 10 g from each chicken piece was removed before microbial analysis, diluted with 90 ml of distilled water (1:10 dilution), and homogenized in a blender for 2 min. Measurements were taken with a Corning model 430 pH meter and electrode (Corning Inc, Canton, NY).



Figure 15. pH meter and assembly used to determine pH of chicken samples

2.7.5. Color measurement

From the consumer preference, chicken should not change color due to antimicrobial treatment. Thus, the color of the breast meat treated with antimicrobial was investigated. The surface colors from three random locations on the chicken breast were monitored using a colorimeter (Hunter Color Machine Hunter Associates Laboratory INC, Reston VA) during the storage period, and an average value was then calculated from the three random locations for "L", "a", and "b" values. Color measurements were made every three days. Three trays from each treatment were removed and evaluated prior to the microbial test (0, 3, 6, 9, 12, 15, 18, and 21 days). Figure 16 shows a picture of the colorimeter used for this test.



Figure 16. Colorimeter used to measure the color of antimicrobial treated chicken breast

2.8. Physical property changes due to ClO_2 treatment

The same ClO_2 sachet from ICA (previously discussed in section 2.2) was used for the ClO_2 treatment. A concentrated aqueous ClO_2 solution was diluted with distilled water to make 100-2000 ppm of ClO_2 headspace concentration in 4 liter glass containers. The dilution amount of the concentrated aqueous ClO_2 solution was varied depending on the vapor-liquid equilibrium relationship of Henry's law. Each headspace concentration in each prepared ClO_2 solution was confirmed by titration (Appendix I). LDPE, PS, and PP films were pre-conditioned at $23 \pm 2^\circ\text{C}/50\% \pm 5\% \text{ RH}$, and inserted into the container containing prepared ClO_2 solution. Films were hung by an aluminum wire hook in the middle of a metal lid, to prevent direct contact with the aqueous ClO_2 mixture. The lid contained a 1/8 in hole, which was filled with silicone as a septum. For the physical properties tests, the films were removed after 1, 12, 24, 48, and 72 hours, and mechanical, barrier, and optical property tests were performed on the test films. An aluminum foil coated metal plate was placed immediately over the mouth of the jar after the lid was opened, so that the loss of ClO_2 vapor was

minimized while sample films were being removed periodically. Figure 17 shows a schematic of the ClO_2 treatment system used with these films.

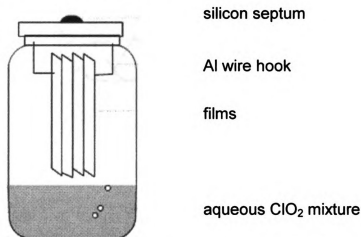


Figure 17. Schematic of ClO_2 film treatment system

2.8.1. Mechanical properties

Tensile strength (TS), elongation at break (EB), and toughness were measured using an Instron 4201 (Instron Corp., Canton, MA) Universal Testing Machine (Figure 18) according to ASTM D 882. Films were cut into strips (1 in width) using a Precision Sample Cutter (Thawing Albert Instrument Co., Philadelphia, PA), and conditioned according to ASTM D 618 at $23 \pm 2^\circ\text{C}/50\% \pm 5\% \text{ RH}$. The thickness of each film (PP, PS, and LDPE) was determined at three locations with a TMI 549M micrometer (Testing Machines, Inc., Amityville, New York) according to ASTM D 374-99.



Figure 18. Instron 4201 used for mechanical properties evaluation of packaging films

2.8.2. Measurement of oxygen transmission rate (OTR)

The oxygen transmission rate (OTR) was determined using an Oxtran Model 8001 unit (MOCON/Modern Controls, Inc., Minneapolis, MN) (Figure 19), according to ASTM D 3985, at 0% RH and 23 °C. The area of the test film was 5 cm². The test was continued until steady state was reached.



Figure 19. Oxtran 8001 unit used for oxygen transmission testing of film samples

2.9. Statistical analysis

Statistical evaluation of the data was performed using SPSS (SPSS Inc, 2004). Microbial growth depends on antimicrobial treatment and storage time was analyzed using the ANOVA procedure. Significance levels were reported at 95% confidence ($p=0.05$) using the Tukey's honestly significant difference (HSD) multiple comparison. The results of statistical analysis are shown as mean values $\pm$ standard deviation.

CHAPTER III. RESULTS AND DISCUSSION

3.1. Inhibition performance of AITC and ClO₂

3.1.1. Inhibition effectiveness of AITC against *Listeria monocytogenes* and *Salmonella* Typhimurium

The inhibitory affect of AITC vapor on *Listeria monocytogenes* and *Salmonella* Typhimurium was established in a series of microbial tests with different inoculum densities ranging from 10⁰ to 10⁶ CFU/dish. *Salmonella* Typhimurium and *Listeria monocytogenes* were stored in the presence of various amounts of impregnated AITC (on filter paper). Storage times were 48 hr and 7 days at 37°C and 7°C respectively.

The antimicrobial activity of AITC depends on dosage and temperature, as shown in Figures 20-21. *Salmonella* Typhimurium which had been inoculated on the agar plates was completely inhibited by AITC concentrations of 160 µg/l or more. At 7°C, it was completely inhibited with 70 µg/l. *Listeria monocytogenes* was more resistant to AITC vapor. At an AITC concentration of 160 µg, there was a 3 log reduction at 37°C. However, the same concentration (160µg/l AITC) reduced *Listeria monocytogenes* 6 log at 7°C. Complete inhibition was obtained by 360µg/l AITC at both temperatures. Overall, AITC was more lethal to both pathogens at 7°C. Delaquis and Sholberg (1997), Ward (1998), and Isshiki et al. (1992) used similar model systems to determine the antimicrobial activity of AITC vapor against various pathogenic bacteria. Delaquis and Sholberg (1997) reported that *Listeria monocytgoenes* was strongly inhibited by an AITC

concentration of 1500 µg/l while *Salmonella Typhimurium* and *E.coli* were barely effected. Thus, the results of Delaquis and Sholberg are in conflict with these test results. However, Ward (1998) showed that *Salmonella Typhimurium* was more susceptible to AITC vapor than *Listeria monocytogenes*. *Salmonella Typhimurium* was inhibited with concentrations of 4000 to 20000 µg/l. In Ward's experiment, the AITC vapor was distributed among 21 agar plates in a glass jar. There was only one agar plate per glass jar, in this test. The range of minimum inhibitory vapor concentrations in this research corresponded to the results from Isshiki et al. (1992). They reported that 37 -110 µg/l inhibited bacteria growth on agar. Figure 22 shows the gas headspace concentration profile during storage. Decomposition of AITC in aqueous media is known to be suppressed in the presence of dextrans and/or polysaccharides in the growth medium (Ohta et al., 1995). The results indicate that AITC decomposed substantially during 7 days of storage at 37°C. After 1 day, residual AITC concentration had decreased more than 50% while AITC concentration at 7°C was down by 40%. On day 2, the original 360 µg/l AITC was down by 90%. The difference in the reduction between the two temperatures can therefore be ascribed to the difference in decomposition rate. At 7°C, AITC concentration decomposition was substantial but approximately 20% of the initial input remained until 6 days when the initial level was 360 µg/l.

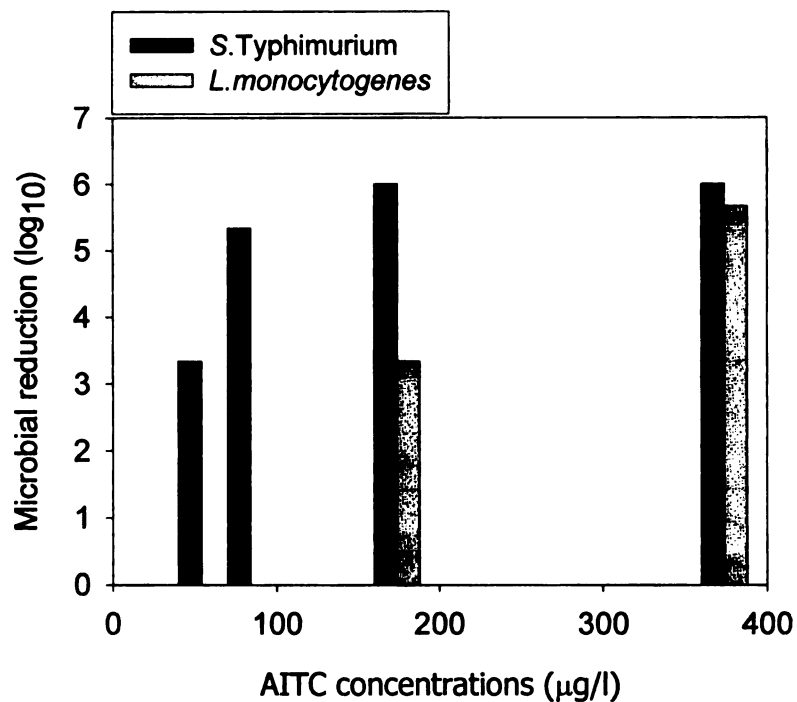


Figure 20. Reduction of *Salmonella* Typhimurium and *Listeria monocytogenes* on agar due to AITC treatment (37°C for 2 days)

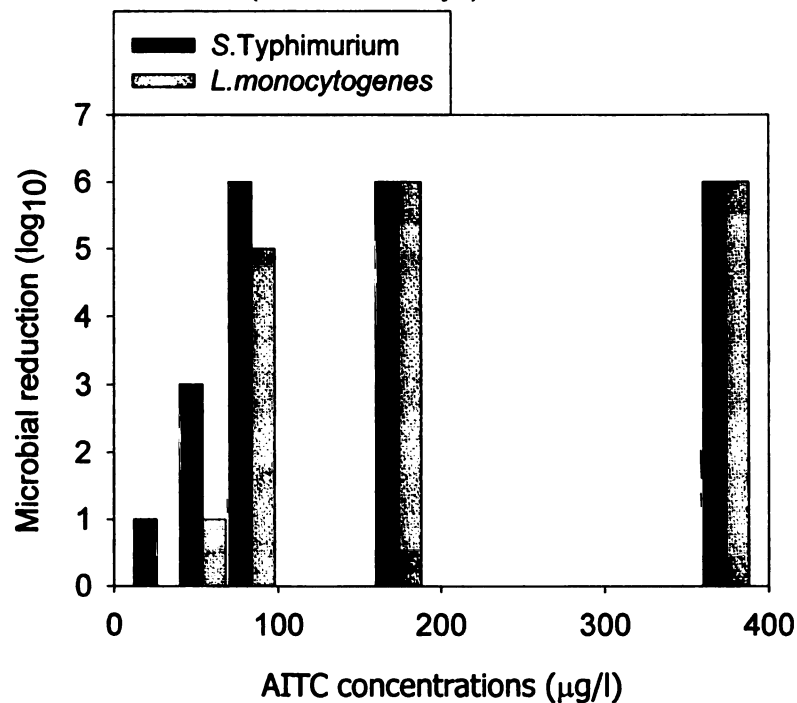


Figure 21. Reduction of *Salmonella* Typhimurium and *Listeria monocytogenes* on agar due to AITC treatment (7°C for 7 days)

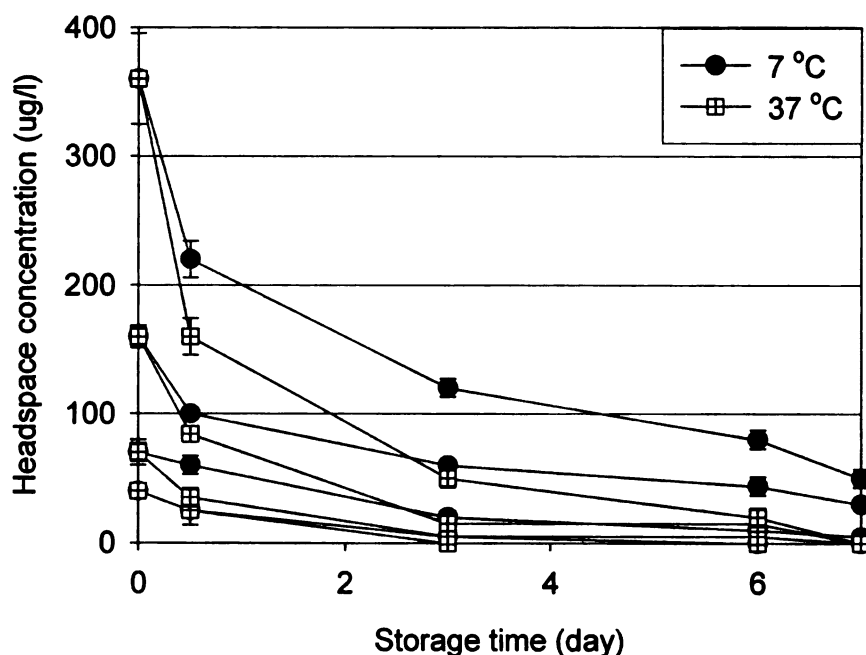


Figure 22. AITC headspace concentrations in glass jars (950 ml) with growth media during storage.

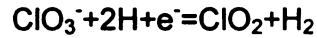
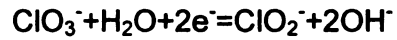
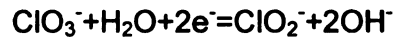
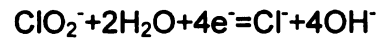
3.1.2. Inhibition effectiveness of ClO_2 against *Listeria monocytogenes* and *Salmonella* Typhimurium

For ClO_2 treatment, an experiment was performed with a known amount of ClO_2 gas to yield desirable headspace concentrations. The inhibition performance of ClO_2 on *Listeria monocytogenes* and *Salmonella* Typhimurium is shown in Figures 23 and 24, respectively. ClO_2 treatment was shown to result in a significant reduction of both pathogens. *Listeria monocytogenes* and *Salmonella* Typhimurium were completely inhibited by 120-180 $\mu\text{g/l}$. At 120 $\mu\text{g/l}$ or more ClO_2 treatment, *Listeria monocytogenes* was inhibited completely at both storage temperatures. At 37°C, 60 $\mu\text{g/l}$ of ClO_2 reduced the *Listeria*

monocytogenes population by 4.0 log CFU. The strong lethality of ClO₂ against *Listeria monocytogenes* has been also reported by other researches. Lee (2004) used a gas sachet which generated 4.3-8.7 mg ClO₂ after 30 min, and reduced the number of *Listeria monocytogenes* on lettuce leaves by 5.0 log, and Kaye et al. (2005) reported a 5.8 log CFU/g maximum reduction of *Listeria monocytogenes* on fresh vegetables with 4.1 mg/l ClO₂ gas. *Salmonella* Typhimurium was more resistant to ClO₂ treatment than *Listeria monocytogenes*. In order to eliminate *Salmonella* Typhimurium, 180 µg/l or more of ClO₂ was used, and 2 log reductions were obtained using a 90 µg/l treatment regardless of the storage temperature.

At 37°C, the pathogens were more inhibited more than at 7°C. The reductions in numbers of *Listeria monocytogenes* at 60 and 90 µg/l were 2 and 1 log higher at 37°C. *Salmonella* Typhimurium also suffered 1 log more destruction at 120 µg/l than at the lower temperature. The main reason is probably loss of ClO₂ from the headspace. As is shown in Figure 23, the initial inputs were quickly reduced at both temperatures. Regardless of storage temperature, all of the ClO₂ in the headspace was reduced to zero within 1 day. The water solubility of ClO₂ is 2.63 g/L at 40°C, and it is approximately 10 times more soluble in water than chlorine (Lenntech, 2006). Both XLD and MOX agar have low density and are water-based mediums. It is assumed that the heavy ClO₂ gas was absorbed into the growth media, and then dissociated to Cl⁻, ClO⁻, ClO₃⁻ as is shown in equation 3 (EPA, 1999). These ClO₂ byproducts are known to be weak antimicrobials (Lenntech, 2006).

Equation 3: Decomposition reactions: $\text{ClO}_2 + \text{e}^- = \text{ClO}_2^-$



At 7°C, the growth mediums (XLD and MOX) samples were held 5 days longer than at 37°C. The pathogens would have had more time to recover from ClO_2 treatment, particularly a psychrophilic bacteria such as *Listeria monocytogenes*. A constant release of ClO_2 during storage may be more effective than a onetime treatment. Ellis et al. (2005) reported that the low release ClO_2 (2.5 mg for 22 day) from sachets was more effective in reducing *Salmonella* Typhimurium on chicken than fast releasing sachets (6.6 mg for 12hr).

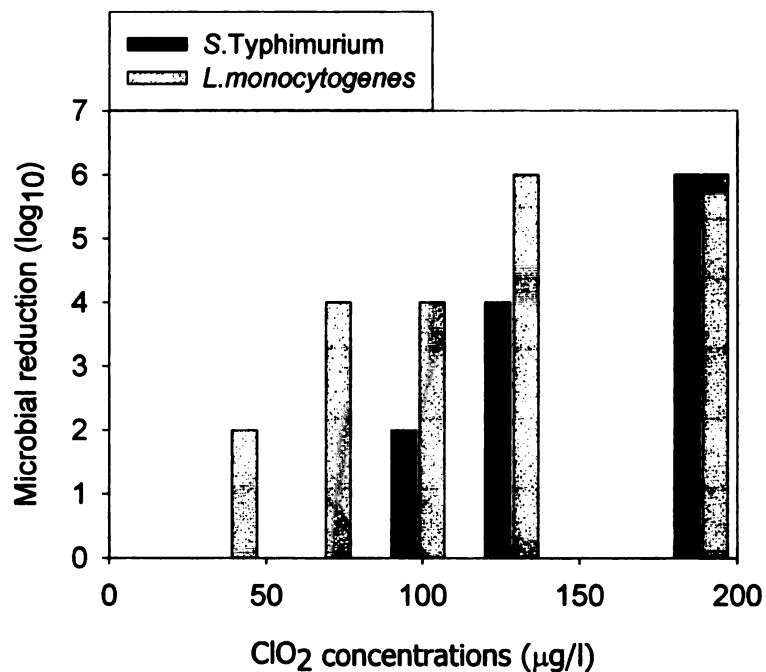


Figure 23. Reduction of *Salmonella* Typhimurium and *Listeria monocytogenes* on agar due to ClO₂ treatments (37°C for 2 days)

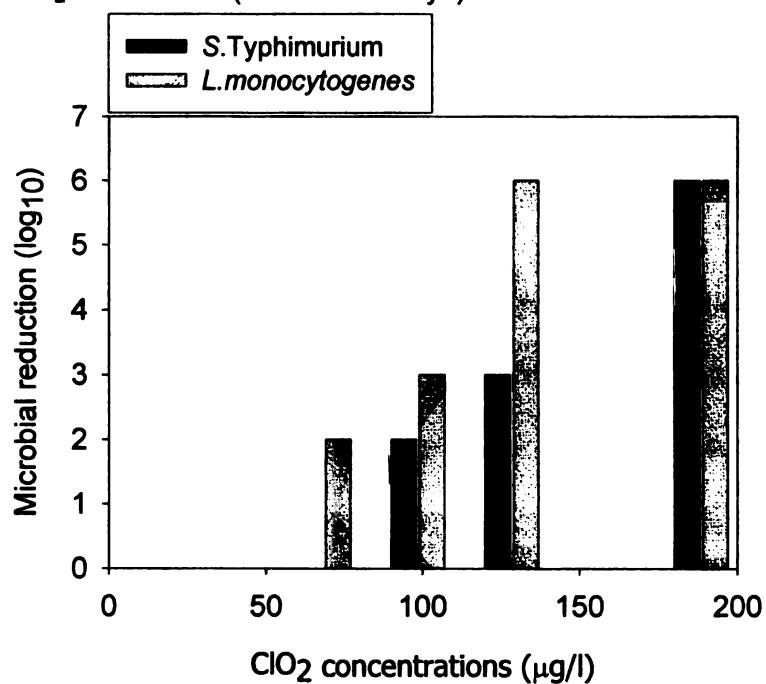


Figure 24. Reduction of *Salmonella* Typhimurium and *Listeria monocytogenes* on agar due to ClO₂ treatments (7°C for 7 days)

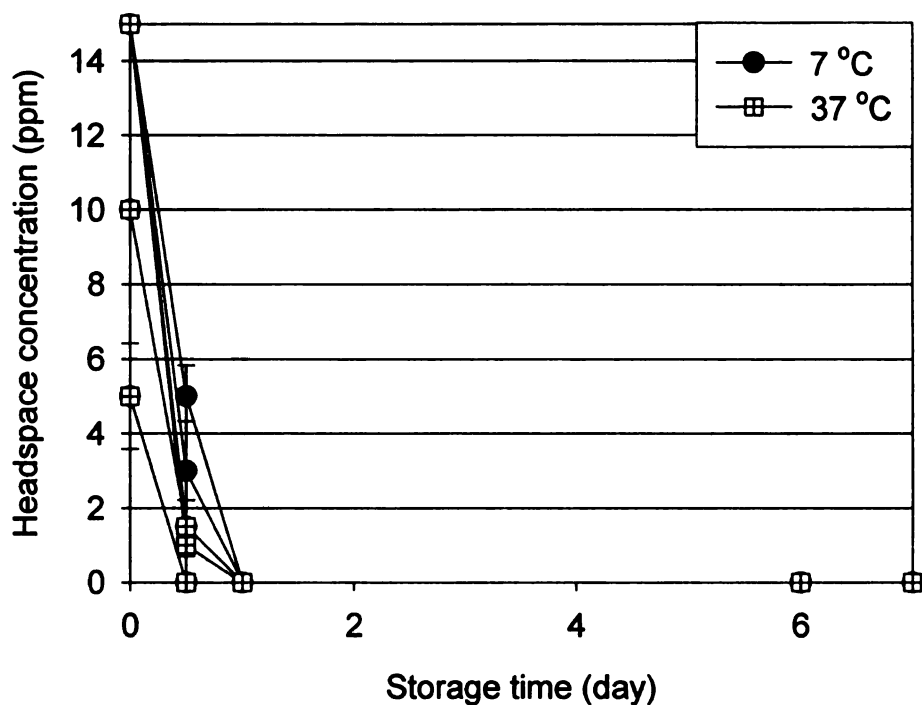


Figure 25. ClO_2 headspace concentrations in glass jar (950 ml) containing growth medium.

3.2. Development of AITC release system

3.2.1. AITC vapor pressure control

The objective of the test was to develop a model to predict the vapor-liquid equilibrium (VLE) of a binary (AITC-triglyceride) mixture as a function of temperature. In an ideal situation, the partial vapor pressure of a component in a mixture is equal to the vapor pressure of the pure component multiplied by its mole fraction in the mixture. Raoult's law was used to develop the VLE model, and an experimental study was conducted to verify the VLE (mixture of AITC and triglyceride) model.

Firstly, P_1^0 (vapor pressure of the pure solvent) as a function of temperature was determined using the Clausius-Clapeyron equation. The unknown temperature dependent vapor pressures were predicted using the following equation.

$$\ln\left(\frac{P_1}{P_2}\right) = \frac{\Delta H_{vap}}{R} \left(\frac{1}{T_2} - \frac{1}{T_1}\right)$$

where,

P = pressure, ΔH_{vap} = enthalpy of vaporization, T = temperature in Kelvin (K)

R = ideal gas constant ($8.3125 \text{ J mol}^{-1} \text{ K}^{-1}$)

P was determined using gas chromatography (GC) experiments. The heat of vaporization (ΔH_{vap}) was determined from the slope of the natural log of the vapor pressure ($\ln P$) versus the inverse of temperature in Kelvin ($1/T$). The slope of the plots was 47.53 KJ/mol with an R^2 of 0.998 . The ΔH_{vap} of AITC was very close to the values determined by other researchers. Chickos and Acree (2003) found it to be 47.6 KJ/mol and Lim and Tung (1997) obtained the same values using a GC experiment. P_1 and P_2 are the pressures at temperatures T_1 and T_2 . Figure 26 shows the predicted vapor pressure as a function of temperature, and demonstrates that AITC vapor pressures are highly dependent on temperature. The experimental data correspond well to the predicted values.

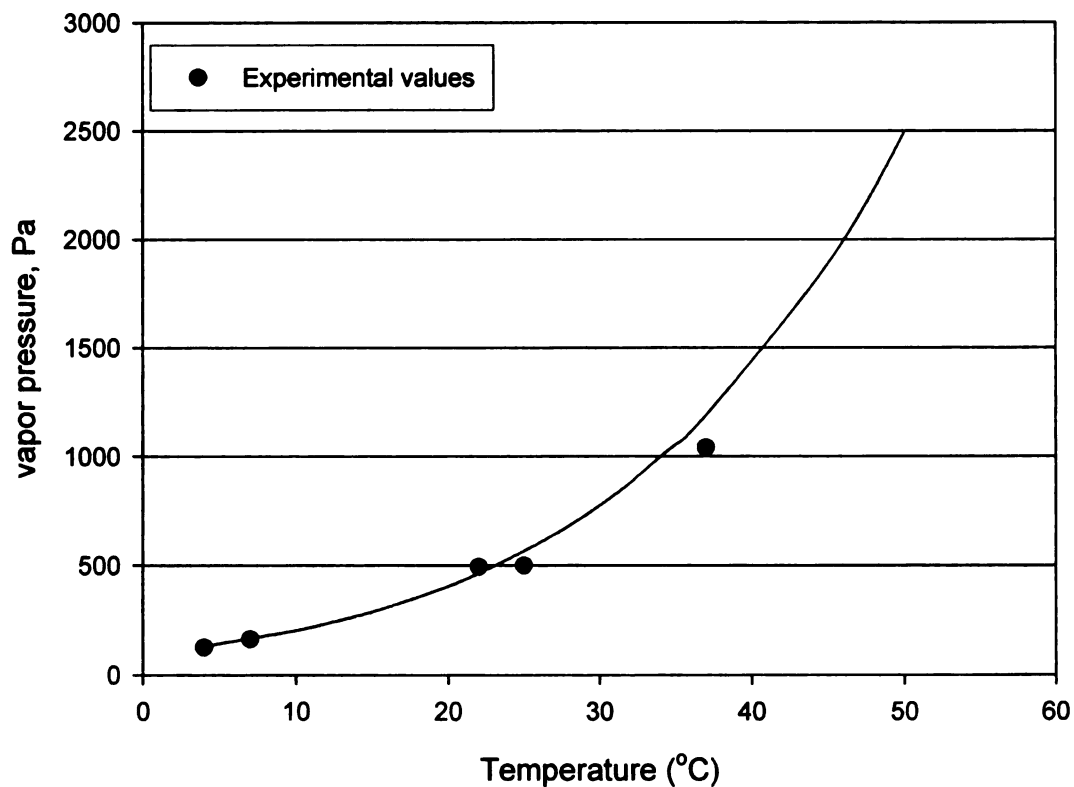


Figure 26. Vapor pressure of AITC as a function of temperature

The AITC partial vapor pressure-temperature-mole fraction relationship was obtained based on Raoult's law. The vapor pressures of pure AITC (p_a^0) at specific temperatures and experimental validation are shown in Figure 27. The vapor depression effects of the triglyceride diminished as temperature increased. In order to achieve an equal vapor pressure at a lower temperature, a much higher AITC mole fraction would be needed.

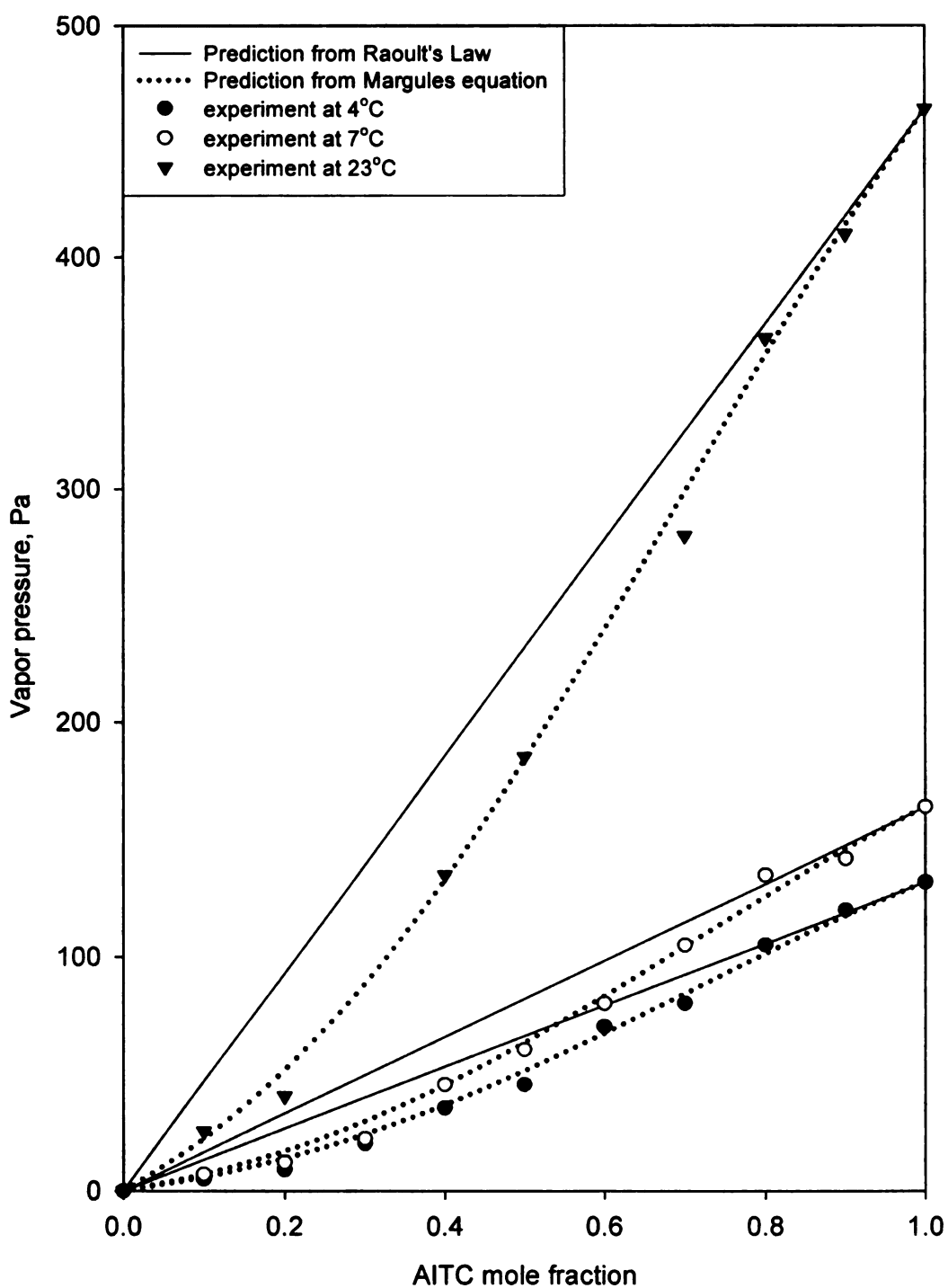


Figure 27. Vapor pressures of AITC in a AITC-Triglyceride mixture at temperatures 4, 7, and 23 °C

Results at 37 °C were out of scale so are not shown.

In order to verify the pressure-mole fraction model, the vapor pressure was determined by GC analysis (described previously). The results showed that the AITC-triglyceride mixtures deviated negatively from Raoult's law. Specifically, the deviation between model and experimental results was higher at lower temperature. Lim and Tung (1997) reported similar results with an AITC-canola oil mixture at 45, 35, 25, and 15°C. The author reported that there were larger negative deviations of vapor pressure from Raoult's Law at lower temperature.

Raoult's law is strictly valid only under the assumption that the chemical interactions between the two liquids are equal to the bonding within the liquids such as with an ideal solution. Therefore, by comparing the actual measured vapor pressures, it showed that the relative bonding strength between AITC and triglyceride was greater than the bond strength within the individual liquids (AITC-AITC). The deviations between Raoult's ideal relationship and the real experimental results were larger at lower temperature.

The non-ideal vapor-liquid equilibrium (VLE) relationship (p vs. x_1) was modeled using Margules equation (Metiu 2004). This is a widely used equation to predict mixed component's vapor pressures at a given temperature.

$$p = p_1^0 \cdot x_1(l)r_1 + p_2^0 \cdot x_2(l) \cdot r_2$$

where, p = vapor pressure, $x_1(l), x_2(l)$ = mole fraction of component x_1 and x_2 in the solution, p_1^0, p_2^0 = vapor pressure of pure liquid (x_1 and x_2), $r_1(x_1(l)), r_2(x_2(l))$ = activity coefficients of component of x_1 and x_2 in the solution, $r_1 = \exp[(A_{12} + 2(A_{21} - A_{12})x_1) \cdot x_2^2]$, $r_2 = \exp[(A_{21} + 2(A_{12} - A_{21})x_2) \cdot x_1^2]$

A_{12} and A_{21} are called Margules parameters. Reference for these parameters was not available for AITC. Therefore, the activity coefficient (α) was obtained through experimental work (Figure 27). Since the vapor pressure of oil is negligibly low, the vapor phase of the binary mixture can be taken to consist only of pure AITC vapor, and x_2 can be substituted for $(1 - x_1)$. Therefore, the equation yielded the following:

$$p_1 = p_1^0 \cdot x_1(l) \cdot \gamma_1 = p_1^0 \cdot x_1(l) \cdot \exp[\alpha(1 - x_1)^2]$$

The non-linear regression procedure of Sigmaplot (Systat software, 2004) was used to determine α from the experimental data, and the α values varied depending on temperature. p_1^0 was calculated from the previous vapor pressure-temperature graph shown in Figure 26. The model had a much more satisfactory fit than did Raoult's Law in Figure 27. As is shown in Table 2, vapor pressure predictions using Margules equation were shown to have R^2 values greater than 0.98 at all temperatures.

As the temperature of the system increased, α values also increased. The relationship between α and temperature was linear, as shown in Figure 28. Therefore, by using the predicted α , a non-ideal relationship (AITC vapor pressure vs. AITC mole fraction) may be able to be modeled at temperatures between 4 and 37°C.

Table 2. Estimated values of α estimated using a non-linear regression procedure and the coefficient of determination (R^2)

Temperature (°C)	α	R^2 value
4	-1.60	0.98
7	-1.57	0.99
22	-0.95	0.99
37	-0.57	0.99

$\alpha = -\Delta H_{mix} / RT$ (the activity coefficient from experimental data in Figure 27)

where, ΔH_{mix} =heat of mixture, $R=8.3143 \text{ m}^3\cdot\text{Pa}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$, T = Kelvin temperature

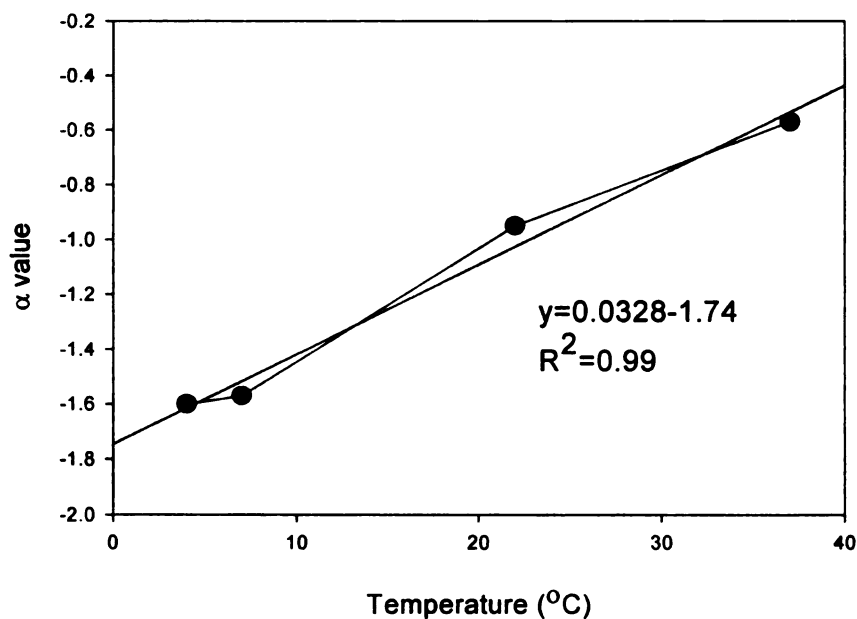


Figure 28. Estimated α values as a function of temperature

3.2.2. AITC permeability of PE film and controlled release rate

The AITC permeability coefficient for PE film (1 mil) was determined, and Figure 29 shows the permeation curve at ambient temperature (23 °C). The AITC transmission rate through PE film was obtained from the slope of the straight line once steady state was reached. The AITC permeability coefficient (P)

(($ug \cdot l$)/($in^2 \cdot hr \cdot pa$)) was calculated as follows:

$$P = \frac{Q \cdot l}{A \cdot t \cdot \Delta p}$$

where,

P = permeability, Q = the rate of permeation during the steady state

l = film thickness, A = area of the film exposed to permeant

t = time, Δp = partial vapor pressure

The equilibrated partial vapor pressure of AITC in the lower cell chamber was 83 pa, and the permeability of AITC through PE film was determined to be 60.76 $\mu g \cdot mil / in^2 \cdot day \cdot pa$. Despite the finite amount of AITC in the lower cell, the change in the AITC mole fraction due to losses from permeation was very low (less than 0.1%). Therefore, the AITC pressure drop within the lower permeation cell was ignored.

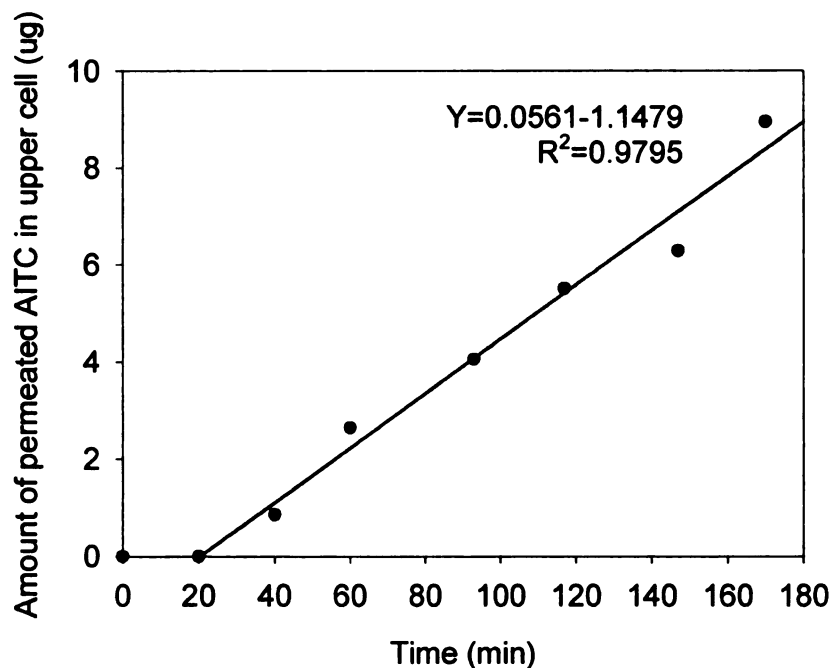


Figure 29. Quasi-isotatic permeation curves for AITC and PE film at 23 °C as a function of time

The AITC release rate was designed using the vapor pressure (P_a) vs. mole fraction (X_a) relationship (Figure 28). Various AITC mole fractions in triglyceride were prepared, and the AITC headspace concentrations in the glass jar verified using GC analysis. Table 3 shows the controlled AITC release rate from the canister system. The size of the orifice on the PE cap of the vial was 0.09 in. At a constant temperature, vapor pressure is the only factor needed to control the AITC release rate. In the test, mole fractions (X_a) varied from 0.3-0.8 to yield release rates from 0.3 to 1.8 μg AITC per hr. Figure 30 shows the AITC concentration in the headspace of the empty container as a function of time.

Table 3. AITC vapor release system as related to AITC mole fractions in the triglyceride mixture at 4°C

AITC (ml)	X_a	P_a	release rate ($\mu\text{g}/\text{day}$)	release rate ($\mu\text{g}/\text{hr}$)
0.5	0.3	20	8	0.3
0.8	0.4	35	15	0.6
1.5	0.6	70	29	1.2
2	0.7	80	34	1.4
3	0.8	105	44	1.8

Total volume (AITC (ml) + Triglyceride (ml))=10 ml

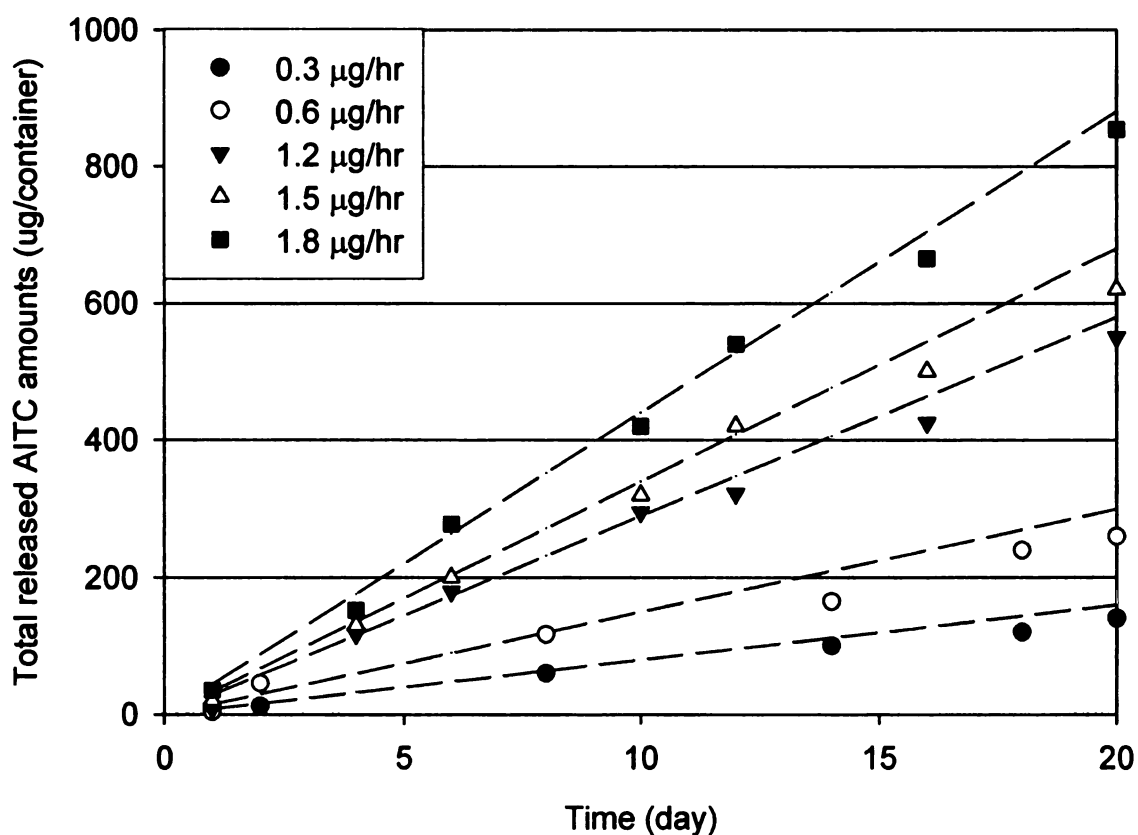


Figure 30. Theoretical vs. experimental AITC headspace concentrations of the different release systems

The headspace AITC concentrations in the container consistently increased as a function of time. The results indicate that the sachets continued to emit AITC gas for more than 20 days. However, the concentration data from the

later tests (after 12~15 days) had lower concentrations than expected. Since the empty container was flushed with 100% nitrogen gas, there was no decomposition from oxidation or product interaction with AITC. It is suspected that AITC suffered some amount of decomposition to the thiocyanate ion (SCN⁻). Radomir (1997) found that 5% of AITC was converted to SCN⁻ after 10 days of storage. Another possible cause could have been sample loss during needle penetration of the container (canning jar) during headspace sampling of AITC by gas chromatography. The possibility of absorption into the package was considered minor because the glass jar and metal lid provide almost perfect barrier properties.

3.3. Development of ClO₂ release system

ClO₂ gas was generated from a powder mixture supplied by ICA. The two different active compounds generated ClO₂ gas almost instantly with mixing, and its release rate was designed to be 3 µg/hr.g at room temperature (23°C) and 0.4 µg/hr.g at 4°C.

Therefore, the release rate was controlled by implementing the following simple equation at constant temperature;

$$\frac{dC}{dt} = K_g \times M$$

Where,

C=gas phase concentration, t=time, K_g=gas generation rate

M=weight of sachet

Using this relationship, the ClO₂ release rate in the closed container was controlled by the mass of powder placed into the sachet. The released ClO₂ gas quickly decomposed during storage. Table 4 shows the concentration difference between total released ClO₂ and remaining ClO₂ in the 1 qt glass jar.

Table 4. Total released ClO₂ amounts vs. experimental headspace concentration as a function of time at 4°C

Time(hour)	4g (calculation)	4g (experiment)	8g (calculation)	8g (experiment)
5	3.6	0.2	7.2	1.0
10	7.2	0.8	14.5	2.5
24 (1 day)	17.4	0.8	34.8	2.5
48 (2 day)	34.8	1.0	69.6	3.0
72 (3 day)	52.2	0.8	104.3	2.5
96 (4 day)	69.6	0.8	139.1	2.5

1. The unit of all data are parts per million (ppm)
2. Calculated concentration was determined by ClO₂ conversion factor (1 ppm= 2.76 mg/m³)

The ClO₂ concentration in both 4 and 8 g treatments reached steady state in 10 - 24 hours. After the ClO₂ concentration reached steady state, it remained constant during storage, though the concentration differed from the total amount released. This shows that ClO₂ continuously decomposed during storage. There are many possible reasons for this decomposition. The study was performed in an empty container after nitrogen flushing. Thus, little or no oxygen remained. However, some oxidation may still have occurred. In the gas state, as discussed in equation 3 on page 56, ClO₂ is unstable, and free radicals are evenly formed and dissociated. Thus, ClO₂ is converted to chlorite (ClO₂⁻), chlorate (ClO₃⁻) and chloride (Cl⁻) by oxidation. Another possible reason for the lower detected

amounts may be due to the instrument. When the detector tube absorbed headspace gas, it needed to be vented using another Tygon tube opening to avoid creating a partial vacuum. Thus, the sample gas might be mixed with air and thus diluted during the extraction.

3.4. Determination of minimum release rate to inhibit target bacteria

The purpose of this study was to determine the AITC and ClO₂ release rates which would inhibit *Salmonella* Typhimurium, *Listeria monocytogenes*, and total aerobic bacteria growth on fresh chicken breast. The previous test results showed that both AITC and ClO₂ either decomposed in the headspace or were absorbed into the agar plates. Also, fast antimicrobial vapor release rates have been known to cause adverse sensory problems. Muthukumarasamy et al. (2003) mixed 0.7 ml of AITC with vegetable oil, and packaged it with ground beef. The package system generated 1400 µg/l of AITC in the headspace immediately, and reduced by 6 log the inoculated level of *Listeria monocytogenes*. The packaged beef was observed to have a pungent flavor. In order to maintain the antimicrobial inhibition, a constant slow release treatment was considered to be an effective way to accomplish this with minimum sensory property changes. Thus, several different release rates were selected and tested with chicken in this study. The minimum effective release rates for both AITC and ClO₂ were selected to be the lowest release rates at which microbial growth was significantly lower compared to control samples. All antimicrobial treatments were combined with modified atmosphere packaging (MAP) (30%CO₂/70% N₂). For control samples,

ambient air flushed packages and MAP were used. The tests were conducted at a temperature of 7°C, a slightly abusive storage temperature for poultry products. The pathogenic organisms grow faster at this temperature than at a more normal storage temperature (3–4°C) for fresh chicken meat.

3.4.1. Target bacteria growth with AITC treatment

Four different constant AITC release systems (0.3, 0.6, 1.2 and 1.4 µg/hr) were developed and evaluated to determine their inhibition effectiveness. Growth of *Salmonella* Typhimurium was lower in all AITC systems than for samples which were packaged in modified atmosphere or ambient air packaging except for the 0.3 µg/hr release system (Figure 31). On day 12, the level of *Salmonella* Typhimurium in the 0.6 µg/hr release system was significantly lower than that without AITC treatment ($p < 0.05$) (Table 5). For the 1.2 µg/hr and 1.4 µg/hr AITC release system, total counts of *Salmonella* Typhimurium were within 1.0 log on day 12. No statistically significant difference between the 1.2 and 1.4 µg/hr release rate systems was observed.

The inhibition performance of AITC was also shown on chicken breast meat inoculated with *Listeria monocytogenes* (Figure 32). The results show that only the 1.2 and 1.4 µg/hr release rate systems had a significant inhibition effect. After 12 days, *Listeria monocytogenes* counts in the 1.2 and 1.4 µg/hr AITC release rate system were approximately 0.3 and 0.5 log lower than in the MA package.

The antimicrobial effectiveness of AITC on total aerobic bacteria was clear. Any treatment containing more than 0.6 µg/hr AITC significantly inhibited total aerobic bacteria compared to MA packages. On day 12, the reduction in total bacteria with 0.6, 1.2, and 1.4 µg/hr AITC treatments was 1.03, 1.41, and 1.73 logs compared to the MA package, respectively. The total bacteria counts of the 0.3 µg/hr AITC release samples were statistically lower than the MA packaging on day 6. However, the effectiveness did not last until day 8.

Overall, 0.6 µg/hr of AITC was the lowest dosage rate shown to significantly inhibit *Salmonella* Typhimurium inoculated on the fresh chicken breast. However, the minimum release rate which was effective against *Listeria monocytogenes* was considerably higher. *Listeria monocytogenes* was inhibited with 1.2 µg/hr or higher AITC release.

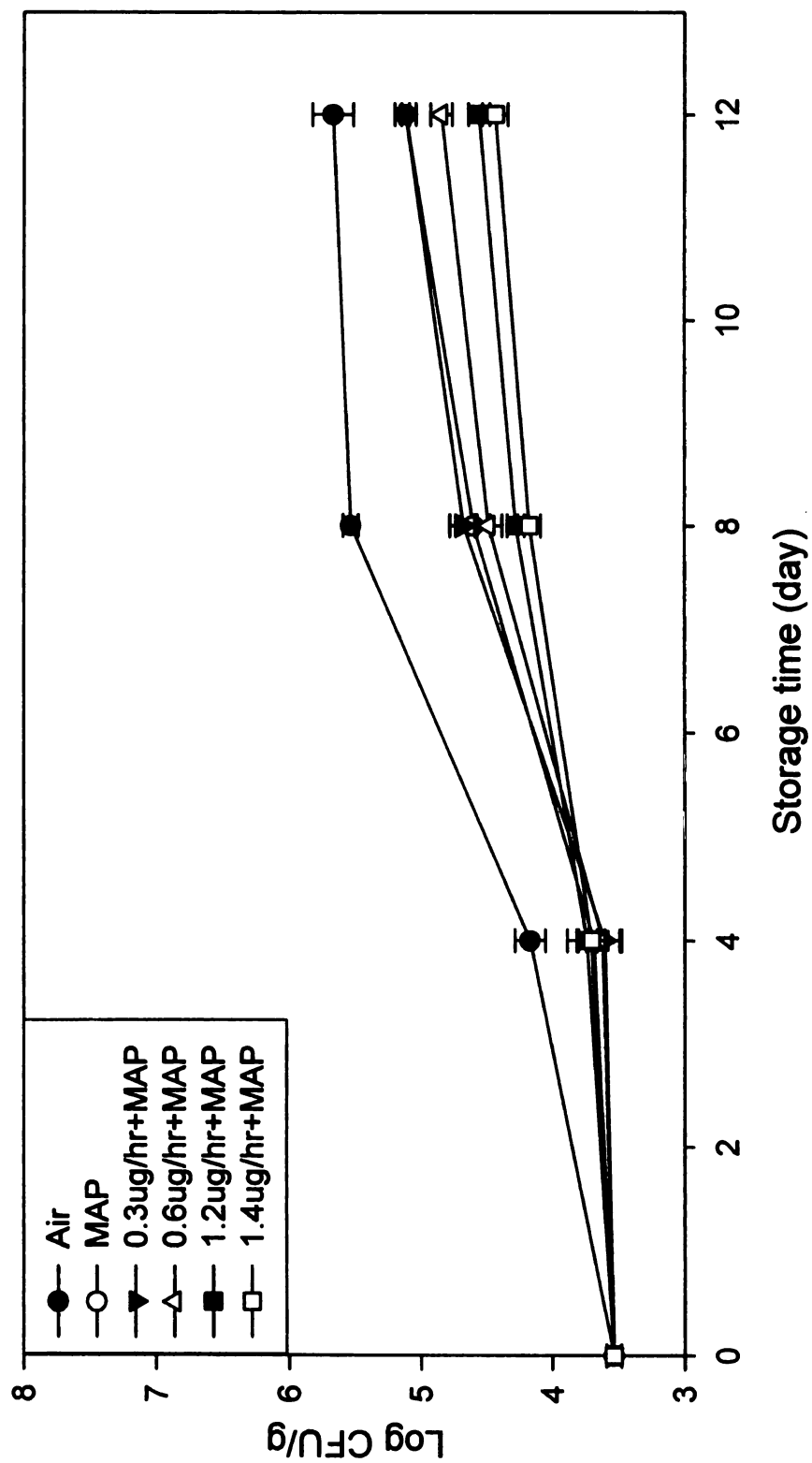


Figure 31. Effect of AITC release rate on *Salmonella* Typhimurium inoculated on chicken breast and refrigerated at 7°C

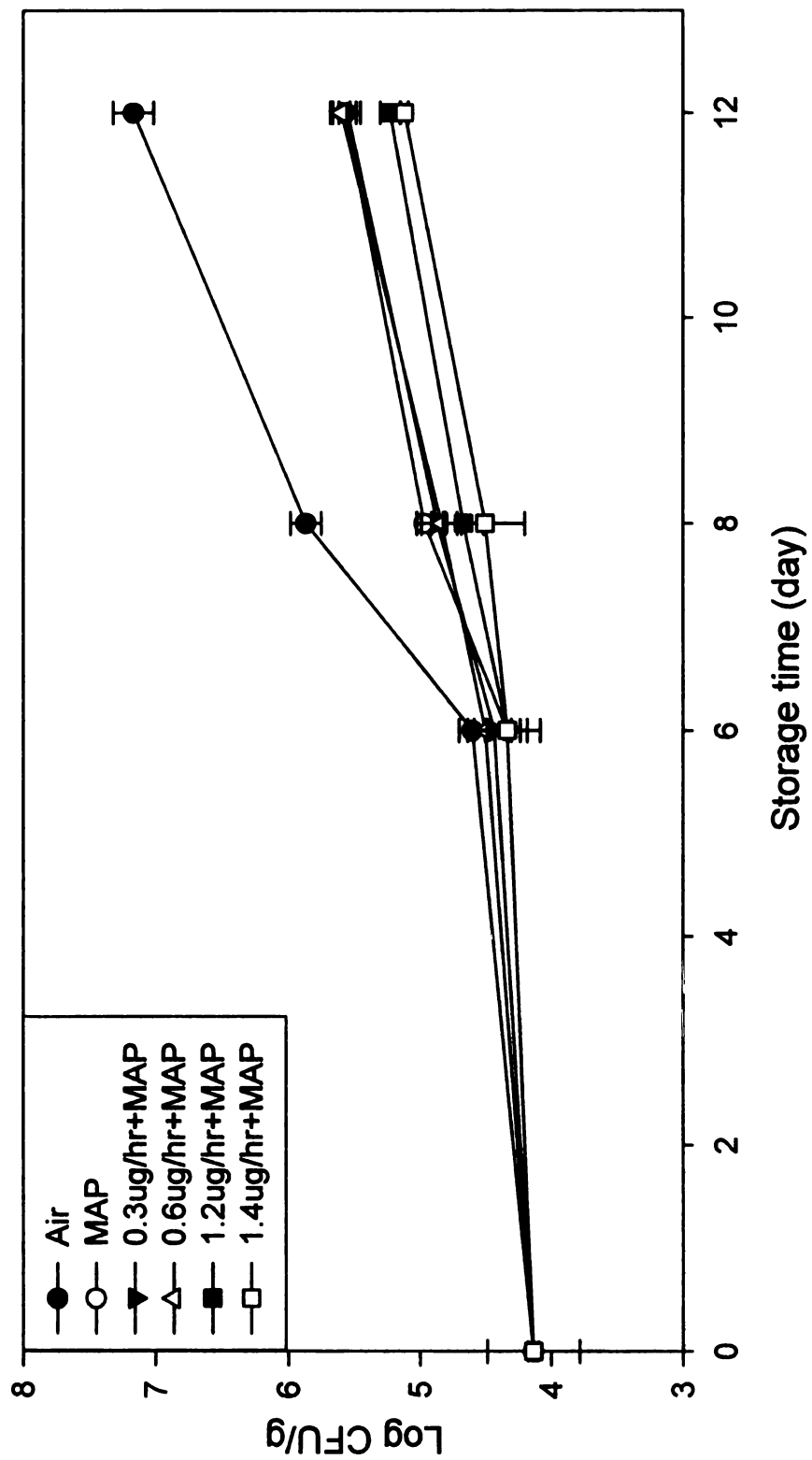


Figure 32. Effect of AITC release rate on *Listeria monocytogenes* inoculated on chicken breast and refrigerated at 7°C

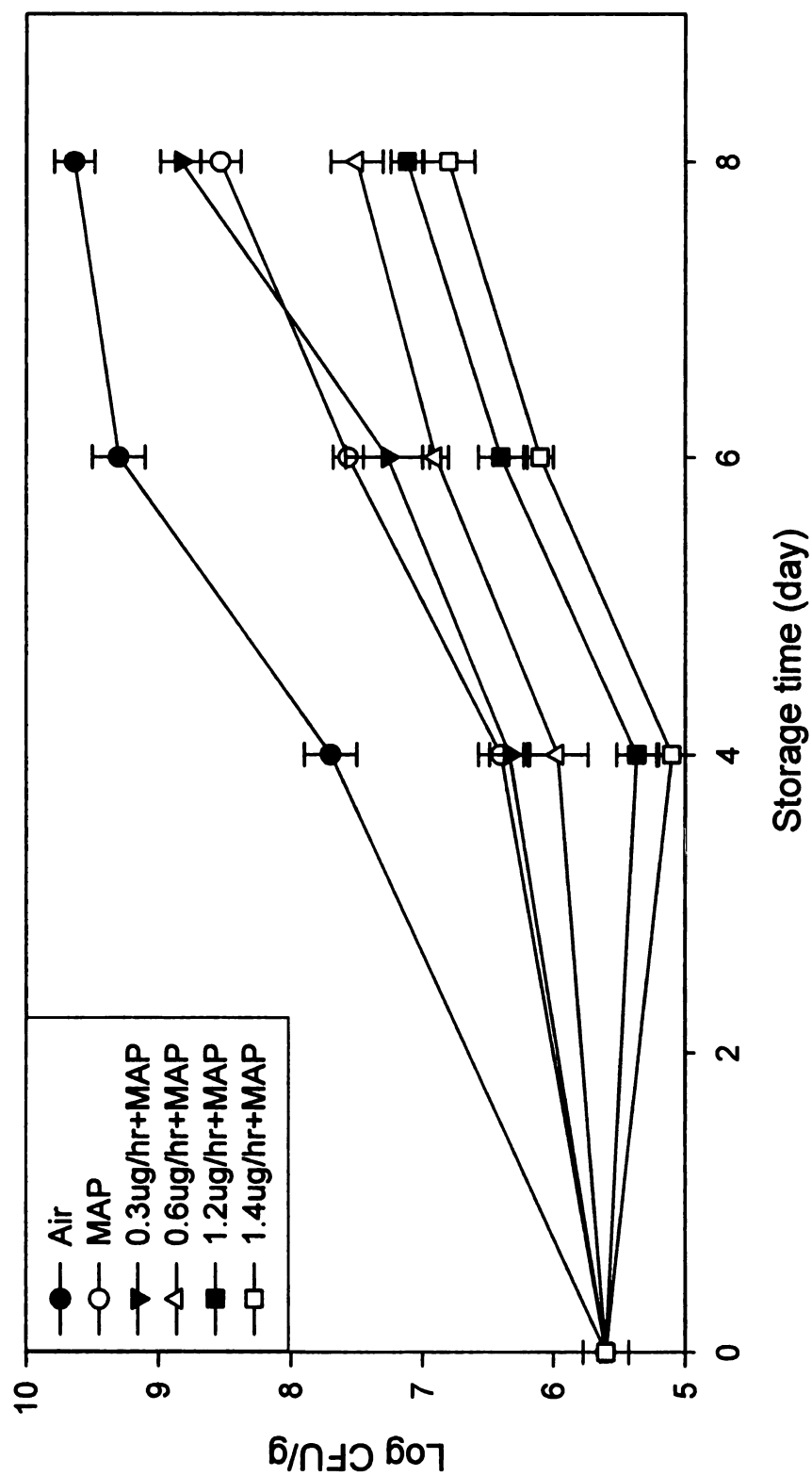


Figure 33. Effect of AITC release rate on total aerobic bacteria on chicken breast and refrigerated at 7°C

Table 5. Inhibition effectiveness of AITC treatment on *Salmonella* Typhimurium, *Listeria monocytogenes*, and total aerobic bacteria on fresh chicken at 7°C

Microorganism	PKG	0 day	4 day	8 day	12 day
<i>Salmonella</i> Typhimurium	AIR	3.53±0.06 _u	4.17±0.12 _v	5.53±0.06 _x	5.67±0.15 _x
	MAP	3.53±0.06 _u	3.68±0.20 _u	4.60±0.13 _w	5.12±0.13 _w
	0.3µg/hr AITC	3.53±0.06 _u	3.60±0.13 _u	4.67±0.10 _w	5.12±0.10 _w
	0.6µg/hr AITC	3.53±0.06 _u	3.62±0.13 _u	4.48±0.10 _{ww}	4.84±0.10 _v
	1.2µg/hr AITC	3.53±0.06 _u	3.74±0.08 _u	4.28±0.06 _{uv}	4.56±0.10 _u
	1.4µg/hr AITC	3.53±0.06 _u	3.70±0.10 _u	4.17±0.09 _u	4.43±0.10 _u
<i>Listeria</i> monocytogene	AIR	4.13±0.35 _u	4.60±0.10 _u	5.87±0.12 _v	7.17±0.15 _w
	MAP	4.13±0.35 _u	4.33±0.15 _u	4.97±0.10 _u	5.57±0.12 _v
	0.3µg/hr AITC	4.13±0.35 _u	4.43±0.20 _u	4.87±0.15 _u	5.55±0.10 _v
	0.6µg/hr AITC	4.13±0.35 _u	4.50±0.20 _u	4.83±0.15 _u	5.60±0.10 _v
	1.2µg/hr AITC	4.13±0.35 _u	4.33±0.15 _u	4.67±0.15 _u	5.23±0.10 _u
	1.4µg/hr AITC	4.13±0.35 _u	4.33±0.25 _u	4.50±0.30 _u	5.12±0.10 _u
Total aerobic bacteria	PKG	0 day	4 day	6 day	8 day
	AIR	5.60±0.17 _u	7.70±0.20 _w	9.30±0.20 _y	9.63±0.15 _x
	MAP	5.60±0.17 _u	6.40±0.17 _v	7.57±0.12 _x	8.53±0.15 _w
	0.3µg/hr AITC	5.60±0.17 _u	6.33±0.15 _v	7.27±0.32 _{xxw}	8.83±0.15 _w
	0.6µg/hr AITC	5.60±0.17 _u	5.97±0.23 _v	6.90±0.10 _{ww}	7.50±0.20 _v
	1.2µg/hr AITC	5.60±0.17 _u	5.37±0.15 _u	6.40±0.17 _{uv}	7.12±0.13 _{uv}
	1.4µg/hr AITC	5.60±0.17 _u	5.10±0.10 _u	6.10±0.10 _u	6.80±0.20 _u

Unit=log cfu/g. Mean±standard deviation.

Means with different letters in same columns are significantly different (p<0.05)

3.4.2. Target bacteria growth with ClO₂ treatment

The same microbial tests were performed on fresh chicken breast treated with ClO₂. The results obtained with *Salmonella* Typhimurium and *Listeria monocytogenes* inoculated chicken are shown in Figures 34 to 36. The tested organisms were significantly inhibited by ClO₂ treatment. However, a higher level of dosage (4 µg to 8 µg/hr) was required to get the same inhibition effectiveness as with AITC.

The growth of *Salmonella* Typhimurium in the presence of 3, 4, and 6 µg/hr release sachets did not show any significant difference ($p>0.05$) from that of the MA package (Table 6). 8 µg/hr release was shown to have a significant effect. The growth of *Salmonella* Typhimurium was 0.6 log lower during 12 days storage. The inhibition effectiveness of ClO₂ was greater against *Listeria monocytogenes*. At 4 µg/hr or higher ClO₂, the growth of *Listeria monocytogenes* was significantly lower than in MA packaging. On day 21, the reductions due to ClO₂, 4.0, 6.0, and 8.0 µg/hr were 0.5, 1.0, and 1.0 logs compared to the MA package (Table 6). The effectiveness of 6 and 8 µg/hr ClO₂ treatments was statistically similar. The population counts in both release rate systems became significantly lower than the other packages at 8 days.

The inhibition effectiveness of total aerobic bacteria in response to ClO₂ treatment is shown in Figure 36. Even though a higher dosage was used, its effectiveness was lower than that of the corresponding AITC treatment. Total bacteria on chicken samples with 4 µg/hr or higher release had significantly lower ($p<0.05$) counts than those in MA packaging. At the end of storage, packages

with 4 µg/hr sachets had 0.7 log lower counts than that of the MA package. Total aerobic bacteria treated with 3 µg/hr ClO₂ did not suffer any statistical increase in inhibition during the storage period. Doubling the ClO₂ release rate did not double the log reduction. Moreover, the effectiveness of 8 µg/hr ClO₂ was statistically the same as 6 µg/hr ClO₂. There may be a rate limiting effect other than concentration. A similar trend was observed in microbial counts for AITC treatment. Chicken subjected to more than 1.2 µg/hr of AITC (1.2 -1.4 µg/hr) had statistically similar results. This may be due to mass transfer of the antimicrobials, or higher microbial growth might have occurred on the bottom side of the chicken in the glass jar. The contact area between the chicken and glass jar may have been less accessible to the antimicrobials (either AITC and ClO₂). A negative side effect was observed at the highest (8.0 µg/hr) ClO₂ release rate. The surface color nearest to the sachet became brown-black. Details concerning color change are discussed in the next section (3.6.6).

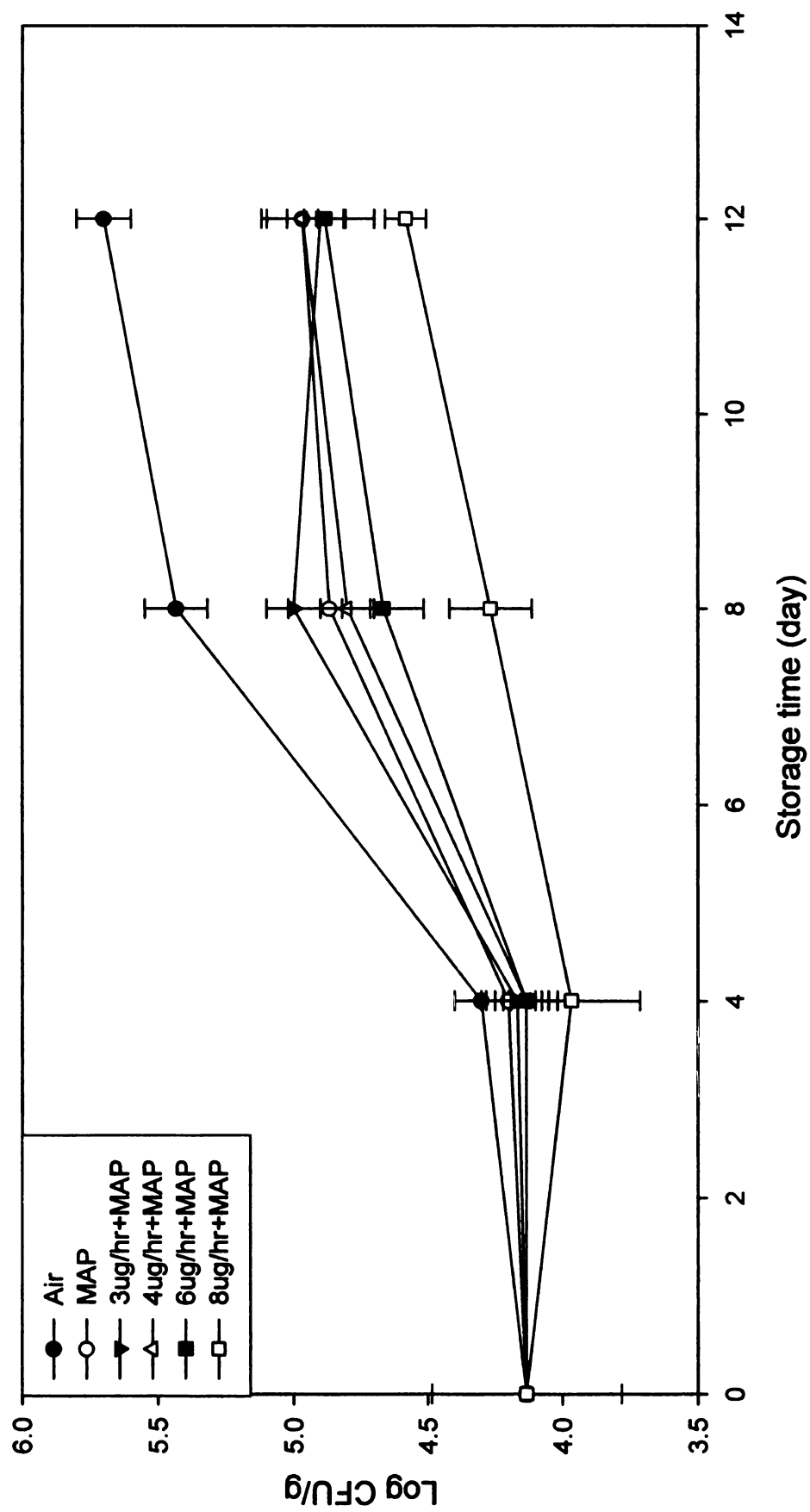


Figure 34. Effect of ClO_2 release rate on *Salmonella Typhimurium* inoculated onto chicken breast and refrigerated at 7°C

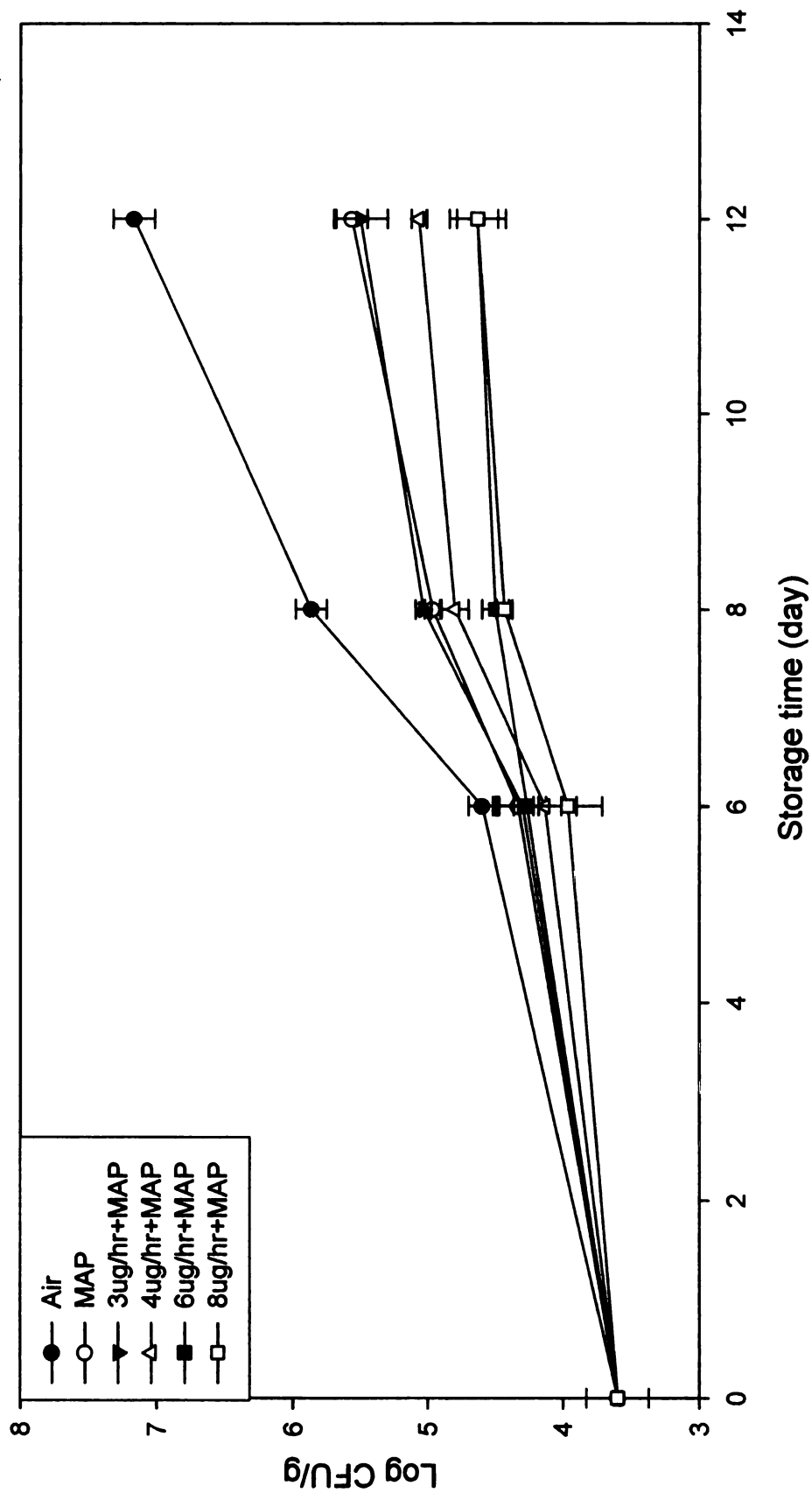


Figure 35. Effect of ClO_2 release rate on *Listeria monocytogenes* inoculated on chicken breast and refrigerated at 7°C

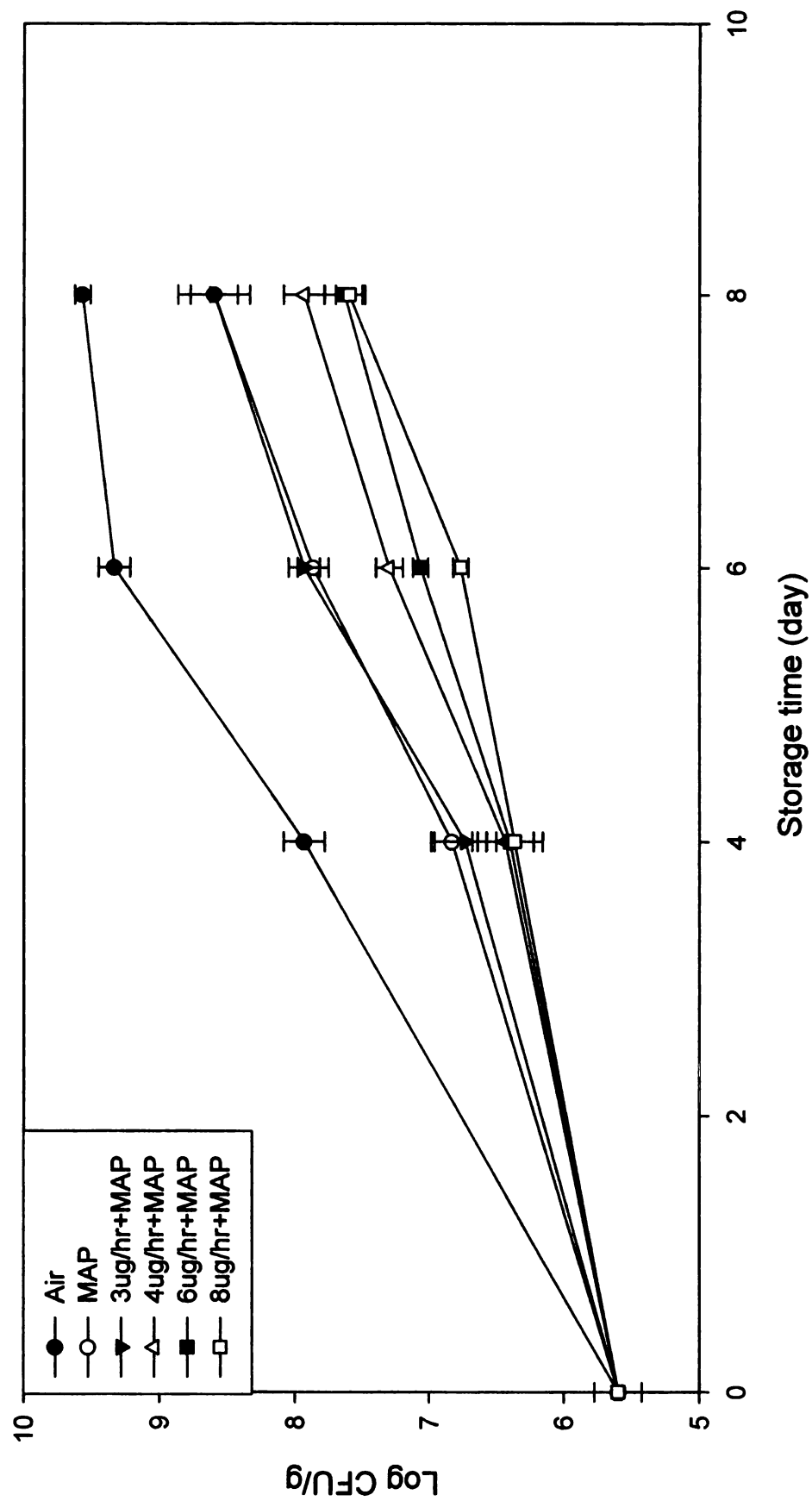


Figure 36. Effect of ClO_2 release rate on total aerobic bacteria on chicken breast and refrigerated at 7°C

Table 6. Inhibition effectiveness of ClO₂ on *Salmonella* Typhimurium, *Listeria monocytogenes*, and total aerobic bacteria on fresh chicken at 7°C

Microorganism	PKG	0 day	4 day	8 day	12 day
Salmonella Typhimurium	AIR	4.13±0.35 _u	4.30±0.10 _u	5.43±0.12 _w	5.70±0.10 _w
	MAP	4.13±0.35 _u	4.20±0.10 _u	4.87±0.15 _v	4.97±0.15 _v
	3.0µg/hr ClO ₂	4.13±0.35 _u	4.17±0.10 _u	5.00±0.10 _v	4.90±0.20 _{uv}
	4.0µg/hr ClO ₂	4.13±0.35 _u	4.13±0.12 _u	4.80±0.10 _v	4.97±0.06 _v
	6.0µg/hr ClO ₂	4.13±0.35 _u	4.13±0.06 _u	4.67±0.15 _v	4.88±0.08 _{uv}
	8.0µg/hr ClO ₂	4.13±0.35 _u	3.97±0.25 _u	4.27±0.15 _u	4.58±0.08 _u
Listeria monocytogene	AIR	3.60±0.23 _u	4.60±0.10 _v	5.87±0.12 _x	7.17±0.15 _x
	MAP	3.60±0.23 _u	4.33±0.15 _u	4.97±0.06 _{ww}	5.57±0.12 _w
	3.0µg/hr ClO ₂	3.60±0.23 _u	4.30±0.17 _u	5.03±0.06 _w	5.50±0.20 _w
	4.0µg/hr ClO ₂	3.60±0.23 _u	4.13±0.23 _u	4.80±0.10 _v	5.07±0.06 _v
	6.0µg/hr ClO ₂	3.60±0.23 _u	4.27±0.25 _u	4.50±0.10 _u	4.63±0.21 _u
	8.0µg/hr ClO ₂	3.60±0.23 _u	3.97±0.25 _u	4.43±0.06 _u	4.63±0.15 _u
Total aerobic bacteria	PKG	0 day	4 day	6 day	8 day
	AIR	5.60±0.17 _u	7.93±0.15 _v	9.33±0.12 _x	9.57±0.06 _x
	MAP	5.60±0.17 _u	6.83±0.15 _u	7.87±0.12 _w	8.60±0.17 _v
	3.0µg/hr ClO ₂	5.60±0.17 _u	6.73±0.23 _u	7.93±0.12 _w	8.60±0.26 _v
	4.0µg/hr ClO ₂	5.60±0.17 _u	6.43±0.21 _u	7.30±0.10 _v	7.93±0.15 _u
	6.0µg/hr ClO ₂	5.60±0.17 _u	6.40±0.17 _u	7.07±0.06 _v	7.63±0.15 _u
	8.0µg/hr ClO ₂	5.60±0.17 _u	6.37±0.21 _u	6.77±0.06 _u	7.60±0.10 _u

Unit=log cfu/g, Mean±standard deviation.

Means with different letters in same columns are significantly different (p<0.05)

3.5. Comparison of the antimicrobial treatment with MAP and antimicrobial packaging

The purpose of the study was to estimate the antimicrobial effectiveness of the antimicrobial in combination with MAP. Microbial enumeration was performed on chicken packaged with an antimicrobial, with and without MAP. If the effectiveness is not decreased, then the atmosphere for the chicken sample would not have to be modified. N₂ gas is substituted for ambient air to prevent aerobic bacteria growth, and CO₂ gas is used to extend the shelf life of food by hindering the growth of spoilage microorganisms (Jimenez et al., 1997) Thus, it was hypothesized that an MAP-antimicrobial combination may be more effective, than either alone.

Half the samples of each selected antimicrobial release system (0.6 µg/hr AITC, 1.2 µg/hr AITC, 4 µg/hr ClO₂, and 8 µg/hr ClO₂) were combined with an MAP gas mixture (30% CO₂/70% N₂), and the other half were sealed in air. The populations of *Salmonella Typhimurium*, *Listeria monocytogenes*, and total aerobic bacteria were investigated. Figures 37-39 show the growth of these organisms after 8 days. The results indicate that antimicrobial treated samples with MAP had significantly more of an inhibition effect than those without MAP (Table 7 and 8). Almost all antimicrobially treated samples packaged in ambient atmosphere had more growth than samples in MAP. The difference in effectiveness with/without MAP was more distinguishable for total aerobic bacteria growth with slow release antimicrobials. At the end of storage, total bacteria counts treated with 0.6 µg/hr AITC with/without MAP were shown to be

statistically different. The bacteria growth was 8.1 log cfu/g in air and 4.6 log cfu/g in MA packages. The total viable counts in 4 $\mu\text{g/hr}$ ClO_2 were 7.8 log cfu/g in air and 5.5 log cfu/g in MAP. The results indicate that the antimicrobial and MAP combination is more effective than antimicrobial treatment alone. Without MAP, a higher antimicrobial dosage is needed to get the same effectiveness as the antimicrobial plus MAP combination.

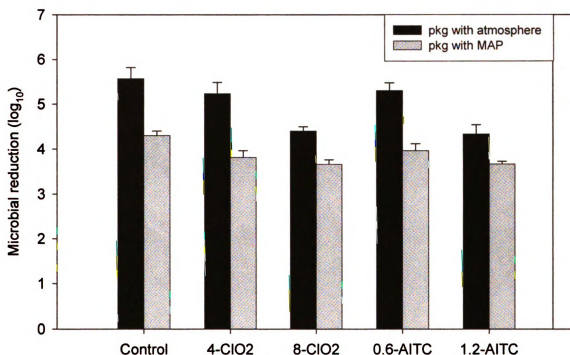


Figure 37. Comparison of inhibition effectiveness of antimicrobial treatments with MAP and without MAP on *Salmonella Typhimurium* on fresh chicken (after 8 days, at 4°C)

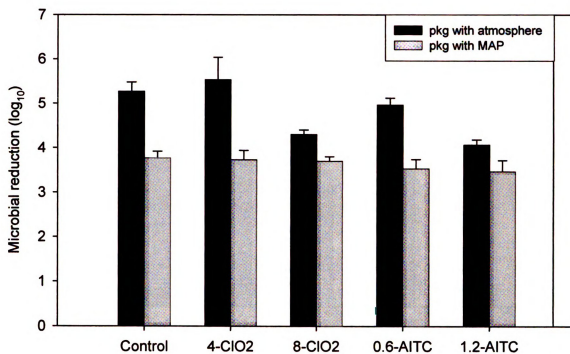


Figure 38. . Comparison of inhibition effectiveness of antimicrobial treatments with MAP and without MAP on *Listeria monocytogenes* on fresh chicken (after 8 days, at 4°C)

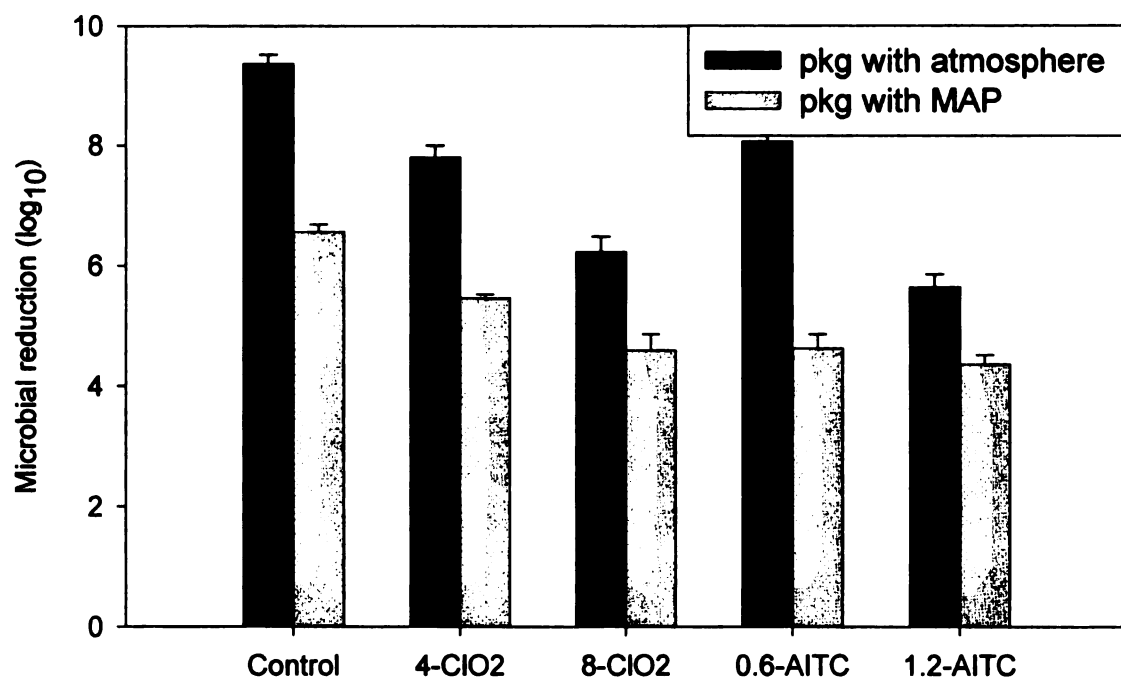


Figure 39. . Comparison of inhibition effectiveness of antimicrobial treatments with MAP and without MAP on total aerobic bacteria on fresh chicken (after 8 days, at 4°C)

Table 7. Comparison of inhibition effectiveness of AITC treatments on *Salmonella* Typhimurium, *Listeria monocytogenes*, and total aerobic bacteria growth on fresh chicken at 4 °C

Microorganism	PKG	0 day	4 day	6 day	8 day
<i>Salmonella</i> Typhimurium	AIR	U ^{3.63±0.06A}	W ^{5.00±0.20B}	W ^{5.33±0.15BC}	W ^{5.57±0.25C}
	MAP	U ^{3.63±0.06A}	W ^{3.90±0.10B}	W ^{4.00±0.10B}	W ^{4.30±0.10C}
	0.6-AITC-AIR	U ^{3.63±0.06A}	W ^{4.00±0.10B}	W ^{4.40±0.10C}	W ^{5.30±0.17D}
	0.6-AITC-MAP	U ^{3.63±0.06A}	U ^{3.63±0.15A}	U ^{3.80±0.10B}	U ^{3.97±0.15C}
	1.2-AITC-AIR	U ^{3.63±0.06A}	W ^{3.93±0.06AB}	W ^{4.03±0.21BC}	W ^{4.33±0.21C}
	1.2-AITC-MAP	U ^{3.63±0.06B}	U ^{2.90±0.10A}	U ^{3.50±0.20B}	U ^{3.67±0.21B}
<i>Listeria</i> <i>monocytogenes</i>	AIR	U ^{3.23±0.25A}	W ^{4.03±0.06B}	W ^{4.73±0.25C}	W ^{5.27±0.21D}
	MAP	U ^{3.23±0.25A}	W ^{3.30±0.20AB}	U ^{3.47±0.06BC}	W ^{3.77±0.15C}
	0.6-AITC-AIR	U ^{3.23±0.25A}	W ^{4.13±0.12B}	W ^{4.30±0.10B}	W ^{4.97±0.15C}
	0.6-AITC-MAP	U ^{3.23±0.25A}	U ^{3.00±0.10A}	U ^{3.20±0.30A}	U ^{3.53±0.21A}
	1.2-AITC-AIR	U ^{3.23±0.25A}	U ^{3.43±0.12A}	U ^{3.40±0.10A}	U ^{4.07±0.12B}
	1.2-AITC-MAP	U ^{3.23±0.25A}	U ^{3.07±0.12A}	U ^{3.03±0.06A}	U ^{3.47±0.25A}
Total aerobic bacteria	AIR	U ^{3.93±0.06A}	W ^{6.10±0.10B}	W ^{7.73±0.25C}	W ^{9.37±0.15D}
	MAP	U ^{3.93±0.06A}	W ^{5.17±0.15B}	W ^{6.00±0.10C}	W ^{6.57±0.12D}
	0.6-AITC-AIR	U ^{3.93±0.07A}	W ^{5.73±0.21B}	W ^{7.03±0.12C}	W ^{8.07±0.15D}
	0.6-AITC-MAP	U ^{3.93±0.07A}	U ^{4.27±0.06AB}	U ^{4.17±0.35AB}	U ^{4.63±0.23B}
	1.2-AITC-AIR	U ^{3.89±0.07A}	U ^{4.45±0.26B}	U ^{5.15±0.21B}	U ^{5.65±0.21C}
	1.2-AITC-MAP	U ^{3.93±0.07A}	U ^{4.23±0.25A}	U ^{4.27±0.21A}	U ^{4.37±0.15A}

Unit=log cfu/g. Mean±standard deviation.

Means with different superscripts in same columns and subscripts in same row are significantly different (p<0.05)

Table 8. Comparison of inhibition effectiveness of ClO₂ treatments on *Salmonella* Typhimurium, *Listeria monocytogenes*, and total aerobic bacteria growth on fresh chicken at 4°C

Microorganism	PKG	0 day	4 day	6 day	8 day
<i>Salmonella</i> Typhimurium	AIR	u ³ 63±0.06 _A	w ⁵ 00±0.20 _{BC}	v ⁵ 33±0.15 _C	w ⁵ 57±0.25 _C
	MAP	u ³ 63±0.06 _A	v ³ 90±0.10 _B	w ⁴ 4.00±0.10 _B	v ⁴ 30±0.10 _C
	4-ClO ₂ -AIR	u ³ 63±0.06 _A	v ³ 90±0.10 _B	x ⁴ 96±0.06 _B	w ⁵ 23±0.25 _B
	4-ClO ₂ -MAP	u ³ 63±0.06 _{AB}	uv ³ 3.37±0.21 _A	uv ⁴ 3.68±0.16 _{AB}	u ³ 81±0.15 _B
	8-ClO ₂ -AIR	u ³ 63±0.06 _A	uv ³ 3.80±0.44 _{AB}	w ⁴ 4.10±0.10 _{AB}	v ⁴ 40±0.10 _B
	8-ClO ₂ -MAP	u ³ 63±0.06 _B	u ³ 3.20±0.20 _A	u ³ 40±0.10 _{AB}	u ³ 66±0.10 _B
<i>Listeria</i> <i>monocytogenes</i>	AIR	u ³ 23±0.25 _A	v ⁴ 03±0.06 _B	x ⁴ 73±0.25 _C	v ⁵ 27±0.21 _C
	MAP	u ³ 23±0.25 _A	v ⁴ 3.30±0.20 _{AB}	w ⁴ 3.47±0.06 _{AB}	v ³ 77±0.15 _B
	4-ClO ₂ -AIR	u ³ 23±0.25 _A	v ⁴ 05±0.73 _{AB}	x ⁴ 60±0.20 _{BC}	v ⁵ 53±0.50 _C
	4-ClO ₂ -MAP	u ³ 23±0.25 _{AB}	u ³ 03±0.25 _A	w ² 93±0.21 _A	u ³ 73±0.21 _B
	8-ClO ₂ -AIR	u ³ 23±0.25 _A	uv ³ 3.60±0.26 _A	w ³ 73±0.15 _A	v ⁴ 30±0.10 _B
	8-ClO ₂ -MAP	u ³ 23±0.25 _{AB}	u ³ 03±0.25 _A	u ³ 3.10±0.10 _A	u ³ 70±0.10 _B
Total aerobic bacteria	AIR	u ³ 93±0.06 _A	w ⁶ 10±0.10 _B	x ⁷ 73±0.25 _C	v ⁹ 37±0.15 _B
	MAP	u ³ 93±0.06 _A	v ⁵ 17±0.15 _B	w ⁶ 00±0.10 _C	x ⁶ 57±0.12 _D
	4-ClO ₂ -AIR	u ³ 93±0.06 _A	u ⁴ 43±0.32 _B	w ⁶ 00±0.10 _C	w ⁷ 80±0.20 _B
	4-ClO ₂ -MAP	u ³ 93±0.06 _A	u ⁴ 27±0.25 _A	v ⁴ 93±0.21 _B	v ⁵ 47±0.06 _C
	8-ClO ₂ -AIR	u ³ 93±0.06 _A	u ⁴ 57±0.12 _B	w ⁵ 57±0.06 _C	w ⁶ 23±0.25 _D
	8-ClO ₂ -MAP	u ³ 93±0.06 _A	u ⁴ 07±0.12 _A	u ⁴ 30±0.20 _{AB}	u ⁴ 60±0.26 _B

Unit=log cfu/g. Mean±standard deviation.

Means with different superscripts in same columns and subscripts in same row are significantly different (p<0.05)

3.6. Fresh chicken packaged with antimicrobials for 21 days

The purpose of this test was to investigate the effect of antimicrobial packaging on the growth of pathogen/spoilage bacteria, pH, and color of such chicken breast samples stored at 4°C. The package systems used in this research experiment are presented in Table 9.

Table 9. Gas mixture sample, and antimicrobial release rates used

Code	Release rate	Gas mixture
AIR	none	Ambient air
MAP	none	30%CO ₂ /70% N ₂
4-CIO ₂	4 µg/hr ClO ₂	30%CO ₂ /70% N ₂
8-CIO ₂	8 µg/hr ClO ₂	30%CO ₂ /70% N ₂
0.6-AITC	0.6 µg/hr AITC	30%CO ₂ /70% N ₂
1.2-AITC	1.2 µg/hr AITC	30%CO ₂ /70% N ₂

Total package volume was 1010 ml

3.6.1. *Listeria monocytogenes* with antimicrobial treatments

Chicken samples were initially inoculated to contain 10³ cfu/g of *Listeria monocytogenes* and examined during 21 days of storage. The population results are shown in Table 10. The growth of *Listeria monocytogenes* at 4°C was slow. The population in all packages studied was about the same until day 9 (except for the samples in AIR). A significant effect was observed ($p < 0.05$) for *Listeria monocytogenes* in the 4-CIO₂, 8-CIO₂, and 1.2-AITC samples from 15 to 21 days. The most inhibition effectiveness was shown by the ClO₂ treatments. At day 15, the population of *Listeria monocytogenes* with 8-CIO₂ was 0.94 logs lower than MAP (Figure 40). On day 21, the total counts on chicken in the 8-CIO₂ were 2.75 and 1.87 logs lower compared to AIR and MAP respectively, There was also a

significant difference as a function of the ClO₂ release rates between 4-ClO₂ and 8-ClO₂. From 12 to 21 days, the growth of *Listeria monocytogenes* in the 4-ClO₂ was higher than in the 8-ClO₂. On day 21, the microbial counts in 4-ClO₂ was 5.60 log cfu/g while 8-ClO₂ had 4.84 log cfu/g.

The growth of *Listeria monocytogenes* in the 1.2-AITC had a statistically similar growth trend compared to 4-ClO₂ throughout the storage period. After 15 days, the growth of *Listeria monocytogenes* in 1.2-AITC was significantly lower than in either AIR or MAP. On day 21, the counts in 1.2-AITC were approximately 1.65 and 0.77 logs lower than in AIR and MAP respectively. A statistical difference between 0.6-AITC and MAP was not observed ($p>0.05$). However, samples in 0.6-AITC had lower microbial counts than the controls on days 12, 15, and 21.

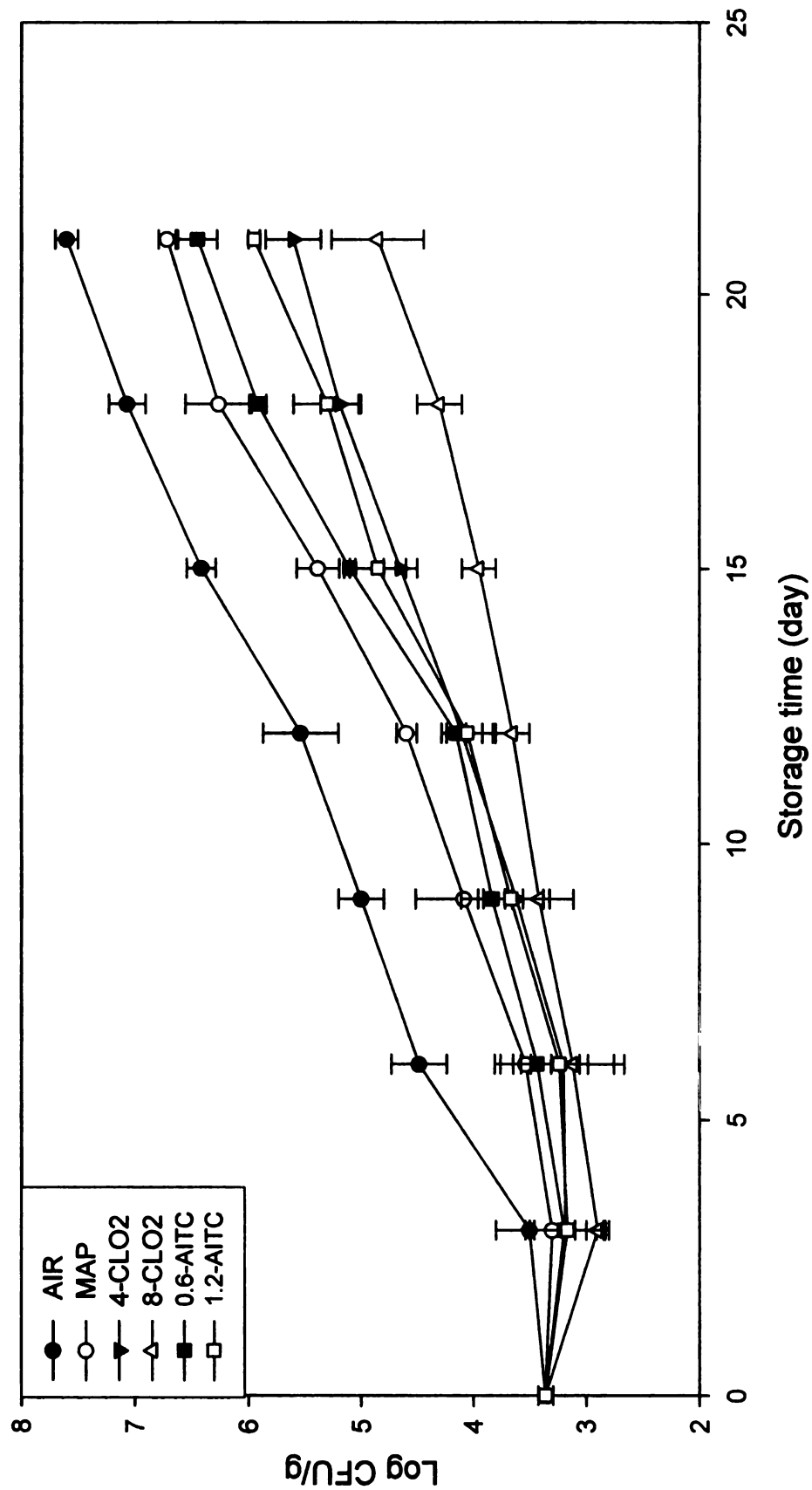


Figure 40. Growth of *Listeria monocytogenes* on fresh chicken breast as a function of storage time, at 4°C.

Table 10. Growth of *Listeria monocytogenes* (log cfu/g) on fresh chicken breast with constant release antimicrobial treatment (ClO₂ and AITC) during 21 days of storage at 4°C

	0 day	3 day	6 day	9 day	12 day	15 day	18 day	21 day
AIR	0.36± 0.07 ^A	0.50± 0.30 ^A	0.48± 0.25 ^B	0.50± 0.20 ^B	0.54± 0.34 ^{BC}	0.42± 0.13 ^D	0.70± 0.16 ^E	0.60± 0.10 ^E
MAP	0.36± 0.07 ^A	0.30± 0.20 ^A	0.53± 0.23 ^A	0.48± 0.43 ^{AB}	0.59± 0.09 ^{BC}	0.38± 0.19 ^{CD}	0.26± 0.29 ^E	0.72± 0.08 ^E
4-ClO ₂	0.36± 0.07 ^A	0.18± 0.28 ^A	0.20± 0.44 ^A	0.62± 0.29 ^{AB}	0.10± 0.18 ^{BC}	0.65± 0.15 ^{CD}	0.19± 0.17 ^{DE}	0.50± 0.25 ^E
8-ClO ₂	0.36± 0.07 ^{AB}	0.19± 0.10 ^A	0.12± 0.46 ^A	0.42± 0.30 ^{AB}	0.65± 0.15 ^{ABC}	0.95± 0.15 ^{BC}	0.30± 0.20 ^{CD}	0.85± 0.41 ^D
0.6-AITC	0.36± 0.07 ^{AB}	0.35 ^A 0.17±	0.38 ^{AB} 0.24±	0.28 ^{BC} 0.67±	0.09 ^C 0.40±	0.05 ^C 0.85±	0.08 ^D 0.30±	0.17 ^D 0.59±
1.2-AITC	0.07 ^A	0.33 ^A	0.25 ^A	0.29 ^{AB}	0.23 ^B	0.25 ^C	0.30 ^{CD}	0.05 ^D

Unit=log cfu/g. Mean ± standard deviation.

Means with different superscripts in same columns and subscripts in same row are significantly different (p<0.05)

3.6.2. *Salmonella* Typhimurium with antimicrobial treatments

As seen in Table 11, the level of *Salmonella* Typhimurium was significantly reduced ($p < 0.05$) by the antimicrobial treatments. No matter the release rate, the level of *Salmonella* Typhimurium present in the antimicrobial plus MAP was significantly lower than the control, and the reduction difference increased as a function of time. Inoculated *Salmonella* Typhimurium chicken breast delayed its lag phase until 9 days while control packages were significantly higher at 6 days. The greater inhibition effectiveness was found with the AITC treatment. On day 21, the microbial counts in 1.2-AITC were approximately 2.1 and 1.3 logs lower than AIR and MAP, respectively. 0.6-AITC was shown to have a statistically similar effect to 1.2-AITC. Except for day 18, the growth of *Salmonella* Typhimurium on the chicken in both packages (0.6-AITC and 1.2-AITC) was not significantly different. AITC treatment has been shown to have more antimicrobial effectiveness on Gram-negative bacteria (such as *Escherichia Coli*, *Samonella*, *Peudomonas*, etc) than Gram-positive bacteria (such as *Listeria* spp., *Staphylococcus* spp., etc) (Issiki et al., 1992; Kim et al., 2002; Delaquis et al., 1997). The results show that AITC had better antimicrobial performance against *Salmonella* Typhimurium than *Listeria monocytogenes*.

ClO_2 sachet packages were also significant different ($p < 0.05$) than the control packages (AIR and MAP) against *Salmonella* Typhimurium as storage time increased. However, overall inhibition performance was significantly lower than with the AITC treatments. The amount of *Salmonella* Typhimurium in the 8- ClO_2 package was approximately 1.40 and 0.70 logs lower than in the AIR and

MAP respectively. ClO_2 had shown strong antimicrobial performance against the pathogens in the agar test. Both *Salmonella* Typhimurium and *Listeria monocytogenes* treated with ClO_2 , were eliminated with 160 $\mu\text{g/l}$ while 360 $\mu\text{g/l}$ AITC was required to eliminate these pathogens. With fresh chicken, however, a much higher ClO_2 treatment was required to get any significant inhibition. The released ClO_2 gas may be absorbed into the moist product mass due to its high water solubility (2.63 g/l), where it will quickly decompose.

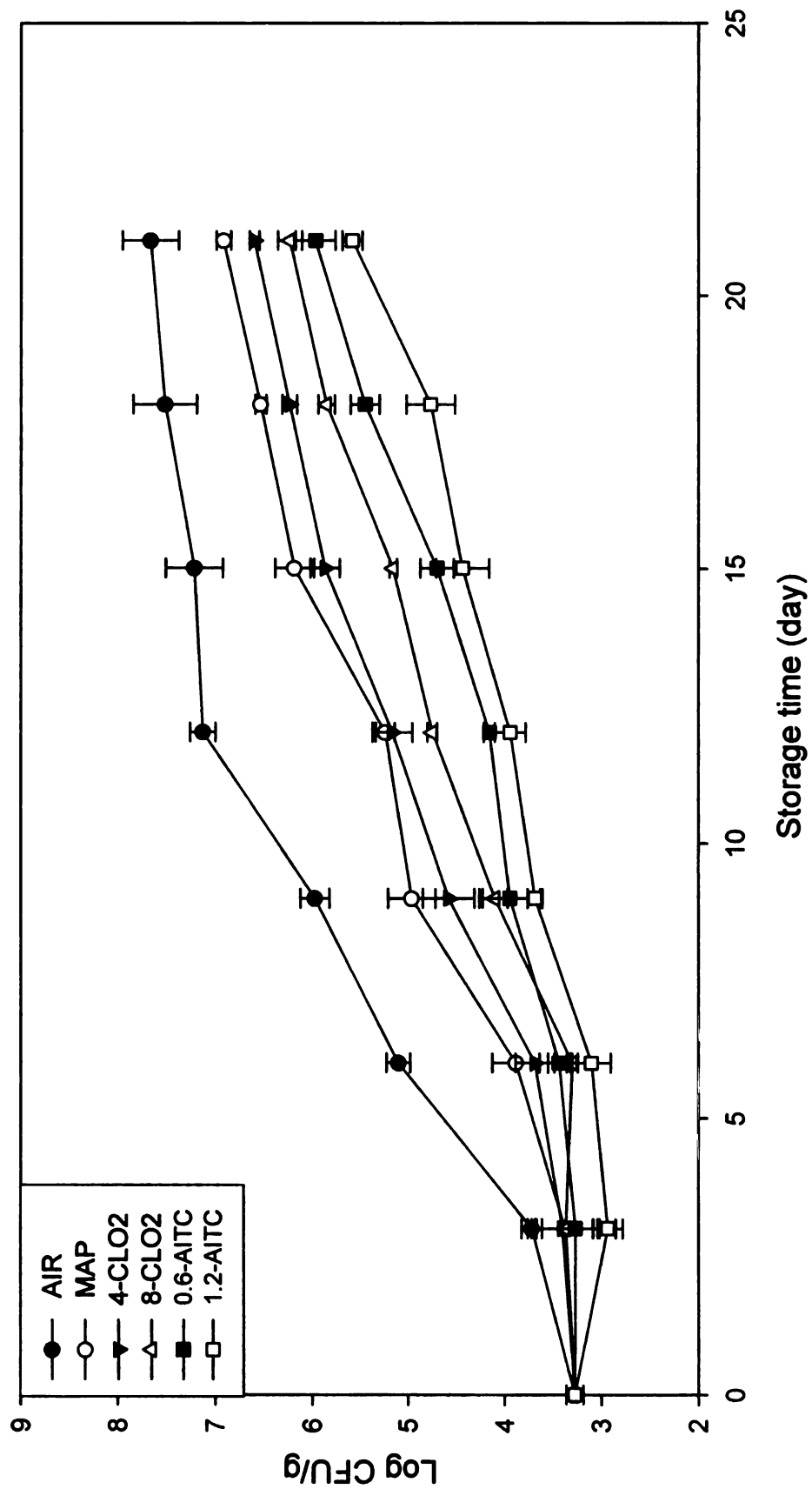


Figure 41. Growth of *Salmonella Typhimurium* on fresh chicken breast as a function of storage time, at 4°C.

Table 11. Growth of *Salmonella* Typhimurium (log cfu/g) on fresh chicken breast with constant release antimicrobial treatments (ClO₂ and AITC) during 21 days of storage at 4°C

	0 day	3 day	6 day	9 day	12 day	15 day	18 day	21 day
AIR	0.327± 0.09 _A	0.372± 0.10 _A	0.510± 0.12 _B	0.597± 0.15 _C	0.713± 0.13 _D	0.722± 0.29 _D	0.752± 0.33 _D	0.767± 0.29 _D
MAP	0.327± 0.09 _A	0.337± 0.29 _{AB}	0.388± 0.24 _B	0.496± 0.25 _C	0.524± 0.10 _C	0.618± 0.20 _D	0.653± 0.06 _{DE}	0.692± 0.08 _E
4-ClO ₂	0.327± 0.09 _A	0.340± 0.36 _A	0.368± 0.20 _A	0.458± 0.27 _B	0.517± 0.21 _C	0.587± 0.15 _D	0.623± 0.08 _{DE}	0.660± 0.05 _E
8-ClO ₂	0.327± 0.09 _A	0.337± 0.36 _A	0.330± 0.06 _A	0.410± 0.13 _B	0.475± 0.05 _C	0.516± 0.04 _C	0.585± 0.09 _D	0.623± 0.13 _D
0.6-AITC	0.327± 0.09 _A	0.327± 0.42 _A	0.343± 0.12 _{AB}	0.394± 0.06 _{BC}	0.416± 0.06 _{CD}	0.470± 0.17 _D	0.45± 0.15 _E	0.597± 0.21 _E
1.2-AITC	0.327± 0.09 _A	0.293± 0.15 _A	0.310± 0.20 _A	0.368± 0.08 _{BC}	0.394± 0.16 _{CD}	0.43± 0.28 _{DE}	0.477± 0.25 _E	0.58± 0.10 _F

Unit=log cfu/g. Mean±standard deviation.

Means with different superscripts in same columns and subscripts in same row are significantly different (p<0.05)

3.6.3. Total viable counts with antimicrobial treatments

The total plate counts of packaged chicken breasts, not inoculated with any pathogenic organism, were also determined to investigate the effect of the antimicrobials' on broad spoilage bacteria. The results are shown in Table 12. Antimicrobial treatments (ClO_2 and AITC) and their relative release rates had a significant inhibition effect on total viable counts ($p < 0.05$).

The highest inhibition effectiveness on chicken breast was obtained with 1.2-AITC. On day 3, the initial population decreased 0.7 logs with 1.2-AITC. The largest difference between MAP and 1.2-AITC was 2.7 log cfu/g on day 15. 0.6-AITC was also shown to have a significant inhibition effect. On day 21, the total viable counts on chicken breast were 9.3, 9.0, and 8.0 log cfu/g in AIR, MAP, and 0.6-AITC. The results show that both AITC release rates inhibited total aerobic bacteria growth during the storage period.

The 8- ClO_2 had a statistically similar effect to 1.2-AITC, until day 15. Then, the total aerobic bacteria increased rapidly. At the end of storage, there was 1.5 and 1.2 log cfu/g less than in AIR and MAP respectively. No significant effectiveness was observed in 4- ClO_2 until day 12. On day 15, the bacterial counts in 4- ClO_2 were 1.4 and 0.8 logs lower ($p < 0.05$) than samples in the controls (AIR and MAP).

Overall, AITC performed better with smaller dosage than ClO_2 treatment. ClO_2 is able to kill a broad range of bacteria with a very small concentration (less than 1 ppm) on a hard surface (Speronello, 2005). However, a much greater ClO_2 dosage is required to get a significant antimicrobial effect on chicken breast.

ClO_2 is broadly effective against microorganisms at a wide range of pHs, but its performance is reduced in high moisture food (Brody, 2006).

At a microbial population of more than 10^8 cfu/g, the product developed ester-like odors and numerous small translucent colonies on the surface of the chicken. At this level of microorganisms, the product shelf life had ended (Mead, 2004). From a microbial standpoint, 0.6, 1.2-AITC, and 8- ClO_2 packages extended the microbial critical point to more than 21 days. Many other factors for shelf life evaluation (such as flavor, texture, color, and so on) were not considered in this study. Further research will be required to clarify the time effect of antimicrobial treatment on product shelf life of fresh chicken breast.

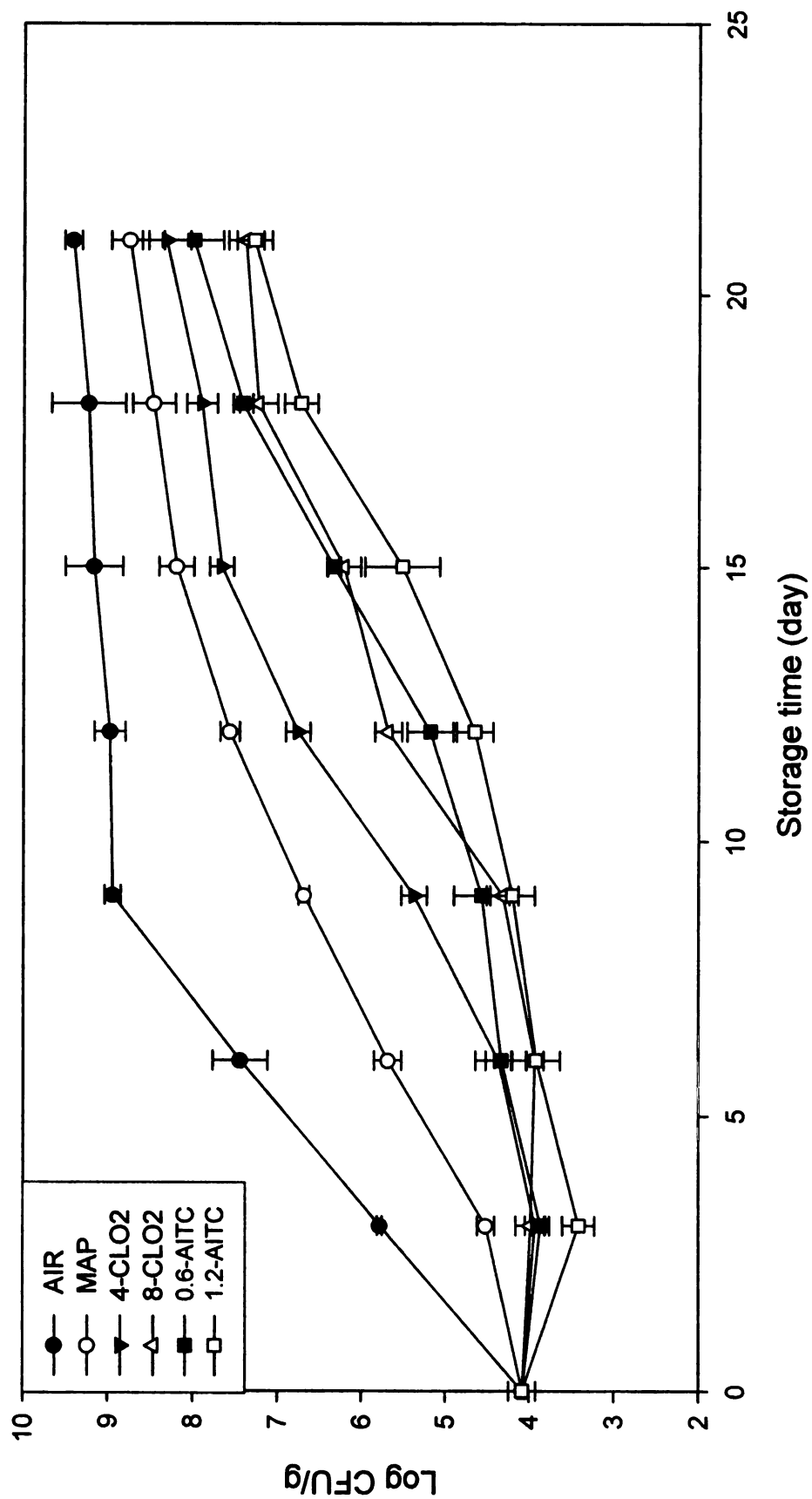


Figure 42. Total aerobic bacteria on fresh chicken breast as a function of storage time at 4°C

Table 12. Total aerobic bacteria counts (log cfu/g) on fresh chicken breast with constant release antimicrobials (ClO₂ and AITC) during 21 days of storage at 4°C

	0	3	6	9	12	15	18	21
AIR	U4.08± 0.16 _A	U5.78± 0.03 _B	U7.44± 0.32 _C	U8.95± 0.09 _D	U8.98± 0.18 _D	U9.17± 0.34 _D	U9.23± 0.44 _D	U9.42± 0.10 _D
MAP	U4.08± 0.16 _A	V4.52± 0.10 _A	V5.69± 0.16 _B	V6.69± 0.07 _C	V7.57± 0.12 _D	V8.20± 0.21 _E	V8.47± 0.25 _{FG}	V8.75± 0.22 _G
4-ClO ₂	U4.08± 0.16 _A	W3.93± 0.12 _A	W4.37± 0.15 _A	W5.38± 0.15 _B	W6.76± 0.14 _C	W7.66± 0.14 _D	W7.90± 0.18 _{DE}	W8.32± 0.29 _E
8-ClO ₂	U4.08± 0.16 _A	W3.98± 0.18 _A	X3.93± 0.10 _A	X4.32± 0.19 _A	X5.68± 0.16 _B	WX6.22± 0.20 _C	XY7.23± 0.23 _D	XY7.38± 0.21 _D
0.6-AITC	U4.08± 0.16 _A	W3.87± 0.10 _A	X4.33± 0.31 _{AB}	X4.57± 0.33 _{BC}	X5.18± 0.28 _C	W6.33± 0.08 _D	WX7.42± 0.12 _E	WX8.00± 0.35 _E
1.2-AITC	U4.08± 0.16 _A	X3.42± 0.19 _A	X3.92± 0.28 _{AB}	X4.20± 0.26 _B	Y4.65± 0.22 _B	X5.52± 0.45 _C	Y6.73± 0.20 _D	Y7.28± 0.21 _D

Unit=log cfu/g. Mean±standard deviation.

Means with different superscripts in same columns and subscripts in same row are significantly different (p<0.05)

3.6.4. pH of chicken breast during 21 days of storage

Many elements can affect the pH of a food during storage. Mead (2004) said that the pool of amino acids (meat) may become depleted, by microbes or spoilage bacteria. There may be microbial proteolysis and lipolytic activity. Under these conditions, oxygen content in the package may be greatly diminished and carbon dioxide increased, and thus, the bacteria population ultimately is dominated by slow growing lactic acid bacteria which are tolerant to CO₂. Therefore, pH value may increase at first, and then decrease due to the growth of lactic acid bacteria. Dunnick et al. (1982) mentioned that increase in meat pH during storage mainly comes from microbial growth. Thus, little change in the pH of chicken meat may be an indication that little microbial growth has occurred in storage. The average pH of the chicken breast in the different packages (AIR, MAP, 4-CIO₂, 8-CIO₂, 0.6-AITC, and 1.2-AITC) and stored at 4°C for up to 21 days is shown in Table 13. Two sets of chicken breasts were obtained from different sources, and each source had a different initial pH value (5.79 vs. 6.33). Therefore, the results are reported separately in the table. Both had similar pH profiles during storage.

As is shown in Table 13, the release rate of the CIO₂ treatments had a significant effect on pH of the chicken samples. The pH values of chicken in 8-CIO₂ was significantly lower from day 6, and then decreased as a function of time. The pH of the chicken taken from the last day of storage was 5.45 and 5.93 (for the 1st and 2nd sets, respectively). 4 µg/hr CIO₂ also had some effect on pH of the chicken. On days 15-18, the chicken pH in the 4-CIO₂ package was significantly

lower than initially. In contrast, no significant pH changes were observed in the chicken treated with AITC. The pH profiles over 21 days were statistically the same for both 0.6-AITC and 1.2-AITC. However, ClO₂ treatments significantly decreased ($p < 0.05$) the pH values of the fresh chicken. As shown in Table 13, chicken breast exposed to the higher ClO₂ treatment had lower pH values. pH of the chicken samples in 8-ClO₂ decreased from 5.79 to 5.45 in the first set, and from 6.33 to 5.93 in the second after 21 days. No significant difference ($p < 0.05$) was observed with 4-ClO₂ in the first set, and a slight decrease from 6.33 to 6.25 was noted in the second set. The pH drop may be due to ClO₂ gas absorption into the product. ClO₂ can penetrate into the moist and tender chicken. The absorbed ClO₂ may then be broken down, leaving chloride ion (Cl⁻) on the chicken (Ellis et al., 2005). This is likely the reason why the pH value of chicken meat treated with ClO₂ was significantly lower than in other packages.

The average pH values of chicken in AIR significantly increased during storage ($p < 0.05$). The pH levels (initial 5.79 and 6.33) began to increase at 6 days, and rose to 6.31 and 6.54 (in the first and second sets, respectively) on day 21. The pH in MAP was also affected by the storage time but the rate of increase was significantly slower than in AIR. The pH values from each test were statistically higher after day 12, and increased from 6.01 to 6.45. Total aerobic bacteria in these samples (Table 12) in MAP and AIR became very high ($> 10^8$ cfu/g) within 9 to 12 days. It is likely that there is a strong relationship between microbial growth and pH as shown in the chicken breast samples. Aksu (2006) also showed that the pH values of chicken breast and drumstick

significantly increased ($p < 0.01$) during storage. Quio et al. (2002) showed that the pH values of broiler breast were 5.82 to 6.23. The authors assumed the reason to be the production of proteases by psychotropic bacteria during storage.

Allen et al. (1998) said that the ultimate pH of meat is highly dependent upon the amount of glycogen present in the muscle. Chicken meat with high pH is darker and redder than meat with low pH. Higher pH meat had a lower lightness value (data will be shown in the next section). Phillips (1996) stated that CO_2 from MAP is able to change the original pH values by subsequent ionization of carbonic acid which can form on the surface of the food by CO_2 absorption. However, it was a very small change, and did not cause any significant effect compared to the pH of the samples in AIR.

Table 13. The pH values of fresh chicken breast during 21 days of storage, from 2 different local stores

PKG		0	3	6	9	12	15	18	21
1 st Test (A)	AIR	5.79± 0.01 ^A	5.81± 0.02 ^A	6.01± 0.02 ^{BC}	5.95± 0.06 ^C	6.08± 0.05 ^C	6.22± 0.03 ^D	6.25± 0.04 ^{DE}	6.31± 0.01 ^E
		5.79± 0.01 ^A	5.80± 0.03 ^A	5.81± 0.01 ^A	5.80± 0.03 ^A	5.95± 0.06 ^B	5.99± 0.07 ^B	5.95± 0.06 ^B	6.01± 0.05 ^B
	MAP	5.79± 0.01 ^A	5.77± 0.04 ^A	5.78± 0.02 ^A	5.79± 0.02 ^A	5.81± 0.02 ^A	5.79± 0.12 ^A	5.75± 0.04 ^A	5.74± 0.02 ^A
		5.79± 0.01 ^A	5.76± 0.07 ^A	5.59± 0.05 ^B	5.46± 0.04 ^{BC}	5.43± 0.04 ^C	5.41± 0.14 ^C	5.51± 0.03 ^{BC}	5.45± 0.07 ^{BC}
	4-ClO ₂	5.79± 0.01 ^A	5.81± 0.02 ^A	5.75± 0.07 ^A	5.82± 0.04 ^A	5.78± 0.11 ^A	5.79± 0.09 ^A	5.83± 0.02 ^A	5.84± 0.04 ^A
		5.79± 0.01 ^A	5.81± 0.02 ^A	5.78± 0.07 ^A	5.78± 0.04 ^A	5.81± 0.03 ^A	5.76± 0.08 ^A	5.81± 0.01 ^A	5.85± 0.03 ^A
	0.6-AITC	5.79± 0.01 ^A	5.81± 0.02 ^A	5.78± 0.08 ^A	5.78± 0.04 ^A	5.81± 0.03 ^A	5.76± 0.08 ^A	5.81± 0.01 ^A	5.85± 0.03 ^A
		5.79± 0.01 ^A	5.81± 0.02 ^A	5.78± 0.08 ^A	5.78± 0.04 ^A	5.81± 0.03 ^A	5.76± 0.08 ^A	5.81± 0.01 ^A	5.85± 0.03 ^A
	1.2-AITC	5.79± 0.01 ^A	5.81± 0.02 ^A	5.78± 0.08 ^A	5.78± 0.04 ^A	5.81± 0.03 ^A	5.76± 0.08 ^A	5.81± 0.01 ^A	5.85± 0.03 ^A
		5.79± 0.01 ^A	5.81± 0.02 ^A	5.78± 0.08 ^A	5.78± 0.04 ^A	5.81± 0.03 ^A	5.76± 0.08 ^A	5.81± 0.01 ^A	5.85± 0.03 ^A
	AIR	6.33± 0.02 ^A	6.38± 0.05 ^A	6.41± 0.05 ^{AB}	6.48± 0.02 ^{BC}	6.47± 0.01 ^{BC}	6.48± 0.04 ^{BC}	6.49± 0.06 ^{BC}	6.54± 0.02 ^C
		6.33± 0.02 ^A	6.29± 0.03 ^A	6.34± 0.01 ^A	6.31± 0.01 ^A	6.41± 0.05 ^B	6.43± 0.01 ^B	6.45± 0.03 ^B	6.45± 0.03 ^B
2 nd Test (B)	MAP	6.33± 0.02 ^A	6.35± 0.03 ^A	6.29± 0.01 ^A	6.30± 0.02 ^A	6.26± 0.04 ^A	6.26± 0.08 ^A	6.25± 0.07 ^A	6.25± 0.05 ^A
		6.33± 0.02 ^A	6.38± 0.03 ^A	6.29± 0.01 ^A	6.30± 0.02 ^A	6.26± 0.04 ^A	6.26± 0.08 ^A	6.25± 0.07 ^A	6.25± 0.05 ^A
	4-ClO ₂	6.33± 0.02 ^A	6.38± 0.10 ^A	6.09± 0.04 ^B	6.06± 0.03 ^{BC}	6.02± 0.05 ^{BCD}	6.00± 0.03 ^{BCD}	5.95± 0.06 ^{CD}	5.93± 0.05 ^D
		6.33± 0.02 ^A	6.36± 0.03 ^A	6.33± 0.07 ^A	6.35± 0.07 ^A	6.32± 0.06 ^A	6.30± 0.12 ^A	6.34± 0.04 ^A	6.35± 0.04 ^A
	8-ClO ₂	6.33± 0.02 ^A	6.33± 0.03 ^A	6.35± 0.07 ^A	6.35± 0.07 ^A	6.34± 0.06 ^A	6.35± 0.12 ^A	6.35± 0.04 ^A	6.35± 0.04 ^A
		6.33± 0.02 ^A	6.33± 0.03 ^A	6.35± 0.07 ^A	6.35± 0.07 ^A	6.34± 0.06 ^A	6.35± 0.12 ^A	6.35± 0.04 ^A	6.35± 0.04 ^A
	0.6-AITC	6.33± 0.02 ^A	6.33± 0.03 ^A	6.35± 0.07 ^A	6.35± 0.07 ^A	6.34± 0.06 ^A	6.35± 0.12 ^A	6.35± 0.04 ^A	6.35± 0.04 ^A
		6.33± 0.02 ^A	6.33± 0.03 ^A	6.35± 0.07 ^A	6.35± 0.07 ^A	6.34± 0.06 ^A	6.35± 0.12 ^A	6.35± 0.04 ^A	6.35± 0.04 ^A
	1.2-AITC	6.33± 0.02 ^A	6.33± 0.03 ^A	6.35± 0.07 ^A	6.35± 0.07 ^A	6.34± 0.06 ^A	6.35± 0.12 ^A	6.35± 0.04 ^A	6.35± 0.04 ^A
		6.33± 0.02 ^A	6.33± 0.03 ^A	6.35± 0.07 ^A	6.35± 0.07 ^A	6.34± 0.06 ^A	6.35± 0.12 ^A	6.35± 0.04 ^A	6.35± 0.04 ^A
	AIR	6.33± 0.02 ^A	6.38± 0.05 ^A	6.41± 0.05 ^{AB}	6.48± 0.02 ^{BC}	6.47± 0.01 ^{BC}	6.48± 0.04 ^{BC}	6.49± 0.06 ^{BC}	6.54± 0.02 ^C
		6.33± 0.02 ^A	6.29± 0.03 ^A	6.34± 0.01 ^A	6.31± 0.01 ^A	6.41± 0.05 ^B	6.43± 0.01 ^B	6.45± 0.03 ^B	6.45± 0.03 ^B

A: source from Goodrich store, East Lansing, MI, B: Source from Sam's club, Lansing, MI

Means with different superscripts in same columns and subscripts in same row are significantly different (p<0.05)

3.6.5. Headspace gas analysis of the chicken packages

The evolution of gas in the chicken packages is shown in Table 14.

Wolfe (1980) observed that dynamic changes in the composition of a package atmosphere can be due to permeation, leakage and biochemical conversion due to microbial respiratory activity. During microbial growth, oxygen concentration in a meat package usually diminishes rapidly and carbon dioxide (CO₂) will be increased. Without oxygen, anaerobic bacteria will grow in the package and also produce CO₂. Therefore, the dynamic gas composition in a package can be used to investigation the microbial growth during storage. The gas composition can be affected by modified atmosphere or controlled atmosphere packaging systems.

Carbon dioxide (CO₂) concentration in antimicrobially treated packages decreased with increasing time of storage until day 12, likely because the meat absorbed the CO₂ gas via the surface of the fresh chicken. After 12 days, the CO₂ levels were increased. However, the increase was significantly lower than that of the MAP control. CO₂ in AIR increased greatly during storage. The initial concentration was 0.1%, and it rose to 18.55% on day 21. The results indicate that there must have been a lot of microbial growth. Lipid oxidation could be another reason for these results in the AIR.

Table 14. Change in gas composition in the package headspace during storage of chicken breasts (n=3) at 4°C

		0	6	12	18	21
AIR	O ₂ (%)	20.1	18.3	10.2	5.5	3.3
	CO ₂ (%)	0.1	4.7	12.5	17.3	18.5
MAP	O ₂ (%)	0.5	0.0	0.0	0.0	0.0
	CO ₂ (%)	29.3	24.1	25.0	31.0	29.70
4-CIO ₂	O ₂ (%)	0.5	0.0	0.0	0.0	0.0
	CO ₂ (%)	29.0	22.1	22.0	23.2	25.0
8-CIO ₂	O ₂ (%)	0.5	0.0	0.0	0.0	0.0
	CO ₂ (%)	28.5	20.1	16.5	22.9	23.4
0.6-AITC	O ₂ (%)	0.5	0.0	0.0	0.0	0.0
	CO ₂ (%)	29.9	19.0	21.0	23.8	24.0
1.2-AITC	O ₂ (%)	0.5	0.0	0.0	0.0	0.0
	CO ₂ (%)	29.0	19.2	18.0	22.9	23.0

3.6.6. Surface color of fresh chicken during 21 days of storage

To the consumer, appearance is the major criterion leading to purchase of meat, and is used to evaluate meat quality (Allen, 1998). Thus, meat color plays a critical role in the consumer's perception in deciding upon whether to purchase a meat product. Other studies have shown that microbial growth did not result in any significant color change (Allen et al., 1998; Quio et al., 2002; Sams, 2001). The purpose of this test was to investigate whether any discoloration occurred due to ClO₂ or AITC treatment. The surface color of the chicken meat was observed using an analytical instrument (colorimeter) as a function of storage time, and the results are described using three parameters (L, a, and b), as shown in Tables 15 -20.

The analytical color test was performed on two separate sets of chicken, which had some initial color difference. The difference was too small to discern with the naked eye, however, the difference was statistically significant. The

chicken in the second set also had a higher initial pH value. Thus, the results are provided in separate tables for the first and second tests.

8-ClO₂ and 1.2-AITC were shown to cause significant differences in surface color of the chicken. The "L" values in 8-ClO₂ decreased by 4.88 (49.37 to 44.49) and 4.66 (44.14 to 39.49). At high ClO₂ treatment, the part of the chicken nearest the ClO₂ sachet became brown black, and the discoloration became more intense as time went on. Thus, the "L" value was reduced during storage. The color changes in 1.2-AITC were nearly opposite to that of the ClO₂ treatment. The "L" value was statistically higher at 18 days in both sets. On day 21, the overall "L" value increased to 5.3 (49.37 to 54.67) for the first set and 3.1 (44.15-->48.68) for the second. No significant difference was observed in 4-ClO₂, 0.6- AITC, and MAP during storage. At the edge of the chicken breast nearest to the 4µg/hr release ClO₂ sachet, chicken meat samples were shown to be slightly yellow black in color. However, overall test results did not show any statistical difference.

The chicken breast in the second set was a little bit darker than the first. Overall lightness (L) was lower than for the chicken of the first set. Even though there was an initial color difference, the results were very similar in terms of color change due to the antimicrobial treatments. Ngoka and Froning (1982) reported that the pH of meat is highly dependent upon the amount of glycogen present in the muscle. Yang and Chen (1993) confirmed that chicken meat with high pH was darker, redder, and yellower in color than meat with lower pH. Thus, the initial color difference may be due to the pH difference (table 13).

Both chicken samples (from the 1st and 2nd batches) in AIR were shown to have higher “L” values than MAP. On the last day, the “L” values in AIR were slightly decreased, 3.22 (53.51 to 50.29) and 2.32 (46.14 to 43.23) for the 1st and 2nd sets respectively, and the values were the same for 4-ClO₂, 0.6- AITC, and MAP. Lawrie (1998) stated that meat in an aerobic condition has a brighter red color (high “L” and “a” value) than MAP due to the formation of oxymyoglobin, and it is eventually oxidized to form metmyoglobin which is a dark color. Unlike fresh red meat, however, fresh chicken breasts usually have a pinkish-white to yellow color and color change by oxidation will not have a significant impact on consumer perception. (Sams, 2001)

Table 17 - 18 show the “a” values recorded for chicken samples packaged in the 1st and 2nd sets. The “a” value from chicken breast treated with 8-ClO₂ was shown to be a positively correlated with the amount of dosage. Chicken breast stored in 8-ClO₂ had a significantly increased “a” value ($p < 0.05$) from 9th day for the first test and 6th day for the second test. The values increased as storage time increased. On day 21, The “a” in 8-ClO₂ increased by 1.54 (1.65 to 3.19) and 1.14 (3.54 to 4.68) for the 1st and 2nd tests, respectively. Oppositely, “a” of chicken in 1.2-AITC was shown to have a negative correlation with amount released and “a” value. After 18 days, “a” in 1.2-AITC was significantly lower ($p < 0.05$) than “a” on day 3. On day 21, the total decrease in “a” in 1.2-AITC was 1.35 (1.75 to 0.40) and 1.07 (3.62 to 2.63) for the first and second test, respectively. No significant difference in “a” values were observed on chicken breasts in 4-ClO₂, 0.6-AITC, MAP, and AIR during storage.

There was a significant effect on the “b” values of chicken samples in 8-ClO₂ and 1.2-AITC. As shown in Table 19 and 20, “b” values in these packages increased significantly as a function of time. The chicken in 8-ClO₂ had higher “b” values after 6 days in both tests. On days 21, “b” values had increased 1.04 (2.39 to 3.43) in the 1st test and 2.43 (1.81 to 4.24) in the 2nd test. All chicken samples in 8-ClO₂ exhibited the same discoloration. A black/greenish color developed in areas close in proximity to the ClO₂ sachet, and the outside of the main discolored portion had more yellow color. It is likely that the ClO₂ gas was quickly absorbed into the chicken nearest the sachets. The reason for this discoloration of meat has not yet been clearly investigated. Ellis et al. (2005) said that the green/dark color (on chicken) could be due to choleglobin formation. The iron atom in the center of a hematic nucleus of myoglobin may be reduced through exposure to the reducing agent (ClO₂).

The “b” value in 1.2-AITC significantly increased after 18 days. On day 21, “b” values in the 1st and 2nd tests increased by 1.22 (2.23 to 3.50) and 1.16 (1.82 to 2.98), respectively. At the end of storage, the surface color of the chicken was observed to be a bright and yellowish green over the whole surface. The color change occurred over a wide area of the chicken in a slower manner while the discoloration in 8-ClO₂ was localized in areas close to the sachet. It is likely that the AITC was distributed more evenly on the surface of the chicken breast. The color compounds in chicken treated with AITC were not identified in this experiment. AITC breakdown reaction schemes involve amino acids or disulfides in proteins. Under these reactions, byproducts such as diallyl disulfide which has

a pale yellow color, allyl thiourea (cobalt color), 2-thiohydrantione (orange-red), and N-allyl thiocarbamoyl amino acid were produced (Ohta et al., 1995, OHSA, 2006, Pechacek et al., 1997, and Winther & Nielsen, 2006). These byproducts may have accumulated on the chicken breasts, and caused the color change. Chicken breast in 4-ClO₂, 0.6-AITC, MAP, and AIR samples did not suffer any significant change in “b” value throughout 21 days storage.

The overall color results clearly show that color changes occurred on chicken breast treated with 8-ClO₂ and 1.2-AITC. Taking into consideration what the consumer’s perception is of what the color should be, it is recommended that the minimum effective concentration of AITC be used, and thus, it is noted that 0.6 µg/hr of AITC did not cause any color change.

The ClO₂ system was designed to release the gas from two sides to reduce the effect on color. Figure 43 shows a color comparison between the one side and two side release systems. The discoloration by the 4 µg/hr sachet was clearly reduced using the two side release system. The results indicate that development of a ClO₂ releasing film may be able to provide microbial inhibition, without causing a negative color change.

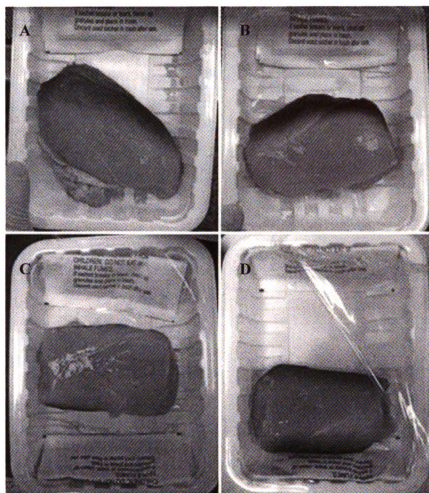


Figure 43. Appearance of chicken breast after 21 days at 4°C
 A: 4 μ g/hr from 1 sachet, B: 8 μ g/hr from 1 sachet
 C: 4 μ g/hr from 2 sachet, D: 8 μ g/hr from 2 sachet

Table 15. Color "L" values of fresh chicken breast during 21 days of storage (Day 0 "a" value was 53.18± 0.24) at 4°C

	3	6	9	12	15	18	21
AIR	v53.51± 0.53A u49.42± 1.84A u49.22± 1.76A u49.37± 2.15A u49.92± 1.13A u49.37± 0.69A	v53.28± 0.86A u49.83± 1.32A u49.50± 1.51A u49.40± 1.45A u49.58± 2.25A u49.99± 0.86A	w53.48± 1.33A vu49.03± 1.27A vu49.03± 1.35A u47.72± 1.09AB vu50.29± 0.95A v50.72± 1.17AB	w53.94± 1.17A v48.95± 1.71A v49.37± 1.77A u45.94± 0.96BC v50.38± 0.86A v50.36± 1.98AB	w52.04± 0.45AB v49.45± 0.90A v49.31± 1.37A u44.88± 0.57BC w50.53± 0.97A w50.96± 1.19AB	w51.24± 0.17B v48.46± 0.58A v47.81± 0.62A u43.86± 0.44C w49.95± 0.72A x53.03± 0.25BC	v50.29± 0.68B v48.02± 1.04A v47.97± 1.27A u44.49± 1.27C v49.54± 1.16A w54.67± 1.42C
MAP							
4-ClO ₂							
8-ClO ₂							
0.6-AITC							
1.2-AITC							

A: chicken source from Goodrich store, East Lansing, MI

Means with different superscripts in same columns and subscripts in same row are significantly different (p<0.05)

Table 16. Color "L" values of fresh chicken breast during 21 days of storage (Day 0 "L" value was 46.55± 0.99) at 4°C

PKG		3	6	9	12	15	18	21
2 nd Test (B)	AIR	v46.14± 0.29 _A	v46.10± 0.34 _A	w46.05± 0.17 _A	w45.65± 0.90 _{BC}	vw44.47± 0.90 _{BC}	v44.19± 0.21 _C	v43.23± 0.75 _C
	MAP	u44.48± 0.46 _A	u44.41± 0.62 _A	u44.06± 0.51 _A	uv44.35± 0.33 _A	vw44.45± 0.90 _A	v44.38± 0.79 _A	v43.90± 0.57 _A
	4-ClO ₂	u44.45± 0.40 _A	u44.45± 0.66 _A	u44.09± 0.57 _A	uv44.46± 0.44 _A	v44.33± 0.43 _A	v43.57± 0.59 _A	v43.14± 0.60 _A
	8-ClO ₂	u44.14± 0.49 _A	u44.44± 0.15 _A	uv44.28± 0.22 _A	u41.69± 0.55 _B	u39.95± 0.43 _C	u39.00± 0.84 _C	u39.48± 0.56 _C
	0.6-AITC	u44.69± 0.43 _A	u44.55± 0.24 _A	uv44.71± 0.48 _A	w45.38± 0.86 _A	vw45.03± 0.50 _A	v44.55± 0.56 _A	v44.54± 1.16 _A
	1.2-AITC	u44.15± 0.43 _A	u44.76± 0.53 _{AB}	v45.01± 0.25 _{ABC}	w45.37± 0.50 _{BC}	w45.97± 0.62 _C	w48.00± 0.56 _D	w48.68± 0.42 _D

B: Source from Sam's club, Lansing, MI

1-∞ Means with different superscripts in same columns and subscripts in same row are significantly different (p<0.05)

Table 17. Color "a" values of fresh chicken breast during 21 days of storage (Day 0 "a" value were 2.68±0.16) at 4°C

PKG		3	6	9	12	15	18	21
1 st Test (A)	AIR	0.270± 0.22A v1.70±	0.270± 0.35A v1.72±	0.268± 0.25A v1.60±	0.278± 0.21A v1.70±	0.271± 0.06A w1.71±	0.273± 0.10A v1.63±	0.275± 0.24A w1.71±
		0.14A v1.70±	0.16A v1.71±	0.26A v1.78±	0.73A v1.74±	0.18A w1.74±	0.34A v1.73±	0.07A w1.98±
	4-ClO ₂	0.34A v1.65±	0.35A v1.73±	0.34A v2.43±	0.20A v2.70±	0.20A v2.85±	0.21A v2.95±	0.21A v3.19±
		0.29A v1.71±	0.13A v1.73±	0.13B v1.70±	0.18BC v1.81±	0.24BC v1.94±	0.13CD v1.69±	0.20D w1.71±
	0.6-AITC	0.23A v1.75±	0.39A v1.68±	0.20A v1.49±	0.35A v1.51±	0.39A w1.27±	0.18A w0.58±	0.11A x0.40±
		0.24B	0.43B	0.07B	0.22B	0.27B	0.17A	0.12A
	1.2-AITC							

A: chicken source from Goodrich store, East Lansing, MI

Means with different superscripts in same columns and subscripts in same row are significantly different (p<0.05)

Table 18. Color "a" values of fresh chicken breast during 21 days of storage (Day 0 "a" value was 4.31±0.14) at 4°C

PKG	3	6	9	12	15	18	21
AIR	4.42 ±0.14A U3.52±	4.29± 0.28A U3.65±	4.24± 0.08A U3.56±	4.25± 0.21A U3.51±	4.18± 0.13A U3.51±	4.23± 0.22A V3.50±	4.21± 0.11A WV3.88±
MAP	0.32A U3.50±	0.24A U3.59±	0.32A U3.45±	0.08A U3.68±	0.21A U3.52±	0.17A V3.60±	0.35A WV3.90±
4-ClO ₂	0.16A U3.54±	0.22A UV3.93±	0.16A WV3.94±	0.23A V4.31±	0.17A V4.31±	0.21A X4.60±	0.12A X4.68±
8-ClO ₂	0.11 A U3.57±	0.11B U3.59±	0.07B U3.55±	0.18C U3.55±	0.07C U3.55±	0.08D WV3.65±	0.13D V3.56±
0.6-AITC	0.06A U3.62±	0.35A U3.64	0.12A UV3.62±	0.19A U3.45±	0.23A U3.15±	0.09A U2.85±	0.17A U2.63±
1.2-AITC	0.04 A	±0.09A	0.12A	0.07A	0.29AB	0.56B	0.09B

B: Source from Sam's club, Lansing, MI

Means with different superscripts in same columns and subscripts in same row are significantly different (p<0.05)

Table 19. Color "b" values of fresh chicken breast during 21 days of storage (Day 0 "b" value was 2.21±0.10) at 4 °C

	3	6	9	12	15	18	21
AIR	2.14± 0.13 _A	2.17± 0.11 _A	2.18± 0.14 _A	2.05± 0.15 _A	2.15± 0.14 _A	2.15± 0.15 _A	2.20± 0.25 _A
MAP	2.29± 0.14 _A	2.33± 0.46 _A	2.15± 0.41 _A	2.33± 0.51 _A	2.33± 0.45 _A	2.38± 0.43 _A	2.33± 0.35 _A
4-ClO ₂	2.30± 0.14 _A	2.23± 0.17 _A	2.13± 0.36 _A	2.33± 0.25 _A	2.15± 0.31 _A	2.55± 0.38 _A	2.66± 0.13 _A
8-ClO ₂	2.39± 0.12 _A	2.83± 0.10 _{AB}	3.06± 0.15 _{BC}	3.06± 0.37 _{BC}	3.34± 0.29 _C	3.44± 0.13 _C	3.43± 0.15 _C
0.6-AITC	2.38± 0.35 _A	2.40± 0.16 _A	2.28± 0.62 _A	2.48± 0.45 _A	2.43± 0.61 _A	2.50± 0.87 _A	2.43± 0.10 _A
1.2-AITC	2.23± 0.46 _A	2.45± 0.21 _A	2.41± 0.14 _A	2.49± 0.17 _A	2.62± 0.21 _{AB}	3.38± 0.48 _{BC}	3.50± 0.58 _C

A: chicken source from Goodrich store, East Lansing, MI

Means with different superscripts in same columns and subscripts in same row are significantly different (p<0.05)

Table 20. Color “b” values of fresh chicken breast during 21 days of storage (Day 0 “b” value was 1.83±0.05) at 4 °C

	3	6	9	12	15	18	21
AIR	1.81± 0.05 _A	1.87± 0.13 _A	2.04± 0.27 _A	1.78± 0.16 _A	1.98± 0.06 _A	1.98± 0.23 _A	2.05± 0.18 _A
MAP	1.87± 0.10 _A	1.93± 0.26 _A	1.78± 0.25 _A	1.87± 0.18 _A	1.72± 0.06 _A	1.85± 0.12 _A	1.71± 0.05 _A
4-ClO ₂	1.80± 0.15 _A	1.83± 0.09 _A	1.83± 0.12 _A	1.81± 0.10 _A	1.81± 0.17 _A	1.94± 0.30 _A	1.95± 0.08 _A
8-ClO ₂	1.81± 0.14 _A	1.91± 0.12 _{AB}	2.21± 0.12 _{AB}	2.33± 0.20 _B	3.23± 0.24 _C	3.69± 0.30 _C	4.24± 0.21 _D
0.6-AITC	1.88± 0.20 _A	1.77± 0.23 _A	1.78± 0.16 _A	1.86± 0.09 _A	1.84± 0.13 _A	1.88± 0.18 _A	1.85± 0.16 _A
1.2-AITC	1.82± 0.15 _A	1.86± 0.23 _A	1.88± 0.12 _A	1.98± 0.11 _A	2.21± 0.10 _A	2.61± 0.19 _B	2.98± 0.25 _B

B: Source from Sam's club, Lansing, MI

Means with different superscripts in same columns and subscripts in same row are significantly different (p<0.05)

3.7. Physical property changes due to ClO₂ treatments

3.7.1. Mechanical properties

Changes in tensile strength (TS) and elongation at break (EB) of conventional package films (PVC, LDPE, and PS) were determined after 1 day. The storage temperature was 23°C and RH was 100% because these films were treated with an equilibrium ClO₂ vapor from the aqueous ClO₂ solution. Since the selected film samples have good water barrier properties, the effect of RH on the mechanical properties was ignored. Figure 44 and 45 show the percentage change in tensile strength and elongation at break, respectively. The tested films did not show any significant mechanical property changes due to the ClO₂ treatment except for PS. The percent EB of PS decreased by approximately 50% at a 250 ppm or higher level of ClO₂. Oppositely, an increase in treatment concentration resulted in an increase in TS strength. PVC and LDPE films had no statistically significant ($p > 0.05$) changes in mechanical properties.

Razumovskii (1983) used ozone (O₃), another highly oxidizing agent, to modify the mechanical properties of PS and PE films. Subjected to the O₃ treatment, the films became brittle and opaque. The decrease in EB might be due to the degradation of the polymer chain. PS is considered more susceptible to oxidation. Ozen et al. (2002) saw significant EB reduction in PE after ozone treatment. The oxidation strength of O₃ (2.07) is much higher than that of ClO₂ (0.95) (Lenntech, 2006). 2000 ppm of ClO₂ did have a significant impact on Cryovac C-1050, LDPE and PVC in this test. The TS of PS was significantly increased by ClO₂ treatment. More than 500 ppm ClO₂ may cause degradation of

polymeric chains and strengthen the intermolecular forces as the result of the formation of polar groups (Ozen et al., 2002). PS became more brittle because of ClO_2 treatments. The functional group changes in PS by ClO_2 treatment should be investigated in a further study.

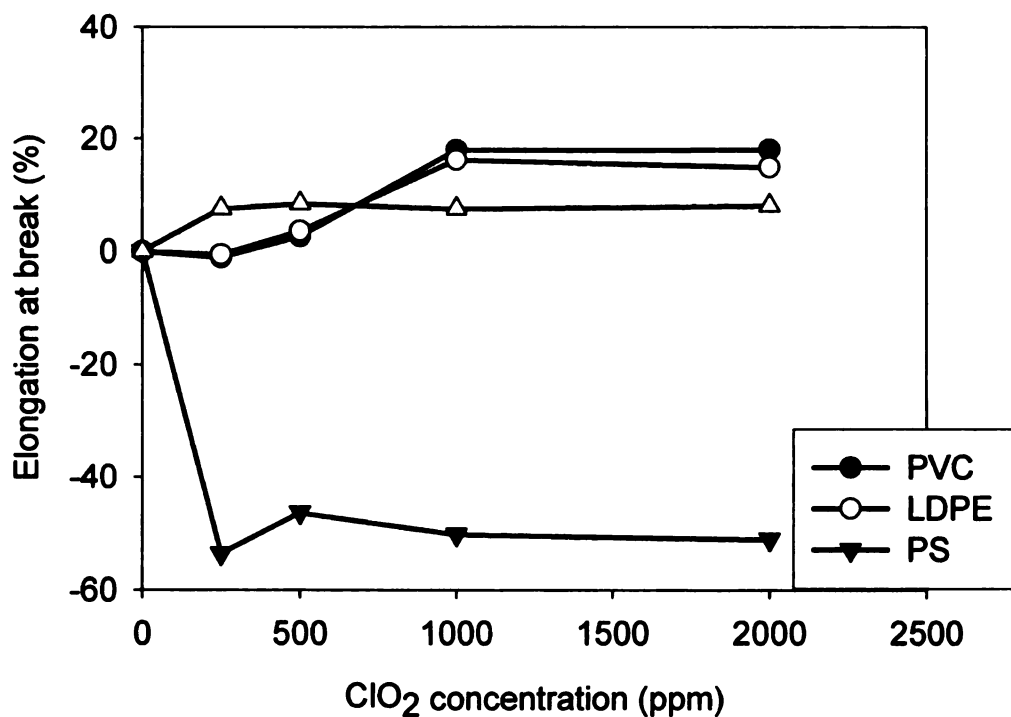


Figure 44. Percent (%) elongation at break with different ClO_2 concentrations for films studied

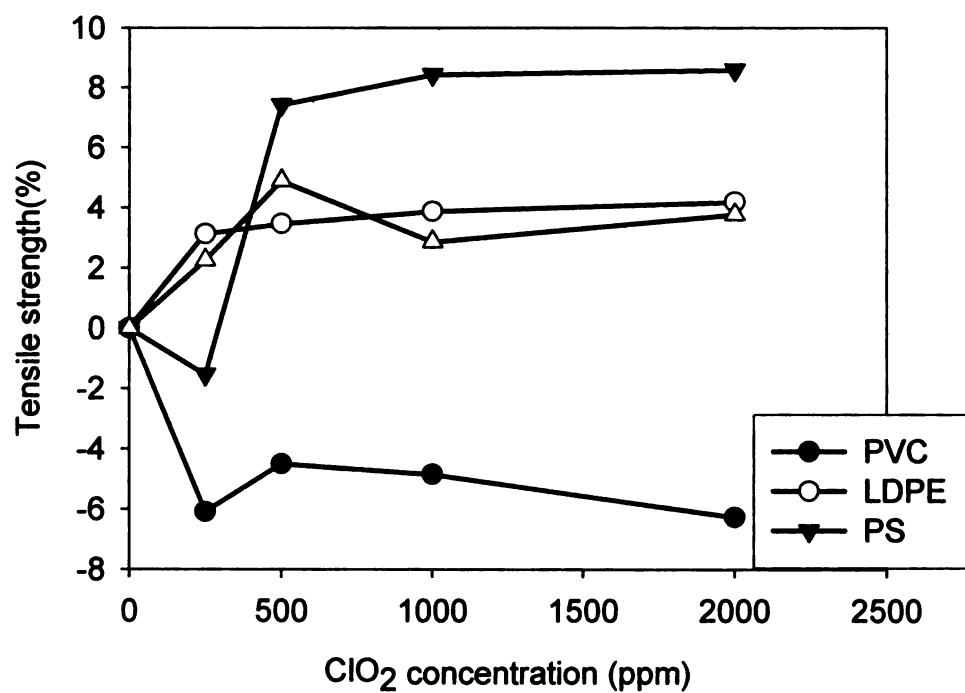


Figure 45. Percent (%) tensile strength at different ClO₂ concentrations used for the films studied

Table 21. Tensile strength and Elongation at break of packaging films (PVC, LDPE, and PS)

	ClO ₂ concentration	PVC	LDPE	PS
EB	0ppm	2.98 _a	9.77 _a	0.47 _a
	250ppm	2.95 _a	9.71 _a	0.22 _b
	500ppm	3.06 _a	10.12 _a	0.25 _b
	1000ppm	3.20 _a	11.35 _a	0.23 _b
	2000ppm	3.14 _a	11.23 _a	0.23 _b
	ClO ₂ concentration	PVC	LDPE	PS
TS	0ppm	15.76 _a	4.44 _a	14.11 _a
	250ppm	14.80 _a	4.58 _a	13.90 _a
	500ppm	15.05 _a	4.59 _a	15.16 _b
	1000ppm	15.00 _a	4.61 _a	15.30 _b
	2000ppm	14.77 _a	4.73 _a	15.33 _b

Means with different letters in the same column are significantly different ($p < 0.05$)

3.7.2. Oxygen barrier properties

The oxygen transmission rate (OTR) of packaging films was determined as a function of the different ClO₂ concentrations. Table 22 shows the permeability results.

At more than 500 ppm ClO₂, the permeability was significantly lower than the control ($p < 0.05$). Tsobkallo et al. (1988) found film (PE) oxidation impairs mechanical properties but improves the molecular ordering of the PE. Further exposure resulted in increased crystallinity and elastic modulus of the PE films. Therefore, the PS barrier properties were enhanced by film oxidation. PVC and LDPE did not show any significant permeability changes due to ClO₂ treatment ($p > 0.05$).

Table 22. Oxygen permeability of conventional packaging films (PS, LDPE, and PVC) after ClO₂ treatment

Films \ Conc.	0ppm (0mg/l)	500ppm (1.38mg/l)	1000ppm (2.76mg/l)	2000ppm (5.52mg/l)
PS(1mil)	323 _a	292 _b	290 _b	271 _c
PVC(1mil)	163 _a	159 _a	164 _a	154 _a
LDPE(1.25mil)	461 _a	460 _a	463 _a	460 _a

Unit: cc.mil/100in².day.atm

Means with different letters in same row are significantly different (p<0.05)

3.7.3. Color

PS, LDPE, and PVC were treated with different ClO₂ concentrations and stored for 24 hr. Change of color by ClO₂ treatment is shown in table 23. No matter the treatment concentration, There were no significant changes observed in LDPE and PVC. However, PS was shown to have a significant color change at 1000 ppm of ClO₂. At 1000 ppm, the L* value decreased by 4.55 and b* increased by 2.86.

Table 23. Color changes of conventional packaging films (PS, LDPE, and PVC) after ClO₂ treatment (n=3)

Films	Conc.	L*	a*	b*	ΔE
PS (1mil)	0 ppm ClO ₂	91.75 _a	-0.92 _a	-0.88 _a	91.76 _a
	500 ppm ClO ₂	90.18 _a	-0.90 _a	-0.24 _a	90.18 _a
	1000 ppm ClO ₂	87.20 _b	-0.83 _a	1.98 _a	87.23 _b
LDPE (1.25mil)	0 ppm ClO ₂	91.88 _a	-0.92 _a	-0.91 _a	91.89 _a
	500 ppm ClO ₂	91.31 _a	-0.92 _a	-0.85 _a	91.32 _a
	1000 ppm ClO ₂	90.45 _a	-0.88 _a	-0.93 _a	90.46 _a
PVC (1mil)	0 ppm ClO ₂	92.53 _a	-0.88 _a	-0.84 _a	92.54 _a
	500 ppm ClO ₂	91.91 _a	-0.89 _a	-0.88 _a	91.92 _a
	1000 ppm ClO ₂	91.87 _a	-0.91 _a	-0.90 _a	91.88 _a

Means with different letters in the same column are significantly different (p<0.05)

CHAPTER IV. CONCLUSION AND FUTURE WORKS

Using a growth medium model system, chlorine dioxide (ClO_2) and allyl isothiocyanate (AITC) were shown to have strong antimicrobial activity against *Salmonella* Typhimurium (G10127, G10601, G10931) and *Listeria monocytogenes* (1002, 1176, 1304). At 7 and 37°C, 160 -360 µg/l of AITC eliminated inoculated *Salmonella* Typhimurium and *Listeria monocytogenes* on growth media. 120-180 µg/l of ClO_2 also eliminated these organisms on growth media.

From the tests (microbial growth, pH, gas composition) with fresh chicken breast, the antimicrobials (ClO_2 or AITC) plus MAP showed potential to enhance the microbial safety of a fresh poultry product. Compared to packaging in AIR, the growth of *Listeria monocytogenes* were reduced by 1.15-2.75 log, and the growth of *Salmonella* Typhimurium were reduced by 1.07-2.09 logs on fresh chicken breast at the end of storage (21 days). The antimicrobials plus MAP combination packages delayed the growth of total bacteria. On day 21, the number of total aerobic bacteria on chicken breasts treated with 0.6-AITC, 1.2-AITC, and 8- ClO_2 was less than 8 log cfu/g. The number of bacteria in AIR and MAP packed chicken breast reached this level on the 9th and 15th days respectively. The pH and gas headspace composition of MAP controls significantly changed over 21 days, with antimicrobial (ClO_2 or AITC) plus MAP combination packages suffered little or no significant change during storage, except for a pH decrease in 8- ClO_2 . The results indicate that microbial growth on the fresh chicken was suppressed by the antimicrobial plus MAP combination.

The fresh chicken color can be affected by high antimicrobial content. Discoloration was observed in the 1.2-AITC, 4-ClO₂, and 8-ClO₂ samples. However, the results also show that discoloration due to ClO₂ treatment can be minimized or avoided by dispersed release. Chicken samples treated with 0.6-AITC did not suffer any negative color change.

AITC or ClO₂ treatment with MAP could improve the stability and shelf life of fresh poultry products. However, the use of high concentrations of AITC or ClO₂ could result in adverse effects on the organoleptic properties of the fresh chicken. Thus, in order to apply these compounds practically, the following research is suggested:

1. Determine the maximum allowable treated concentration for consumer acceptance at conventional fresh chicken storage temperatures
2. Investigate the use of other antimicrobial combinations for fresh chicken to lead to lower dosages.
3. Identify and quantify the AITC or ClO₂ byproducts in toxicological and sensory aspect
4. Develop ClO₂ releasable film for dispersed treatment
5. Investigate a masking technique to minimize or prevent undesirable flavor due to AITC or ClO₂ treatment.

In addition, LDPE and PVC were observed to not suffer any significant physical property changes due to ClO₂ treatment (up to 2000 ppm). These polymers should not be affected by ClO₂ sanitization. However, ClO₂ treatment caused significant changes in mechanical, oxygen barrier properties, and optical

properties (color) of PS. These findings will be helpful in selecting appropriate plastic packaging materials for a food process which uses ClO₂ as a food preservative. Detailed structure and functional group analysis are needed to determine the morphological effect of ClO₂ treatment on PS film.

APPENDIX A
Methods for analyzing chlorine dioxide and related components in gas
phases (ICA titration method))

GAS PHASE SAMPLING AND ANALYSIS

1. SCOPE & FIELD OF APPLICATIONS

This method describes the analytical procedure for withdrawing gas samples from a ClO₂ gas treatment container (such as a HDPE bucket), and analyzing the gas.

2. APPARATUS

50 ml Beaker
50 ml Burette and Stand
Gas-Tight Syringe (60 cc) with Slip-Tip end

3. REAGENTS

Potassium Iodide	KI	10 wt% Solution
Sodium Thiosulfate	Na ₂ S ₂ O ₃	0.001 N from Certified Solution
Sulfuric Acid	H ₂ SO ₄	2 N – 4N (1 -2 M)
Starch indicator		

4. PROCEDURE

- 4.1. Draw exactly 10 mL of 10 wt% KI solution into a syringe.
- 4.2. Hold the syringe upright and expel air from the syringe.
- 4.3. Draw syringe plunger back very slightly so that liquid meniscus is not right at the tip.
- 4.4. Remove tape from the sampling port of the bucket (or other treatment container).
- 4.5. Insert the tip of the syringe into the sampling port to form a gas tight seal.
- 4.6. Sample 50 mL of gas by slowly drawing back the plunger to the 60 mL mark.
- 4.7. Hold VERY slight backward pressure on plunger to prevent KI from entering the container and remove from sampling port.
- 4.8. Place finger over tip of syringe and mix by shaking the contents for about 5 seconds. (ClO₂ will rapidly transfer to the liquid).
- 4.9. Repeat steps 2 through 8 until the desired cumulative volume of gas has been sampled. Record this volume of gas as VS. (Typical volumes are 200 – 500 ml).
- 4.10. Transfer liquid from the syringe into a 50 mL beaker and add 5 to 25 mL of distilled water.
- 4.11. Add 8-10 drops of starch indicator to the solution.
- 4.12. Titrate the solution to a colorless endpoint using 0.001 N sodium thiosulfate.
- 4.13. Record the volume of Thio used for the titration as VN (neutral titration).
- 4.14. Using the pH probe to stir the solution, add H₂SO₄ slowly to a pH of <2.0.
- 4.15. Allow the solution to stand for 5 minutes in the dark.

4.16. Titrate the solution to a colorless end point using 0.001 N sodium thiosulfate.

4.17. Record the volume of Thio used for the titration as V_A (acid titration). Discard the solution.

5. CALCULATIONS

5.1. Calculate chlorine dioxide concentration in bucket:

5.2. To obtain results in mg per L of gas,

$$ClO_2, (mg / L) = \frac{V_A \times N \times 67,500}{4 \times V_S}$$

$$Cl_2, (mg / L) = \frac{\left(V_N - \frac{V_A}{4} \right)}{2 \times V_S} \times N \times 71,000$$

5.3. To obtain results in ppmv (parts per million by volume of gas), assuming room temperature gas:

$$ClO_2, (ppmv) = 358 \times ClO_2, (mg / L)$$

$$Cl_2, (ppmv) = 340 \times ClO_2, (mg / L)$$

where N = normality of sodium thiosulfate.

APPENDIX B
Growth model system raw data at 37°C and 7°C

Table 24. The reduction of Salmonella Typhimurium on agar plate (60 mm X 16 mm) in glass bottle (with soaked Allyl-isothiocyanate-oil mixture filter paper) (37°C for 2 days)

Inoculation \ AITC					
	12ug/l	40ug/l	70ug/l	160ug/l	360ug/l
10 ¹	O	X	X	X	X
	O	X	X	X	X
	O	X	X	X	X
10 ²	O	X	X	X	X
	O	X	X	X	X
	O	X	X	X	X
10 ³	O	X	X	X	X
	O	X	X	X	X
	O	X	X	X	X
10 ⁴	O	X	X	X	X
	O	O	X	X	X
	O	O	X	X	X
10 ⁵	O	O	X	X	X
	O	O	X	X	X
	O	O	X	X	X
10 ⁶	O	O	X	X	X
	O	O	X	X	X
	O	O	O	X	X

1. O: microbial growth on the agar, X: No-microbial growth on the agar
2. The soaked mixture of AITC with corn oil was 1 ml
3. Atmosphere in the bottle was 100% N₂

Table 25. The reduction of *Listeria monocytogenes* on agar plate (16 mm x 60 mm) in glass bottle (with soaked Allyl-isothiocyanate-oil mixture filter paper) (37°C for 2 days)

Inoculation	AITC				
	12ug/l	40ug/l	70ug/l	160ug/l	360ug/l
10^1	O	O	X	X	X
	O	O	X	X	X
	O	O	X	X	X
10^2	O	O	O	X	X
	O	O	O	X	X
	O	O	O	X	X
10^3	O	O	O	X	X
	O	O	O	X	X
	O	O	O	X	X
10^4	O	O	O	X	X
	O	O	O	O	X
	O	O	O	O	X
10^5	O	O	O	O	X
	O	O	O	O	X
	O	O	O	O	X
10^6	O	O	O	O	X
	O	O	O	O	X
	O	O	O	O	O

1. O: microbial growth on the agar, X: No-microbial growth on the agar
2. The soaked mixture of AITC with corn oil was 1 ml
3. Atmosphere in the bottle was 100% N₂

Table 26. The reduction of Salmonella Typhimurium on agar plate (60 mm X 16 mm) in glass bottle (with soaked Allylithiocyanate-oil mixture filter paper) (7°C for 7 days)

Inoculation	AITC				
	12ug/l	40ug/l	70ug/l	160ug/l	360ug/l
10 ¹	X	X	X	X	X
	X	X	X	X	X
	X	X	X	X	X
10 ²	O	X	X	X	X
	O	X	X	X	X
	O	X	X	X	X
10 ³	O	X	X	X	X
	O	X	X	X	X
	O	X	X	X	X
10 ⁴	O	O	X	X	X
	O	O	X	X	X
	O	O	X	X	X
10 ⁵	O	O	X	X	X
	O	O	X	X	X
	O	O	X	X	X
10 ⁶	O	O	X	X	X
	O	O	X	X	X
	O	O	X	X	X

1. O: microbial growth on the agar, X: No-microbial growth on the agar
2. The soaked mixture of AITC with corn oil was 1 ml
3. Atmosphere in the bottle was 100% N₂

Table 27. The reduction of *Listeria monocytogenes* on agar plate (60 mm X 16 mm) in glass bottle (with soaked Allylthiocyanate-oil mixture filter paper) (7°C for 7 days)

Inoculation	AITC				
	12ug/l	40ug/l	70ug/l	160ug/l	360ug/l
10^1	O	X	X	X	X
	O	X	X	X	X
	O	X	X	X	X
10^2	O	O	X	X	X
	O	O	X	X	X
	O	O	X	X	X
10^3	O	O	X	X	X
	O	O	X	X	X
	O	O	X	X	X
10^4	O	O	X	X	X
	O	O	X	X	X
	O	O	X	X	X
10^5	O	O	X	X	X
	O	O	X	X	X
	O	O	X	X	X
10^6	O	O	O	X	X
	O	O	O	X	X
	O	O	O	X	X

1. O: microbial growth on the agar, X: No-microbial growth on the agar
2. The soaked mixture of AITC with corn oil was 1 ml
3. Atmosphere in the bottle was 100% N₂

Table 28. The reduction of Salmonella Typhimurium on agar plate (60 mm X 16 mm) in glass bottle (with chlorine dioxide treatment) (37°C for 2 days)

ClO₂(ug) Inoculation	12ug/l	30ug/l	60ug/l	90ug/l	120ug/l	180ug/l
10¹	O	O	O	X	X	X
	O	O	O	X	X	X
	O	O	O	X	X	X
10²	O	O	O	X	X	X
	O	O	O	X	X	X
	O	O	O	X	X	X
10³	O	O	O	O	X	X
	O	O	O	O	X	X
	O	O	O	O	X	X
10⁴	O	O	O	O	X	X
	O	O	O	O	X	X
	O	O	O	O	X	X
10⁵	O	O	O	O	O	X
	O	O	O	O	O	X
	O	O	O	O	O	X
10⁶	O	O	O	O	O	X
	O	O	O	O	O	X
	O	O	O	O	O	X

1. O: microbial growth on the agar, X: No-microbial growth on the agar

2. Atmosphere in the bottle was 100% N₂

Table 29. The reduction of *Listeria monocytogene* on agar plate (60 mm X 16 mm) in glass bottle (with chlorine dioxide treatment) (37°C for 2 days)

ClO₂(ug) Inoculation	12ug/l	30ug/l	60ug/l	90ug/l	120ug/l	180ug/l
10¹	O	X	X	X	X	X
	O	X	X	X	X	X
	O	X	X	X	X	X
10²	O	X	X	X	X	X
	O	X	X	X	X	X
	O	X	X	X	X	X
10³	O	O	X	X	X	X
	O	O	X	X	X	X
	O	O	X	X	X	X
10⁴	O	O	X	X	X	X
	O	O	X	X	X	X
	O	O	X	X	X	X
10⁵	O	O	O	O	X	X
	O	O	O	O	X	X
	O	O	O	O	X	X
10⁶	O	O	O	O	X	X
	O	O	O	O	X	X
	O	O	O	O	X	X

1. O: microbial growth on the agar, X: No-microbial growth on the agar

2. Atmosphere in the bottle was 100% N₂

Table 30. The reduction of Salmonella Typhimurium on agar plate (60 mm X 16 mm) in glass bottle (with chlorine dioxide treatment) (7°C for 7 days)

CIO₂(ug) Inoculation	12ug/l	30ug/l	60ug/l	90ug/l	120ug/l	180ug/l
10¹	O	O	O	X	X	X
	O	O	O	X	X	X
	O	O	O	X	X	X
10²	O	O	O	X	X	X
	O	O	O	X	X	X
	O	O	O	X	X	X
10³	O	O	O	O	X	X
	O	O	O	O	X	X
	O	O	O	O	X	X
10⁴	O	O	O	O	O	X
	O	O	O	O	O	X
	O	O	O	O	O	X
10⁵	O	O	O	O	O	X
	O	O	O	O	O	X
	O	O	O	O	O	X
10⁶	O	O	O	O	O	X
	O	O	O	O	O	X
	O	O	O	O	O	X

1. O: microbial growth on the agar, X: No-microbial growth on the agar

2. Atmosphere in the bottle was 100% N₂

Table 31. The reduction of *Listeria monocytogene* on agar plate (60 mm X 16 mm) in glass bottle (with chlorine dioxide treatment) (7°C for 7 days)

$\text{ClO}_2(\text{ug})$ Inoculation	12ug/l	30ug/l	60ug/l	90ug/l	120ug/l	180ug/l
10^1	O	O	X	X	X	X
	O	O	X	X	X	X
	O	O	X	X	X	X
10^2	O	O	X	X	X	X
	O	O	X	X	X	X
	O	O	X	X	X	X
10^3	O	O	O	X	X	X
	O	O	O	X	X	X
	O	O	O	O	X	X
10^4	O	O	O	O	X	X
	O	O	O	O	X	X
	O	O	O	O	X	X
10^5	O	O	O	O	X	X
	O	O	O	O	X	X
	O	O	O	O	X	X
10^6	O	O	O	O	X	X
	O	O	O	O	X	X
	O	O	O	O	X	X

1. O: microbial growth on the agar, X: No-microbial growth on the agar
2. Atmosphere in the bottle was 100% N_2

Table 32. Headspace concentrations (ppm) of Chlorine dioxide (ClO₂) with growth medium (at 7 °C)

Wt	0 day	0.5 day	1 day	3 day	7 day
60ug/l	6.0	0.0	0.0	0.0	0.0
	4.0	0.0	0.0	0.0	0.0
AVE	5.0	0.0	0.0	0.0	0.0
SD	1.4	0.0	0.0	0.0	0.0
90ug/l	10.0	2.0	0.0	0.0	0.0
	10.0	1.0	0.0	0.0	0.0
AVE	10.0	1.5	0.0	0.0	0.0
SD	0.0	0.7	0.0	0.0	0.0
120ug/l	15.0	5.0	0.0	0.0	0.0
	15.0	1.0	0.0	0.0	0.0
AVE	15.0	3.0	0.0	0.0	0.0
SD	0.0	2.8	0.0	0.0	0.0
180ug/l	15.0	7.0	0.0	0.0	0.0
	15.0	3.0	0.0	0.0	0.0
AVE	15.0	5.0	0.0	0.0	0.0
SD	0.0	2.8	0.0	0.0	0.0

Table 33. Headspace concentrations (ppm) of Chlorine dioxide (ClO₂) with growth medium (at 37 °C)

Wt	0 day	0.5 day	1 day	3 day	7 day
60ug/l	6.0	0.0	0.0	0.0	0.0
	4.0	0.0	0.0	0.0	0.0
AVE	5.0	0.0	0.0	0.0	0.0
SD	1.4	0.0	0.0	0.0	0.0
90ug/l	10.0	2.0	0.0	0.0	0.0
	10.0	1.0	0.0	0.0	0.0
AVE	10.0	1.5	0.0	0.0	0.0
SD	0.0	0.7	0.0	0.0	0.0
120ug/l	15.0	1.0	0.0	0.0	0.0
	15.0	1.0	0.0	0.0	0.0
AVE	15.0	1.0	0.0	0.0	0.0
SD	0.0	0.0	0.0	0.0	0.0
180ug/l	15.0	1.5	0.0	0.0	0.0
	15.0	1.5	0.0	0.0	0.0
AVE	15.0	1.5	0.0	0.0	0.0
SD	0.0	0.0	0.0	0.0	0.0

Table 34. Headspace concentrations (ng/ml) of Allyl isothiocyanate (AITC) with growth medium (at 7 °C)

Wt	0 day	1 day	2 day	4 day	7 day
0.5ug/l	44.0	33.0	0.0	0.0	0.0
	36.0	17.0	0.0	0.0	0.0
AVE	40.0	25.0	0.0	0.0	0.0
SD	5.7	11.3	0.0	0.0	0.0
1.0ug/l	75.0	40.0	7.0	7.0	0.0
	65.0	30.0	3.0	3.0	0.0
AVE	70.0	35.0	5.0	5.0	0.0
SD	7.1	7.1	2.8	2.8	0.0
2.5ug/l	165.0	85.0	22.0	16.0	0.0
	155.0	83.0	8.0	14.0	0.0
AVE	160.0	84.0	15.0	15.0	0.0
SD	7.1	1.4	9.9	1.4	0.0
5ug/l	385.0	170.0	55.0	25.0	0.0
	335.0	150.0	45.0	15.0	0.0
AVE	360.0	160.0	50.0	20.0	0.0
SD	35.4	14.1	7.1	7.1	0.0

wt(ug/ml): Soaked mg of AITC with 1ml corn oil

Table 35. Headspace concentrations (ng/ml) of Allyl isothiocyanate (AITC) with growth medium (at 37 °C)

Wt	0 day	1 day	2 day	4 day	7 day
0.5ug/l	44.0	25.0	6.0	0.0	0.0
	36.0	25.0	4.0	0.0	0.0
AVE	40.0	25.0	5.0	0.0	0.0
SD	5.7	0.0	1.4	0.0	0.0
1.0ug/l	77.0	65.0	23.0	13.0	5.0
	63.0	55.0	17.0	7.0	5.0
AVE	70.0	60.0	20.0	10.0	5.0
SD	9.9	7.1	4.2	4.2	0.0
2.5ug/l	166.0	103.0	60.0	49.0	32.0
	154.0	97.0	60.0	39.0	28.0
AVE	160.0	100.0	60.0	44.0	30.0
SD	8.5	4.2	0.0	7.1	2.8
5ug/l	385.0	230.0	125.0	85.0	55.0
	335.0	210.0	115.0	75.0	45.0
AVE	360.0	220.0	120.0	80.0	50.0
SD	35.4	14.1	7.1	7.1	7.1

wt(ug/ml): Soaked mg of AITC with 1ml corn oil

APPENDIX C
Summary tables of experimental AITC vapor pressures and model verification

Table 36. AITC vapor pressure based on mole fraction at 4°C

AITC (x ₁)	<i>p</i>	<i>p</i> (Margule's equation)	(<i>y_i</i> - <i>y_i</i>) ²	(<i>y</i> - <i>y</i>) ²
0.0	0.0	0.0	0.0	3187.1
0.1	5.0	3.6	1.9	2792.5
0.2	9.0	9.5	0.2	2206.6
0.3	20.0	18.1	3.7	1472.6
0.4	35.0	29.7	28.3	716.9
0.5	42.0	44.2	5.0	149.2
0.6	70.0	61.3	75.5	23.6
0.7	80.0	80.0	0.0	554.9
0.8	105.0	99.1	35.4	1814.4
0.9	110.0	116.9	47.7	3654.9
1.0	145.0	132.0	169.0	5707.1

$$R^2 = 1 - \frac{(\hat{y}_i - y_i)^2}{(\bar{y} - y)^2} = 0.98$$

Table 37. AITC vapor pressure based on mole fraction at 7°C

AITC (x ₁)	<i>p</i>	<i>p</i> (Margule's equation)	(<i>y_i</i> - <i>y_i</i>) ²	(<i>y</i> - <i>y</i>) ²
0.0	0.0	0.0	0.0	3187.1
0.1	7.0	4.6	5.9	2691.0
0.2	12.0	12.0	0.0	1978.9
0.3	22.0	22.7	0.5	1136.7
0.4	45.0	37.2	60.7	370.4
0.5	60.0	55.3	21.9	1.3
0.6	80.0	76.5	12.4	401.0
0.7	105.0	99.6	28.8	1864.1
0.8	135.0	123.2	139.5	4453.6
0.9	142.0	145.3	10.8	7891.7
1.0	164.0	164.0	0.0	11566.0

$$R^2 = 1 - \frac{(\hat{y}_i - y_i)^2}{(\bar{y} - y)^2} = 0.99$$

Table 38. AITC vapor pressure based on mole fraction at 22°C

AITC (x ₁)	<i>P</i>	<i>P</i> (Margule's equation)	$(y_i - \hat{y}_i)^2$	$(y - \bar{y})^2$
0.0	0.0	0.0	0.0	3187.1
0.1	25.0	21.3	13.4	1233.0
0.2	40.0	50.2	104.9	38.6
0.3	-	87.0	-	-
0.4	135.0	131.4	12.8	5619.8
0.5	185.0	182.6	6.0	15900.1
0.6	-	238.8	-	-
0.7	280.0	297.9	321.8	58315.2
0.8	365.0	357.2	60.4	90465.9
0.9	410.0	413.6	13.0	127560.0
1.0	464.0	464.0	0.0	166093.3

$$R^2 = 1 - \frac{(y_i - \hat{y}_i)^2}{(y - \bar{y})^2} = 1.00$$

Table 39. AITC vapor pressure based on mole fraction at 37°C

AITC (x ₁)	<i>P</i>	<i>P</i> (Margule's equation)	$(y_i - \hat{y}_i)^2$	$(y - \bar{y})^2$
0.0	0.0	0.0	0.0	3187.1
0.1	-	74.6	-	-
0.2	200.0	164.4	1270.2	11643.6
0.3	320.0	268.6	2639.9	45014.2
0.4	450.0	385.8	4121.6	108468.4
0.5	570.0	513.6	3185.5	208945.4
0.6	700.0	648.8	2618.4	350908.7
0.7	820.0	787.9	1031.7	534983.2
0.8	950.0	926.6	549.4	757083.5
0.9	1065.0	1060.4	21.0	1007946.6
1.0	0.0	0.0	0.0	3187.1

$$R^2 = 1 - \frac{(y_i - \hat{y}_i)^2}{(y - \bar{y})^2} = 1.00$$

Table 40. The quantity of AITC permeant in the cell through PE film at 23°C as a function of time

Time	RT	q
0	0	0.00
40	50589	0.86
60	155037	2.64
93	237948	4.05
117	323702	5.51
147	369219	6.28
170	525922	8.95

APPENDIX D
Raw data tables of microbial growth, headspace gas composition, pH, and
Color

Table 41. Raw data of *Salmonella* Typhimurium growth (log cfu/g) on fresh chicken based on AITC release rates at 7°C

	No.	0day	4day	8day	12day
AIR	1	3.60	4.10	5.50	5.70
	2	3.50	4.30	5.50	5.50
	3	3.50	4.10	5.60	5.80
	AVE	3.53	4.17	5.53	5.67
	SD	0.06	0.12	0.06	0.15
MAP	1	3.60	3.90	4.50	5.10
	2	3.50	3.50	4.75	5.15
	3	3.50	3.65	4.55	5.10
	AVE	3.53	3.68	4.60	5.12
	SD	0.06	0.20	0.13	0.03
0.3ug/hr	1	3.60	3.60	4.77	5.20
	2	3.50	3.50	4.60	5.00
	3	3.50	3.70	4.65	5.15
	AVE	3.53	3.60	4.67	5.12
	SD	0.06	0.10	0.09	0.10
0.6ug/hr	1	3.60	3.50	4.45	4.75
	2	3.50	3.75	4.40	4.88
	3	3.50	3.60	4.60	4.90
	AVE	3.53	3.62	4.48	4.84
	SD	0.06	0.13	0.10	0.08
1.2ug/hr	1	3.60	3.65	4.35	4.65
	2	3.50	3.76	4.23	4.50
	3	3.50	3.80	4.25	4.52
	AVE	3.53	3.74	4.28	4.56
	SD	0.06	0.08	0.06	0.08
1.4ug/hr	1	3.60	3.80	4.15	4.33
	2	3.50	3.70	4.27	4.52
	3	3.50	3.60	4.10	4.45
	AVE	3.53	3.70	4.17	4.43
	SD	0.06	0.10	0.09	0.10

Table 42. Raw data of *Listeria monocytogenes* growth (log cfu/g) on fresh chicken based on AITC release rates at 7°C

	No.	0day	4day	8day	12day
AIR	1	4.50	4.60	6.00	7.20
	2	4.10	4.50	5.80	7.00
	3	3.80	4.70	5.80	7.30
	AVE	4.13	4.60	5.87	7.17
	SD	0.35	0.10	0.12	0.15
MAP	1	3.35	4.50	5.00	5.50
	2	3.65	4.30	4.90	5.50
	3	3.80	4.20	5.00	5.70
	AVE	3.60	4.33	4.97	5.57
	SD	0.23	0.15	0.06	0.12
0.3ug/hr	1	3.35	4.40	5.00	5.70
	2	3.65	4.10	5.10	5.30
	3	3.80	4.40	5.00	5.50
	AVE	3.60	4.30	5.03	5.50
	SD	0.23	0.17	0.06	0.20
0.6ug/hr	1	3.35	4.40	4.80	5.10
	2	3.65	4.00	4.90	5.10
	3	3.80	4.00	4.70	5.00
	AVE	3.60	4.13	4.80	5.07
	SD	0.23	0.23	0.10	0.06
1.2ug/hr	1	3.35	4.30	4.60	4.40
	2	3.65	4.00	4.40	4.80
	3	3.80	4.50	4.50	4.70
	AVE	3.60	4.27	4.50	4.63
	SD	0.23	0.25	0.10	0.21
1.4ug/hr	1	3.35	4.00	4.40	4.50
	2	3.65	3.70	4.40	4.60
	3	3.80	4.20	4.50	4.80
	AVE	3.60	3.97	4.43	4.63
	SD	0.23	0.25	0.06	0.15

Table 43. Raw data of total aerobic bacteria growth (log cfu/g) on fresh chicken based on AITC release rates at 7°C

	No.	0day	4day	6day	8day
AIR	1	5.50	7.70	9.10	9.50
	2	5.50	7.50	9.50	9.60
	3	5.80	7.90	9.30	9.80
	AVE	5.60	7.70	9.30	9.63
	SD	0.17	0.20	0.20	0.15
MAP	1	5.50	6.50	7.50	8.70
	2	5.50	6.20	7.50	8.40
	3	5.80	6.50	7.70	8.50
	AVE	5.60	6.40	7.57	8.53
	SD	0.17	0.17	0.12	0.15
0.3ug/hr	1	5.50	6.30	7.40	8.70
	2	5.50	6.20	7.50	9.00
	3	5.80	6.50	6.90	8.80
	AVE	5.60	6.33	7.27	8.83
	SD	0.17	0.15	0.32	0.15
0.6ug/hr	1	5.50	6.10	7.00	7.50
	2	5.50	5.70	6.90	7.30
	3	5.80	6.10	6.80	7.70
	AVE	5.60	5.97	6.90	7.50
	SD	0.17	0.23	0.10	0.20
1.2ug/hr	1	5.50	5.40	6.20	7.10
	2	5.50	5.50	6.50	7.25
	3	5.80	5.20	6.50	7.00
	AVE	5.60	5.37	6.40	7.12
	SD	0.17	0.15	0.17	0.13
1.4ug/hr	1	5.50	5.20	6.10	6.60
	2	5.50	5.10	6.20	6.80
	3	5.80	5.00	6.00	7.00
	AVE	5.60	5.10	6.10	6.80
	SD	0.17	0.10	0.10	0.20

Table 44. Raw data of *Salmonella* Typhimurium growth (log cfu/g) on fresh chicken based on ClO₂ release rates at 7°C

	No.	0day	4day	8day	12day
AIR	1	4.50	4.30	5.50	5.60
	2	4.10	4.20	5.30	5.70
	3	3.80	4.40	5.50	5.80
	AVE	4.13	4.30	5.43	5.70
	SD	0.35	0.10	0.12	0.10
MAP	1	4.50	4.20	5.00	5.00
	2	4.10	4.30	4.90	5.10
	3	3.80	4.10	4.70	4.80
	AVE	4.13	4.20	4.87	4.97
	SD	0.35	0.10	0.15	0.15
3ug/hr	1	4.50	4.10	5.00	5.10
	2	4.10	4.30	5.10	4.90
	3	3.80	4.10	4.90	4.70
	AVE	4.13	4.17	5.00	4.90
	SD	0.35	0.12	0.10	0.20
4ug/hr	1	4.50	4.20	4.90	5.00
	2	4.10	4.20	4.70	4.90
	3	3.80	4.00	4.80	5.00
	AVE	4.13	4.13	4.80	4.97
	SD	0.35	0.12	0.10	0.06
6ug/hr	1	4.50	4.20	4.50	4.80
	2	4.10	4.10	4.80	4.90
	3	3.80	4.10	4.70	4.95
	AVE	4.13	4.13	4.67	4.88
	SD	0.35	0.06	0.15	0.08
8ug/hr	1	4.50	4.00	4.10	4.60
	2	4.10	3.70	4.40	4.50
	3	3.80	4.20	4.30	4.65
	AVE	4.13	3.97	4.27	4.58
	SD	0.35	0.25	0.15	0.08

Table 45. Raw data of *Listeria monocytogenes* growth (log cfu/g) on fresh chicken depend on ClO₂ release rates at 7°C

	No.	0day	4day	8day	12day
AIR	1	3.35	4.60	6.00	7.20
	2	3.65	4.50	5.80	7.00
	3	3.80	4.70	5.80	7.30
	AVE	3.60	4.60	5.87	7.17
	SD	0.23	0.10	0.12	0.15
MAP	1	4.50	4.50	5.00	5.50
	2	4.10	4.30	4.90	5.50
	3	3.80	4.20	5.00	5.70
	AVE	4.13	4.33	4.97	5.57
	SD	0.35	0.15	0.06	0.12
3ug/hr	1	4.50	4.50	4.70	5.45
	2	4.10	4.20	4.90	5.55
	3	3.80	4.60	5.00	5.65
	AVE	4.13	4.43	4.87	5.55
	SD	0.35	0.21	0.15	0.10
4ug/hr	1	4.50	4.50	5.00	5.53
	2	4.10	4.30	4.70	5.60
	3	3.80	4.70	4.80	5.66
	AVE	4.13	4.50	4.83	5.60
	SD	0.35	0.20	0.15	0.07
6ug/hr	1	4.50	4.50	4.80	5.23
	2	4.10	4.30	4.70	5.15
	3	3.80	4.20	4.50	5.30
	AVE	4.13	4.33	4.67	5.23
	SD	0.35	0.15	0.15	0.08
8ug/hr	1	4.50	4.30	4.80	5.10
	2	4.10	4.60	4.20	5.15
	3	3.80	4.10	4.50	5.10
	AVE	4.13	4.33	4.50	5.12
	SD	0.35	0.25	0.30	0.03

Table 46. Raw data of total aerobic bacteria growth (log cfu/g) on fresh chicken depend on ClO₂ release rates at 7°C

	No.	0day	4day	6day	8day
AIR	1	5.50	7.70	9.10	9.50
	2	5.50	7.50	9.50	9.60
	3	5.80	7.90	9.30	9.80
	AVE	5.60	7.70	9.30	9.63
	SD	0.17	0.20	0.20	0.15
MAP	1	5.50	6.50	7.50	8.70
	2	5.50	6.20	7.50	8.40
	3	5.80	6.50	7.70	8.50
	AVE	5.60	6.40	7.57	8.53
	SD	0.17	0.17	0.12	0.15
0.3ug/hr	1	5.50	6.30	7.40	8.70
	2	5.50	6.20	7.50	9.00
	3	5.80	6.50	6.90	8.80
	AVE	5.60	6.33	7.27	8.83
	SD	0.17	0.15	0.32	0.15
0.6ug/hr	1	5.50	6.10	7.00	7.50
	2	5.50	5.70	6.90	7.30
	3	5.80	6.10	6.80	7.70
	AVE	5.60	5.97	6.90	7.50
	SD	0.17	0.23	0.10	0.20
1.2ug/hr	1	5.50	5.40	6.20	7.10
	2	5.50	5.50	6.50	7.25
	3	5.80	5.20	6.50	7.00
	AVE	5.60	5.37	6.40	7.12
	SD	0.17	0.15	0.17	0.13
1.4ug/hr	1	5.50	5.20	6.10	6.60
	2	5.50	5.10	6.20	6.80
	3	5.80	5.00	6.00	7.00
	AVE	5.60	5.10	6.10	6.80
	SD	0.17	0.10	0.10	0.20

Table 47. Raw data of *Salmonella* Typhimurium growth on fresh chicken using AITC treatment with MAP and without MAP at 4°C

	No.	0day	4day	8day	12day
AIR	1	3.60	4.80	5.20	5.80
	2	3.60	5.00	5.30	5.60
	3	3.70	5.20	5.50	5.30
	AVE	3.63	5.00	5.33	5.57
	SD	0.06	0.20	0.15	0.25
MAP	1	3.60	3.80	3.90	4.30
	2	3.60	3.90	4.00	4.20
	3	3.70	4.00	4.10	4.40
	AVE	3.63	3.90	4.00	4.30
	SD	0.06	0.10	0.10	0.10
4.0ug/hr+AIR	1	3.60	4.00	4.89	5.00
	2	3.60	3.80	4.98	5.20
	3	3.70	3.90	5.00	5.50
	AVE	3.63	3.90	4.96	5.23
	SD	0.06	0.10	0.06	0.25
4.0ug/hr+MAP	1	3.60	3.30	3.50	3.65
	2	3.60	3.20	3.80	3.84
	3	3.70	3.60	3.75	3.95
	AVE	3.63	3.37	3.68	3.81
	SD	0.06	0.21	0.16	0.15
8.0ug/hr+AIR	1	3.60	4.10	4.00	4.50
	2	3.60	3.30	4.20	4.40
	3	3.70	4.00	4.10	4.30
	AVE	3.63	3.80	4.10	4.40
	SD	0.06	0.44	0.10	0.10
8.0ug/hr+MAP	1	3.60	3.20	3.50	3.75
	2	3.60	3.00	3.30	3.55
	3	3.70	3.40	3.40	3.68
	AVE	3.63	3.20	3.40	3.66
	SD	0.06	0.20	0.10	0.10

Table 48. Raw data of *Listeria monocytogenes* growth on fresh chicken using AITC treatment with MAP and without MAP at 4°C

		0day	4day	8day	12day
AIR	1	3.50	4.00	4.70	5.20
	2	3.00	4.10	4.50	5.50
	3	3.20	4.00	5.00	5.10
	AVE	3.23	4.03	4.73	5.27
	SD	0.25	0.06	0.25	0.21
MAP	1	3.50	3.50	3.50	3.90
	2	3.00	3.10	3.40	3.80
	3	3.20	3.30	3.50	3.60
	AVE	3.23	3.30	3.47	3.77
	SD	0.25	0.20	0.06	0.15
0.6ug/hr+AIR	1	3.50	4.20	4.30	5.10
	2	3.00	4.00	4.20	4.80
	3	3.20	4.20	4.40	5.00
	AVE	3.23	4.13	4.30	4.97
	SD	0.25	0.12	0.10	0.15
0.6ug/hr+MAP	1	3.50	3.00	3.50	3.70
	2	3.00	3.10	2.90	3.30
	3	3.20	2.90	3.20	3.60
	AVE	3.23	3.00	3.20	3.53
	SD	0.25	0.10	0.30	0.21
1.2ug/hr+AIR	1	3.50	3.50	3.50	4.20
	2	3.00	3.30	3.40	4.00
	3	3.20	3.50	3.30	4.00
	AVE	3.23	3.43	3.40	4.07
	SD	0.25	0.12	0.10	0.12
1.2ug/hr+MAP	1	3.50	3.20	3.00	3.50
	2	3.00	3.00	3.10	3.70
	3	3.20	3.00	3.00	3.20
	AVE	3.23	3.07	3.03	3.47
	SD	0.25	0.12	0.06	0.25

Table 49. Comparison of total aerobic bacteria growth on fresh chicken using AITC treatment with MAP and without MAP at 4°C

		0day	4day	6day	8day
AIR	1	3.90	6.20	7.50	9.50
	2	3.90	6.00	8.00	9.20
	3	4.00	6.10	7.70	9.40
	AVE	3.93	6.10	7.73	9.37
	SD	0.06	0.10	0.25	0.15
MAP	1	3.90	5.20	6.00	6.50
	2	3.90	5.00	6.10	6.70
	3	4.00	5.30	5.90	6.50
	AVE	3.93	5.17	6.00	6.57
	SD	0.06	0.15	0.10	0.12
0.6ug/hr+AIR	1	3.90	5.50	7.10	7.90
	2	3.90	5.80	7.10	8.10
	3	4.00	5.90	6.90	8.20
	AVE	3.93	5.73	7.03	8.07
	SD	0.06	0.21	0.12	0.15
0.6ug/hr+MAP	1	3.90	4.30	3.80	4.90
	2	3.90	4.20	4.20	4.50
	3	4.00	4.30	4.50	4.50
	AVE	3.93	4.27	4.17	4.63
	SD	0.06	0.06	0.35	0.23
1.2ug/hr+AIR	1	3.90	4.40	5.00	5.50
	2	3.90	4.50	5.30	5.80
	3	4.00	4.90	4.90	5.90
	AVE	3.93	4.60	5.07	5.73
	SD	0.06	0.26	0.21	0.21
1.2ug/hr+MAP	1	3.90	4.00	4.10	4.20
	2	3.90	4.50	4.20	4.50
	3	4.00	4.20	4.50	4.40
	AVE	3.93	4.23	4.27	4.37
	SD	0.06	0.25	0.21	0.15

Table 50. Comparison of *Salmonella* Typhimurium growth on fresh chicken using ClO₂ treatment with MAP and without MAP at 4°C

		0day	4day	8day	12day
AIR	1	3.60	4.80	5.20	5.80
	2	3.60	5.00	5.30	5.60
	3	3.70	5.20	5.50	5.30
	AVE	3.63	5.00	5.33	5.57
	SD	0.06	0.20	0.15	0.25
MAP	1	3.60	3.80	3.90	4.30
	2	3.60	3.90	4.00	4.20
	3	3.70	4.00	4.10	4.40
	AVE	3.63	3.90	4.00	4.30
	SD	0.06	0.10	0.10	0.10
4.0ug/hr+AIR	1	3.60	4.00	4.89	5.00
	2	3.60	3.80	4.98	5.20
	3	3.70	3.90	5.00	5.50
	AVE	3.63	3.90	4.96	5.23
	SD	0.06	0.10	0.06	0.25
4.0ug/hr+MAP	1	3.60	3.30	3.50	3.65
	2	3.60	3.20	3.80	3.84
	3	3.70	3.60	3.75	3.95
	AVE	3.63	3.37	3.68	3.81
	SD	0.06	0.21	0.16	0.15
8.0ug/hr+AIR	1	3.60	4.10	4.00	4.50
	2	3.60	3.30	4.20	4.40
	3	3.70	4.00	4.10	4.30
	AVE	3.63	3.80	4.10	4.40
	SD	0.06	0.44	0.10	0.10
8.0ug/hr+MAP	1	3.60	3.20	3.50	3.75
	2	3.60	3.00	3.30	3.55
	3	3.70	3.40	3.40	3.68
	AVE	3.63	3.20	3.40	3.66
	SD	0.06	0.20	0.10	0.10

Table 51. Comparison of *Listeria monocytogenes* growth on fresh chicken using ClO₂ treatment with MAP and without MAP at 4°C

		0day	4day	8day	12day
AIR	1	3.50	4.00	4.70	5.20
	2	3.00	4.10	4.50	5.50
	3	3.20	4.00	5.00	5.10
	AVE	3.23	4.03	4.73	5.27
	SD	0.25	0.06	0.25	0.21
MAP	1	3.50	3.50	3.50	3.90
	2	3.00	3.10	3.40	3.80
	3	3.20	3.30	3.50	3.60
	AVE	3.23	3.30	3.47	3.77
	SD	0.25	0.20	0.06	0.15
4.0ug/hr+AIR	1	3.50	3.35	4.80	6.00
	2	3.00	4.80	4.40	5.60
	3	3.20	4.00	4.60	5.00
	AVE	3.23	4.05	4.60	5.53
	SD	0.25	0.73	0.20	0.50
4.0ug/hr+MAP	1	3.50	3.30	3.10	3.80
	2	3.00	2.80	3.00	3.90
	3	3.20	3.00	2.70	3.50
	AVE	3.23	3.03	2.93	3.73
	SD	0.25	0.25	0.21	0.21
8.0ug/hr+AIR	1	3.50	3.70	3.70	4.20
	2	3.00	3.30	3.60	4.30
	3	3.20	3.80	3.90	4.40
	AVE	3.23	3.60	3.73	4.30
	SD	0.25	0.26	0.15	0.10
8.0ug/hr+MAP	1	3.50	3.30	3.20	3.80
	2	3.00	3.00	3.00	3.60
	3	3.20	2.80	3.10	3.70
	AVE	3.23	3.03	3.10	3.70
	SD	0.25	0.25	0.10	0.10

Table 52. Comparison of total aerobic bacteria growth on fresh chicken using ClO₂ treatment with MAP and without MAP at 4°C

		0day	4day	6day	8day
AIR	1	3.90	6.20	7.50	9.50
	2	3.90	6.00	8.00	9.20
	3	4.00	6.10	7.70	9.40
	AVE	3.93	6.10	7.73	9.37
	SD	0.06	0.10	0.25	0.15
MAP	1	3.90	5.20	6.00	6.50
	2	3.90	5.00	6.10	6.70
	3	4.00	5.30	5.90	6.50
	AVE	3.93	5.17	6.00	6.57
	SD	0.06	0.15	0.10	0.12
4.0ug/hr+AIR	1	3.90	4.30	6.00	7.60
	2	3.90	4.80	6.10	8.00
	3	4.00	4.20	5.90	7.80
	AVE	3.93	4.43	6.00	7.80
	SD	0.06	0.32	0.10	0.20
4.0ug/hr+MAP	1	3.90	4.00	5.00	5.50
	2	3.90	4.50	5.10	5.40
	3	4.00	4.30	4.70	5.50
	AVE	3.93	4.27	4.93	5.47
	SD	0.06	0.25	0.21	0.06
8.0ug/hr+AIR	1	3.90	4.50	5.60	6.20
	2	3.90	4.50	5.60	6.50
	3	4.00	4.70	5.50	6.00
	AVE	3.93	4.57	5.57	6.23
	SD	0.06	0.12	0.06	0.25
8.0ug/hr+MAP	1	3.90	4.00	4.30	4.70
	2	3.90	4.20	4.10	4.30
	3	4.00	4.00	4.50	4.80
	AVE	3.93	4.07	4.30	4.60
	SD	0.06	0.12	0.20	0.26

Table 53. Raw data of *Listeria monocytogenes* (log cfu/g) on fresh chicken breast with constant release antimicrobial treatment during 21 days at 4°C

	No.	0day	3day	6day	9day	12day	15day	18day	21day
AIR	1	3.30	3.20	4.20	4.80	5.20	6.40	7.00	7.70
	2	3.43	3.80	4.65	5.20	5.87	6.55	7.25	7.50
	3	3.35	3.50	4.60	5.00	5.54	6.30	6.95	7.60
	AVE	3.36	3.50	4.48	5.00	5.54	6.42	7.07	7.60
	SD	0.07	0.30	0.25	0.20	0.34	0.13	0.16	0.10
MAP	1	3.30	3.10	3.30	4.00	4.50	5.60	6.60	6.80
	2	3.43	3.50	3.55	3.70	4.68	5.24	6.07	6.70
	3	3.35	3.30	3.75	4.55	4.60	5.31	6.12	6.65
	AVE	3.36	3.30	3.53	4.08	4.59	5.38	6.26	6.72
	SD	0.07	0.20	0.23	0.43	0.09	0.19	0.29	0.08
4-CIO2	1	3.30	2.90	2.70	3.50	3.90	4.50	5.00	5.60
	2	3.43	3.20	3.55	3.40	4.25	4.80	5.32	5.85
	3	3.35	3.45	3.35	3.95	4.15	4.65	5.25	5.36
	AVE	3.36	3.18	3.20	3.62	4.10	4.65	5.19	5.60
	SD	0.07	0.28	0.44	0.29	0.18	0.15	0.17	0.25
8-CIO2	1	3.30	2.80	2.60	3.10	3.50	4.10	4.50	5.20
	2	3.43	3.00	3.46	3.70	3.80	3.80	4.10	4.40
	3	3.35	2.90	3.30	3.45	3.65	3.95	4.30	4.96
	AVE	3.36	2.90	3.12	3.42	3.65	3.95	4.30	4.85
	SD	0.07	0.10	0.46	0.30	0.15	0.15	0.20	0.41
0.6-AITC	1	3.30	2.80	3.00	3.70	4.10	5.10	5.90	6.55
	2	3.43	3.45	3.65	3.65	4.25	5.15	6.00	6.55
	3	3.35	3.33	3.65	4.15	4.10	5.05	5.85	6.25
	AVE	3.36	3.19	3.43	3.83	4.15	5.10	5.92	6.45
	SD	0.07	0.35	0.38	0.28	0.09	0.05	0.08	0.17
1.2-AITC	1	3.30	2.80	3.00	3.50	3.80	4.60	5.00	5.90
	2	3.43	3.25	3.21	3.50	4.11	5.10	5.60	6.00
	3	3.35	3.45	3.50	4.00	4.25	4.85	5.30	5.95
	AVE	3.36	3.17	3.24	3.67	4.05	4.85	5.30	5.95
	SD	0.07	0.33	0.25	0.29	0.23	0.25	0.30	0.05

Table 54. Raw data of *Salmonella* Typhimurium (log cfu/g) on fresh chicken breast with constant release antimicrobial treatment during 21 days at 4°C

	No.	0day	3day	6day	9day	12day	15day	18day	21day
AIR	1	3.20	3.60	5.00	5.80	7.00	7.10	7.50	7.50
	2	3.37	3.80	5.07	6.07	7.13	7.00	7.20	7.50
	3	3.25	3.75	5.24	6.05	7.26	7.55	7.85	8.00
	AVE	3.27	3.72	5.10	5.97	7.13	7.22	7.52	7.67
	SD	0.09	0.10	0.12	0.15	0.13	0.29	0.33	0.29
MAP	1	3.20	3.20	3.60	5.20	5.15	6.00	6.50	6.90
	2	3.37	3.21	3.99	4.71	5.22	6.15	6.50	6.85
	3	3.25	3.71	4.05	4.98	5.35	6.40	6.60	7.00
	AVE	3.27	3.37	3.88	4.96	5.24	6.18	6.53	6.92
	SD	0.09	0.29	0.24	0.25	0.10	0.20	0.06	0.08
4-CIO2	1	3.20	3.50	3.65	4.50	5.10	5.70	6.15	6.55
	2	3.37	3.00	3.90	4.36	5.00	6.00	6.25	6.60
	3	3.25	3.70	3.50	4.88	5.40	5.90	6.30	6.65
	AVE	3.27	3.40	3.68	4.58	5.17	5.87	6.23	6.60
	SD	0.09	0.36	0.20	0.27	0.21	0.15	0.08	0.05
8-CIO2	1	3.20	3.40	3.30	4.20	4.80	5.12	5.90	6.10
	2	3.37	3.00	3.24	4.14	4.70	5.20	5.90	6.35
	3	3.25	3.70	3.35	3.95	4.75	5.15	5.75	6.25
	AVE	3.27	3.37	3.30	4.10	4.75	5.16	5.85	6.23
	SD	0.09	0.35	0.06	0.13	0.05	0.04	0.09	0.13
0.6-AITC	1	3.20	3.40	3.50	3.90	4.15	4.90	5.60	5.90
	2	3.37	2.80	3.30	3.65	4.22	4.60	5.30	5.80
	3	3.25	3.60	3.50	4.28	4.10	4.60	5.45	6.20
	AVE	3.27	3.27	3.43	3.94	4.16	4.70	5.45	5.97
	SD	0.09	0.42	0.12	0.32	0.06	0.17	0.15	0.21
1.2-AITC	1	3.20	2.90	2.90	3.70	4.10	4.70	5.00	5.70
	2	3.37	2.80	3.10	3.75	3.78	4.15	4.50	5.50
	3	3.25	3.10	3.30	3.60	3.94	4.45	4.80	5.55
	AVE	3.27	2.93	3.10	3.68	3.94	4.43	4.77	5.58
	SD	0.09	0.15	0.20	0.08	0.16	0.28	0.25	0.10

Table 55. Raw data of total aerobic bacteria (log cfu/g) on fresh chicken breast with constant release antimicrobial treatment during 21 days at 4°C

	No.	0day	3day	6day	9day	12day	15day	18day	21day
AIR	1	3.90	5.80	7.08	8.84	8.79	9.05	8.75	9.50
	2	4.20	5.80	7.70	9.00	9.00	8.90	9.60	9.30
	3	4.15	5.75	7.55	9.00	9.15	9.55	9.35	9.45
	AVE	4.08	5.78	7.44	8.95	8.98	9.17	9.23	9.42
	SD	0.16	0.03	0.32	0.09	0.18	0.34	0.44	0.10
MAP	1	3.90	4.40	5.50	6.62	7.50	7.99	8.20	8.50
	2	4.20	4.60	5.76	6.75	7.50	8.20	8.50	8.90
	3	4.15	4.55	5.80	6.70	7.70	8.40	8.70	8.85
	AVE	4.08	4.52	5.69	6.69	7.57	8.20	8.47	8.75
	SD	0.16	0.10	0.16	0.07	0.12	0.21	0.25	0.22
4-CIO2	1	3.90	4.00	4.50	5.20	6.60	7.74	7.70	8.00
	2	4.20	3.80	4.20	5.45	6.88	7.50	8.05	8.42
	3	4.15	4.00	4.40	5.48	6.80	7.75	7.95	8.55
	AVE	4.08	3.93	4.37	5.38	6.76	7.66	7.90	8.32
	SD	0.16	0.12	0.15	0.15	0.14	0.14	0.18	0.29
8-CIO2	1	3.90	3.80	3.85	4.10	5.50	6.00	7.00	7.15
	2	4.20	4.00	3.90	4.40	5.80	6.40	7.25	7.45
	3	4.15	4.15	4.05	4.45	5.75	6.25	7.45	7.55
	AVE	4.08	3.98	3.93	4.32	5.68	6.22	7.23	7.38
	SD	0.16	0.18	0.10	0.19	0.16	0.20	0.23	0.21
0.6-AITC	1	3.90	3.75	4.00	4.20	4.90	6.25	7.35	7.60
	2	4.20	3.90	4.40	4.65	5.20	6.35	7.35	8.25
	3	4.15	3.95	4.60	4.85	5.45	6.40	7.55	8.15
	AVE	4.08	3.87	4.33	4.57	5.18	6.33	7.42	8.00
	SD	0.16	0.10	0.31	0.33	0.28	0.08	0.12	0.35
1.2-AITC	1	3.90	3.20	3.60	3.90	4.40	5.00	6.50	7.05
	2	4.20	3.50	4.00	4.30	4.80	5.80	6.80	7.35
	3	4.15	3.55	4.15	4.40	4.75	5.75	6.88	7.45
	AVE	4.08	3.42	3.92	4.20	4.65	5.52	6.73	7.28
	SD	0.16	0.19	0.28	0.26	0.22	0.45	0.20	0.21

Table 56. Raw data for pH values of fresh chicken breast for 21 days, 4°C (1st test set)

	No.	0day	3day	6day	9day	12day	15day	18day	21day
AIR	1	5.79	5.82	6.00	5.99	6.05	6.20	6.30	6.32
	2	5.77	5.79	6.03	6.01	6.11	6.25	6.25	6.31
	3	5.80	5.80	5.99	5.89	6.14	6.23	6.20	6.31
	4	5.79	5.82	6.02	5.92	6.03	6.18	6.25	6.30
	AVE	5.79	5.81	6.01	5.95	6.08	6.22	6.25	6.31
	SD	0.01	0.02	0.02	0.06	0.05	0.03	0.04	0.01
MAP	1	5.79	5.83	5.81	5.78	5.89	5.99	6.01	6.01
	2	5.77	5.79	5.82	5.83	5.95	5.94	5.88	6.05
	3	5.80	5.77	5.79	5.77	5.94	6.09	5.91	5.94
	4	5.79	5.82	5.80	5.80	6.03	5.95	5.99	6.05
	AVE	5.79	5.80	5.81	5.80	5.95	5.99	5.95	6.01
	SD	0.01	0.03	0.01	0.03	0.06	0.07	0.06	0.05
4-CIO2	1	5.79	5.79	5.77	5.80	5.81	5.81	5.72	5.75
	2	5.77	5.80	5.76	5.82	5.79	5.77	5.72	5.75
	3	5.80	5.71	5.80	5.77	5.84	5.93	5.80	5.75
	4	5.79	5.77	5.79	5.78	5.79	5.64	5.77	5.71
	AVE	5.79	5.77	5.78	5.79	5.81	5.79	5.75	5.74
	SD	0.01	0.04	0.02	0.02	0.02	0.12	0.04	0.02
8-CIO2	1	5.79	5.77	5.66	5.45	5.42	5.45	5.50	5.40
	2	5.77	5.66	5.60	5.42	5.49	5.24	5.55	5.45
	3	5.80	5.82	5.54	5.50	5.40	5.58	5.50	5.40
	4	5.79	5.79	5.57	5.48	5.42	5.35	5.49	5.55
	AVE	5.79	5.76	5.59	5.46	5.43	5.41	5.51	5.45
	SD	0.01	0.07	0.05	0.04	0.04	0.14	0.03	0.07
0.6-AITC	1	5.79	5.81	5.84	5.82	5.82	5.89	5.82	5.88
	2	5.77	5.84	5.68	5.79	5.79	5.72	5.81	5.83
	3	5.80	5.80	5.71	5.88	5.88	5.71	5.85	5.79
	4	5.79	5.79	5.77	5.78	5.63	5.85	5.83	5.85
	AVE	5.79	5.81	5.75	5.82	5.78	5.79	5.83	5.84
	SD	0.01	0.02	0.07	0.04	0.11	0.09	0.02	0.04
1.2-AITC	1	5.79	5.79	5.88	5.80	5.84	5.88	5.82	5.88
	2	5.77	5.81	5.72	5.72	5.82	5.72	5.82	5.83
	3	5.80	5.83	5.81	5.79	5.77	5.69	5.80	5.85
	4	5.79	5.82	5.72	5.80	5.79	5.75	5.81	5.82
	AVE	5.79	5.81	5.78	5.78	5.81	5.76	5.81	5.85
	SD	0.01	0.02	0.08	0.04	0.03	0.08	0.01	0.03

Table 57. Raw data for pH values of fresh chicken breast for 21 days, 4°C (2st test set)

	No.	0day	3day	6day	9day	12day	15day	18day	21day
AIR	1	6.32	6.43	6.34	6.44	6.45	6.48	6.51	6.52
	2	6.32	6.32	6.39	6.45	6.44	6.42	6.43	6.55
	3	6.36	6.35	6.43	6.42	6.47	6.51	6.55	6.55
	4	6.33	6.40	6.46	6.45	6.45	6.49	6.45	6.53
	AVE	6.33	6.38	6.41	6.44	6.45	6.48	6.49	6.54
	SD	0.02	0.05	0.05	0.01	0.01	0.04	0.06	0.02
MAP	1	6.32	6.30	6.35	x	6.40	6.42	6.44	6.45
	2	6.32	6.26	6.34	6.32	6.47	6.43	6.49	6.44
	3	6.36	6.32	6.32	6.30	6.35	6.44	6.43	6.43
	4	6.33	6.28	6.33	6.31	6.41	6.42	6.45	6.49
	AVE	6.33	6.29	6.34	6.31	6.41	6.43	6.45	6.45
	SD	0.02	0.03	0.01	0.01	0.05	0.01	0.03	0.03
4-CIO2	1	6.32	6.40	6.27	6.30	6.28	6.37	6.34	6.24
	2	6.32	6.34	6.29	6.31	6.22	6.18	6.27	6.18
	3	6.36	6.34	6.29	6.27	6.30	6.22	6.18	6.31
	4	6.33	6.32	6.30	6.31	6.22	6.25	6.20	6.25
	AVE	6.33	6.35	6.29	6.30	6.26	6.26	6.25	6.25
	SD	0.02	0.03	0.01	0.02	0.04	0.08	0.07	0.05
8-CIO2	1	6.32	6.24	6.04	6.05	6.00	6.03	6.02	5.95
	2	6.32	6.44	6.07	6.07	6.00	5.99	5.99	5.89
	3	6.36	6.46	6.12	6.09	6.10	6.03	5.88	6.00
	4	6.33	6.36	6.12	6.03	5.98	6.00	5.92	5.89
	AVE	6.33	6.38	6.09	6.06	6.02	6.01	5.95	5.93
	SD	0.02	0.10	0.04	0.03	0.05	0.02	0.06	0.05
0.6-AITC	1	6.32	6.39	6.28	6.30	6.25	6.28	6.24	6.34
	2	6.32	6.36	6.29	6.32	6.30	6.15	6.25	6.40
	3	6.36	6.33	6.43	6.34	6.33	6.45	6.38	6.34
	4	6.33	6.34	6.30	6.45	6.40	6.33	6.37	6.31
	AVE	6.33	6.36	6.33	6.35	6.32	6.30	6.31	6.35
	SD	0.02	0.03	0.07	0.07	0.06	0.12	0.08	0.04
1.2-AITC	1	6.32	6.32	6.45	6.34	6.34	6.35	6.34	6.33
	2	6.32	6.36	6.32	6.42	6.32	6.37	6.35	6.35
	3	6.36	6.32	6.33	6.33	6.38	6.34	6.38	6.40
	4	6.33	6.33	6.28	6.31	6.33	6.32	6.32	6.31
	AVE	6.33	6.33	6.35	6.35	6.34	6.35	6.35	6.35
	SD	0.02	0.02	0.07	0.05	0.03	0.02	0.02	0.04

Table 58. Raw data for color “L” values of fresh chicken breast for 21 days, 4°C (1st test set)

	No.	0day	3day	6day	9day	12day	15day	18day	21day
AIR	1	53.1	53.21	53.30	53.20	52.63	52.13	51.00	51.30
	2	53.52	54.30	54.20	55.30	55.38	52.50	51.27	50.11
	3	53.15	53.24	52.13	52.10	54.28	51.43	51.30	49.98
	4	52.95	53.30	53.50	53.30	53.46	52.11	51.40	49.80
	AVE	53.18	53.51	53.28	53.48	53.94	52.04	51.24	50.30
	SD	0.24	0.53	0.86	1.33	1.17	0.45	0.17	0.68
MAP	1	53.1	49.00	48.27	47.25	49.00	50.00	48.00	47.50
	2	53.52	47.00	49.38	49.38	48.27	49.25	48.29	48.25
	3	53.15	51.00	50.27	50.25	47.25	48.28	49.30	47.25
	4	52.95	50.68	51.38	49.25	51.28	50.28	48.25	49.56
	AVE	53.18	49.42	49.83	49.03	48.95	49.45	48.46	48.14
	SD	0.24	1.84	1.32	1.27	1.71	0.90	0.58	1.04
4-CIO2	1	53.1	51.44	48.25	49.25	50.25	49.35	49.00	48.00
	2	53.52	49.68	50.25	48.28	49.72	49.73	48.39	49.73
	3	53.15	48.36	48.30	50.83	50.71	50.71	48.40	47.36
	4	52.95	47.38	51.30	47.75	46.78	47.45	47.50	46.79
	AVE	53.18	49.22	49.53	49.03	49.37	49.31	48.32	47.97
	SD	0.24	1.76	1.51	1.35	1.77	1.37	0.62	1.27
8-CIO2	1	53.1	50.36	49.25	48.54	45.13	45.50	44.00	44.30
	2	53.52	49.36	51.50	48.56	47.22	44.50	43.25	43.22
	3	53.15	46.38	48.30	46.25	45.26	45.21	43.90	44.20
	4	52.95	51.36	48.56	47.55	46.13	44.30	44.30	46.25
	AVE	53.18	49.37	49.40	47.72	45.94	44.88	43.86	44.49
	SD	0.24	2.15	1.45	1.09	0.96	0.57	0.44	1.27
0.6-AITC	1	53.1	51.26	50.35	51.28	50.57	50.25	49.50	48.50
	2	53.52	50.26	47.25	49.83	51.37	51.29	51.25	49.25
	3	53.15	49.56	48.35	50.85	49.29	49.28	50.30	51.21
	4	52.95	48.59	52.36	49.19	50.28	51.29	50.28	49.21
	AVE	53.18	49.92	49.58	50.29	50.38	50.53	50.33	49.54
	SD	0.24	1.13	2.25	0.95	0.86	0.97	0.72	1.16
1.2-AITC	1	53.1	49.25	51.00	52.00	53.00	52.46	53.10	56.40
	2	53.52	50.20	50.40	51.26	50.40	50.12	53.30	53.50
	3	53.15	48.52	49.25	49.30	49.78	49.89	52.70	53.52
	4	52.95	49.52	49.30	50.32	48.25	51.36	53.02	55.26
	AVE	53.18	49.37	49.99	50.72	50.36	50.96	53.03	54.67
	SD	0.24	0.69	0.86	1.17	1.98	1.19	0.25	1.42

Table 59. Raw data for color “L” values of fresh chicken breast for 21 days, 4°C (2nd test set)

	No.	0day	3day	6day	9day	12day	15day	18day	21day
AIR	1	45.50	45.70	46.00	46.10	45.60	45.10	44.20	43.50
	2	46.40	46.30	45.70	46.20	44.40	43.20	44.30	44.10
	3	46.54	46.20	46.50	45.80	46.10	44.40	43.90	43.00
	4	47.90	46.30	46.20	46.10	46.50	45.10	44.40	42.30
	AVE	46.59	46.13	46.10	46.05	45.65	44.45	44.20	43.23
	SD	0.99	0.29	0.34	0.17	0.91	0.90	0.22	0.76
MAP	1	45.50	44.70	44.20	43.30	44.00	45.00	44.80	44.10
	2	45.40	44.20	44.40	44.40	44.30	44.30	43.30	43.30
	3	46.54	44.00	45.30	44.30	44.30	43.30	44.30	44.60
	4	47.90	45.00	43.80	44.30	44.80	45.30	45.10	43.60
	AVE	46.34	44.48	44.43	44.08	44.35	44.48	44.38	43.90
	SD	1.16	0.46	0.63	0.52	0.33	0.89	0.79	0.57
4-CIO2	1	45.50	44.40	44.50	44.30	44.60	44.40	43.00	43.00
	2	45.40	44.70	43.70	43.50	44.70	44.70	43.40	43.70
	3	46.54	44.80	44.30	44.80	44.70	43.70	44.40	42.40
	4	47.90	43.90	45.30	43.80	43.80	44.50	43.50	43.50
	AVE	46.34	44.45	44.45	44.10	44.45	44.33	43.58	43.15
	SD	1.16	0.40	0.66	0.57	0.44	0.43	0.59	0.58
8-CIO2	1	45.50	44.40	44.30	44.10	42.10	40.50	38.40	39.30
	2	45.40	44.40	44.50	44.20	42.20	40.50	39.20	39.10
	3	46.54	43.40	44.40	44.20	41.30	39.50	40.10	39.20
	4	47.90	44.40	44.60	44.60	41.10	39.30	38.30	40.30
	AVE	46.34	44.15	44.45	44.28	41.68	39.95	39.00	39.48
	SD	1.16	0.50	0.13	0.22	0.56	0.64	0.84	0.56
0.6-AITC	1	45.50	45.30	44.80	45.30	45.60	45.30	44.00	43.50
	2	45.40	44.30	44.30	44.80	46.40	45.30	45.30	44.30
	3	46.54	44.60	44.40	44.50	44.30	44.30	44.30	46.20
	4	47.90	44.60	44.70	44.20	45.30	45.30	44.60	44.20
	AVE	46.34	44.70	44.55	44.70	45.40	45.05	44.55	44.55
	SD	1.16	0.42	0.24	0.47	0.87	0.50	0.56	1.16
1.2-AITC	1	45.50	44.30	45.00	45.10	46.00	46.50	47.30	48.40
	2	45.40	44.30	45.40	44.80	45.40	45.10	47.80	48.50
	3	46.54	43.50	44.30	44.80	44.80	45.90	48.40	48.50
	4	47.90	44.50	44.40	45.30	45.30	46.40	48.50	49.30
	AVE	46.34	44.15	44.78	45.00	45.38	45.98	48.00	48.68
	SD	1.16	0.44	0.52	0.24	0.49	0.64	0.56	0.42

Table 60. Raw data for color "a" values of fresh chicken breast for 21 days, 4°C
(1st test set)

	No.	0day	3day	6day	9day	12day	15day	18day	21day
AIR	1	2.71	3.00	3.10	2.70	2.80	2.70	2.60	2.80
	2	2.73	2.70	2.40	2.40	3.00	2.70	2.70	2.90
	3	2.51	2.50	2.40	3.00	2.50	2.70	2.80	2.90
	4	2.78	2.60	2.90	2.60	2.80	2.80	2.80	2.40
	AVE	2.68	2.70	2.70	2.68	2.78	2.73	2.73	2.75
	SD	0.12	0.22	0.36	0.25	0.21	0.05	0.10	0.24
MAP	1	2.71	1.70	1.80	1.90	1.90	1.50	1.20	1.70
	2	2.73	1.50	1.70	1.50	0.90	1.90	1.90	1.70
	3	2.51	1.80	1.50	1.70	2.60	1.70	1.50	1.70
	4	2.78	1.80	1.90	1.30	1.40	1.80	1.90	1.80
	AVE	2.68	1.70	1.73	1.60	1.70	1.73	1.63	1.73
	SD	0.12	0.14	0.17	0.26	0.73	0.17	0.34	0.05
4-CIO2	1	2.71	1.50	1.50	1.80	2.00	1.50	1.50	2.00
	2	2.73	2.20	2.20	2.20	1.80	1.80	1.70	1.70
	3	2.51	1.60	1.80	1.40	1.60	2.00	1.80	2.20
	4	2.78	1.50	1.50	1.80	1.60	1.70	2.00	2.00
	AVE	2.68	1.70	1.75	1.80	1.75	1.75	1.75	1.98
	SD	0.12	0.34	0.33	0.33	0.19	0.21	0.21	0.21
8-CIO2	1	2.71	1.80	1.70	2.60	2.50	2.70	3.10	2.90
	2	2.73	2.00	1.60	2.30	2.60	2.60	2.90	3.20
	3	2.51	1.50	1.70	2.40	2.80	3.00	3.00	3.40
	4	2.78	1.40	1.90	2.40	2.90	3.10	2.80	3.30
	AVE	2.68	1.68	1.73	2.43	2.70	2.85	2.95	3.20
	SD	0.12	0.28	0.13	0.13	0.18	0.24	0.13	0.22
0.6-AITC	1	2.71	1.80	1.30	1.60	2.30	2.40	1.80	1.70
	2	2.73	1.50	2.20	2.00	1.50	2.10	1.50	1.80
	3	2.51	2.00	1.80	1.60	1.80	1.50	1.90	1.90
	4	2.78	1.60	1.70	1.70	1.70	1.80	1.70	1.60
	AVE	2.68	1.73	1.75	1.73	1.83	1.95	1.73	1.75
	SD	0.12	0.22	0.37	0.19	0.34	0.39	0.17	0.13
1.2-AITC	1	2.71	2.00	1.50	1.50	1.70	1.00	0.60	0.50
	2	2.73	1.50	2.20	1.40	1.70	1.10	0.80	0.30
	3	2.51	1.90	1.80	1.50	1.20	1.50	0.40	0.50
	4	2.78	1.70	1.20	1.60	1.50	1.50	0.50	0.30
	AVE	2.68	1.78	1.68	1.50	1.53	1.28	0.58	0.40
	SD	0.12	0.22	0.43	0.08	0.24	0.26	0.17	0.12

Table 61. Raw data for color “a” values of fresh chicken breast for 21 days, 4°C (2nd test set)

	No.	0day	3day	6day	9day	12day	15day	18day	21day
AIR	1	4.50	4.50	4.10	4.30	4.15	4.35	4.55	4.22
	2	4.22	4.26	4.70	4.15	4.15	4.06	4.22	4.10
	3	4.60	4.35	4.15	4.30	4.56	4.22	4.10	4.15
	4	3.90	4.56	4.22	4.20	4.12	4.10	4.05	4.35
	AVE	4.31	4.42	4.29	4.24	4.25	4.18	4.23	4.21
	SD	0.31	0.14	0.28	0.08	0.21	0.13	0.22	0.11
MAP	1	3.50	3.80	3.50	3.52	3.50	3.60	3.45	4.30
	2	3.20	3.10	3.40	4.00	3.54	3.75	3.70	3.50
	3	4.60	3.44	3.90	3.22	3.40	3.45	3.30	4.00
	4	3.90	3.75	3.80	3.50	3.60	3.25	3.54	3.70
	AVE	3.80	3.52	3.65	3.56	3.51	3.51	3.50	3.88
	SD	0.61	0.32	0.24	0.32	0.08	0.21	0.17	0.35
4-CIO2	1	3.50	3.50	3.44	3.64	4.00	3.74	3.85	4.02
	2	3.20	3.70	3.80	3.50	3.66	3.33	3.64	3.88
	3	4.60	3.30	3.75	3.30	3.50	3.45	3.55	3.75
	4	3.90	3.50	3.35	3.34	3.54	3.54	3.34	3.95
	AVE	3.80	3.50	3.59	3.45	3.68	3.52	3.60	3.90
	SD	0.61	0.16	0.22	0.16	0.23	0.17	0.21	0.12
8-CIO2	1	3.50	3.65	3.88	3.95	4.12	4.35	4.56	4.65
	2	3.20	3.60	4.05	4.01	4.55	4.35	4.55	4.85
	3	4.60	3.44	3.99	3.85	4.32	4.32	4.55	4.65
	4	3.90	3.45	3.80	3.95	4.24	4.20	4.72	4.55
	AVE	3.80	3.54	3.93	3.94	4.31	4.31	4.60	4.68
	SD	0.61	0.11	0.11	0.07	0.18	0.07	0.08	0.13
0.6-AITC	1	3.50	3.50	3.80	3.65	3.45	3.75	3.54	3.30
	2	3.20	3.56	3.40	3.64	3.33	3.40	3.64	3.64
	3	4.60	3.65	3.20	3.50	3.75	3.30	3.77	3.65
	4	3.90	3.55	3.95	3.40	3.65	3.75	3.65	3.65
	AVE	3.80	3.57	3.59	3.55	3.55	3.55	3.65	3.56
	SD	0.61	0.06	0.35	0.12	0.19	0.23	0.09	0.17
1.2-AITC	1	3.50	3.60	3.55	3.50	3.35	3.45	2.55	2.50
	2	3.20	3.65	3.77	3.65	3.45	3.25	3.10	2.65
	3	4.60	3.58	3.60	3.77	3.50	3.15	3.50	2.70
	4	3.90	3.66	3.64	3.56	3.50	2.75	2.25	2.65
	AVE	3.80	3.62	3.64	3.62	3.45	3.15	2.85	2.63
	SD	0.61	0.04	0.09	0.12	0.07	0.29	0.56	0.09

Table 62. Raw data for color “b” values of fresh chicken breast for 21 days, 4°C
(1st test set)

	No.	0day	3day	6day	9day	12day	15day	18day	21day
AIR	1	2.15	2.15	2.22	2.15	1.90	2.30	2.13	1.98
	2	2.22	2.22	2.30	2.32	1.95	2.10	2.35	2.00
	3	2.35	1.95	2.12	2.00	2.21	1.99	2.00	2.32
	4	2.10	2.25	2.05	2.25	2.15	2.22	2.10	2.50
	AVE	2.21	2.14	2.17	2.18	2.05	2.15	2.15	2.20
	SD	0.11	0.13	0.11	0.14	0.15	0.14	0.15	0.25
MAP	1	2.15	2.20	1.80	2.25	1.90	2.10	2.50	2.20
	2	2.22	2.24	2.40	2.35	1.90	2.10	2.90	2.50
	3	2.35	2.50	2.20	2.50	2.60	2.10	2.20	1.90
	4	2.10	2.20	2.90	2.24	2.90	3.00	1.90	2.70
	AVE	2.21	2.29	2.33	2.34	2.33	2.33	2.38	2.33
	SD	0.11	0.14	0.46	0.12	0.51	0.45	0.43	0.35
4-CIO2	1	2.15	2.30	2.40	2.13	2.40	2.50	2.90	2.60
	2	2.22	2.20	2.00	2.20	2.30	2.30	2.20	2.50
	3	2.35	2.20	2.20	2.34	2.00	2.00	2.85	2.75
	4	2.10	2.50	2.30	2.33	2.60	1.80	2.25	2.77
	AVE	2.21	2.30	2.23	2.25	2.33	2.15	2.55	2.66
	SD	0.11	0.14	0.17	0.10	0.25	0.31	0.38	0.13
8-CIO2	1	2.15	2.50	2.87	3.10	2.90	3.50	3.50	3.50
	2	2.22	2.22	2.90	2.90	2.65	3.45	3.25	3.22
	3	2.35	2.44	2.88	2.99	3.50	3.50	3.55	3.45
	4	2.10	2.40	2.68	3.25	3.20	2.90	3.45	3.55
	AVE	2.21	2.39	2.83	3.06	3.06	3.34	3.44	3.43
	SD	0.11	0.12	0.10	0.15	0.37	0.29	0.13	0.15
0.6-AITC	1	2.15	2.50	2.35	2.40	2.50	3.30	2.80	2.30
	2	2.22	2.80	2.55	2.15	3.00	2.30	2.65	2.40
	3	2.35	2.20	2.20	2.20	2.50	2.20	2.30	2.50
	4	2.10	2.00	2.50	2.30	1.90	1.90	2.53	2.50
	AVE	2.21	2.38	2.40	2.26	2.48	2.43	2.57	2.43
	SD	0.11	0.35	0.16	0.11	0.45	0.61	0.21	0.10
1.2-AITC	1	2.15	2.70	2.50	2.45	2.25	2.90	3.00	3.00
	2	2.22	1.70	2.20	2.50	2.60	2.46	3.50	4.00
	3	2.35	2.00	2.40	2.33	2.60	2.68	3.00	4.00
	4	2.10	2.50	2.70	2.45	2.50	2.45	4.00	3.00
	AVE	2.21	2.23	2.45	2.43	2.49	2.62	3.38	3.50
	SD	0.11	0.46	0.21	0.07	0.17	0.21	0.48	0.58

APPENDIX E
Raw data tables of tensile strength (TS), elongation at break, oxygen
transmission rate, and color

Table 63. Raw data for color "b" values of fresh chicken breast for 21 days, 4°C (2nd test set)

	No.	0day	3day	6day	9day	12day	15day	18day	21day
AIR	1	1.90	1.34	1.55	1.13	1.20	1.40	1.20	1.74
	2	1.65	1.32	1.25	1.64	1.10	1.45	1.56	1.45
	3	2.00	1.23	1.35	1.70	1.32	1.50	1.50	1.64
	4	1.75	1.35	1.33	1.69	1.48	1.55	1.76	1.35
	AVE	1.83	1.31	1.37	1.54	1.28	1.48	1.51	1.55
	SD	0.16	0.05	0.13	0.27	0.16	0.06	0.23	0.18
MAP	1	1.90	1.96	2.30	1.70	1.84	1.69	1.60	1.70
	2	1.65	1.95	1.70	2.40	1.85	1.77	1.75	1.77
	3	2.00	1.80	1.79	1.74	1.84	1.65	1.87	1.73
	4	1.75	1.77	1.82	1.76	1.67	1.76	1.76	1.65
	AVE	1.83	1.87	1.90	1.90	1.80	1.72	1.75	1.71
	SD	0.16	0.10	0.27	0.33	0.09	0.06	0.11	0.05
4-CIO2	1	1.90	1.71	1.70	1.98	1.84	1.59	1.63	1.88
	2	1.65	1.64	1.87	1.80	1.83	2.10	1.86	1.75
	3	2.00	1.88	1.80	1.87	1.86	1.99	1.65	2.04
	4	1.75	1.97	1.84	1.76	1.67	1.84	1.9	1.88
	AVE	1.83	1.80	1.80	1.85	1.80	1.88	1.76	1.89
	SD	0.16	0.15	0.07	0.10	0.09	0.22	0.14	0.12
8-CIO2	1	1.90	1.77	1.78	2.10	2.54	2.95	3.52	4.21
	2	1.65	2.00	1.90	2.31	2.11	3.25	3.35	3.95
	3	2.00	1.80	2.07	2.31	2.22	3.18	3.89	4.44
	4	1.75	1.66	1.89	2.11	2.46	3.54	3.98	4.35
	AVE	1.83	1.81	1.91	2.21	2.33	3.23	3.69	4.24
	SD	0.16	0.14	0.12	0.12	0.20	0.24	0.30	0.21
0.6-AITC	1	1.90	1.65	2.11	1.64	1.98	2.00	1.65	2.04
	2	1.65	1.98	1.61	1.64	1.85	1.78	2.10	1.87
	3	2.00	2.10	1.71	1.88	1.75	1.69	1.89	1.84
	4	1.75	1.77	1.65	1.95	1.87	1.88	1.87	1.65
	AVE	1.83	1.88	1.77	1.78	1.86	1.84	1.88	1.85
	SD	0.16	0.20	0.23	0.16	0.09	0.13	0.18	0.16
1.2-AITC	1	1.90	1.70	1.70	1.87	1.95	2.15	2.50	3.00
	2	1.65	1.70	1.75	2.05	2.01	2.10	2.40	3.33
	3	2.00	2.00	2.20	1.75	1.84	2.33	2.80	2.75
	4	1.75	1.89	1.8	1.86	2.1	2.25	2.75	2.85
	AVE	1.83	1.82	1.86	1.88	1.98	2.21	2.61	2.98
	SD	0.16	0.15	0.23	0.12	0.11	0.10	0.19	0.25

Table 64. Raw data for Tensile strength and Elongation at break of packaging films (PVC, LDPE, and PS) after ClO₂ treatment

ClO ₂ (ppm)	No.	PVC		LDPE		PS	
		E.B	T.S	E.B	T.S	E.B	T.S
Control	1	2.97	15.50	10.17	4.18	0.61	14.3
	2	2.43	13.40	8.50	4.12	0.36	14.3
	3	3.16	16.90	11.38	5.20	0.75	14.2
	4	3.12	15.90	8.68	4.27	0.25	13.87
	5	3.21	17.10	10.10	4.43	0.36	13.9
	AVE	2.98	15.76	9.77	4.44	0.47	14.11
	SD	0.32	1.48	1.19	0.44	0.21	0.21
250 ppm	1	2.89	15.24	9.77	4.57	0.23	13.48
	2	3.05	14.50	9.85	4.56	0.18	13.95
	3	3.01	15.06	9.67	4.55	0.28	14
	4	2.88	15.50	9.50	4.65	0.21	14.2
	5	2.90	13.70	9.78	4.57	0.18	13.85
	AVE	2.95	14.80	9.71	4.58	0.22	13.9
	SD	0.08	0.72	0.14	0.04	0.04	0.27
500 ppm	1	3.12	14.99	10.02	4.59	0.25	15.42
	2	3.17	14.85	10.20	4.60	0.42	15.05
	3	3.25	15.15	9.85	4.60	0.17	15.05
	4	2.89	15.24	10.03	4.55	0.25	15.13
	5	2.85	15.02	10.50	4.63	0.16	15.16
	AVE	3.06	15.05	10.12	4.59	0.25	15.16
	SD	0.18	0.15	0.25	0.03	0.1	0.15
1000 ppm	1	3.51	15.00	10.34	4.62	0.24	15.59
	2	3.25	15.20	10.31	4.66	0.22	15.15
	3	3.64	14.80	11.40	4.65	0.27	15.38
	4	2.75	15.08	11.35	4.56	0.18	15.26
	5	2.85	14.90	9.45	4.57	0.25	15.14
	AVE	3.20	15.00	10.57	4.61	0.23	15.3
	SD	0.39	0.16	0.82	0.05	0.03	0.19
2000 ppm	1	2.85	15.10	9.90	4.43	0.2	15.7
	2	2.64	14.50	10.08	4.70	0.14	15.05
	3	3.33	15.06	10.06	4.94	0.24	15.38
	4	3.59	15.50	10.10	4.54	0.34	15.36
	5	3.27	13.70	10.99	4.52	0.22	15.14
	AVE	3.14	14.77	10.23	4.63	0.23	15.33
	SD	0.38	0.70	0.43	0.20	0.07	0.25

Table 65. Raw data for Oxygen permeability of packaging films (PVC, LDPE, and PS) after ClO₂ treatment

ClO ₂ (ppm)	No.	PS	PVC	LDPE
0 ppm	1	322.0	150.0	450.0
	2	317.0	175.0	470.0
	3	330.0	165.0	465.0
	AVE	323.0	163.3	461.7
	SD	6.6	12.6	10.4
500 ppm	1	292.0	155.0	455.0
	2	302.0	162.0	456.0
	3	282.0	160.0	470.0
	AVE	292.0	159.0	460.3
	SD	10.0	3.6	8.4
1000 ppm	1	287.0	170.0	460.0
	2	285.0	166.0	475.0
	3	300.0	158.0	455.0
	AVE	290.7	164.7	463.3
	SD	8.1	6.1	10.4
2000 ppm	1	271.0	145.0	477.0
	2	275.0	158.0	445.0
	3	267.0	160.0	458.0
	AVE	271.0	154.3	460.0
	SD	4.0	8.1	16.1

Table 66. Raw data for color changes of packaging films ((PVC, LDPE, and PS) after ClO₂ treatment

	ClO ₂ (ppm)	No.	L*	a*	b*	Dele
PS	0 ppm	1	91.48	-0.87	-0.95	91.49
		2	91.48	-0.94	-0.84	91.49
		3	92.29	-0.94	-0.84	92.30
		AVE	91.75	-0.92	-0.88	91.76
		SD	0.47	0.04	0.06	0.47
	500 ppm	1	92.51	-0.87	0.15	92.51
		2	89.56	-0.95	-0.54	89.57
		3	88.46	-0.87	-0.32	88.46
		AVE	90.18	-0.90	-0.24	90.18
		SD	2.09	0.05	0.35	2.09
	1000 ppm	1	86.08	-0.84	1.25	86.09
		2	88.65	-0.85	2.35	88.69
		3	86.87	-0.81	2.33	86.91
		AVE	87.20	-0.83	1.98	87.23
		SD	1.32	0.02	0.63	1.33
PVC	0 ppm	1	92.54	-0.87	-0.78	92.55
		2	93.52	-0.94	-0.88	93.53
		3	91.54	-0.84	-0.86	91.55
		AVE	92.53	-0.88	-0.84	92.54
		SD	0.99	0.05	0.05	0.99
	500 ppm	1	91.48	-0.87	-0.87	91.49
		2	92.29	-0.85	-0.92	92.30
		3	91.96	-0.94	-0.86	91.97
		AVE	91.91	-0.89	-0.88	91.92
		SD	0.41	0.05	0.03	0.41
	1000 ppm	1	91.46	-0.87	-0.95	91.47
		2	92.46	-0.91	-0.84	92.47
		3	91.68	-0.94	-0.92	91.69
		AVE	91.87	-0.91	-0.90	91.88
		SD	0.53	0.04	0.06	0.52

Table 66 (continued)

LDPE	0 ppm	1	91.45	-0.87	-0.97	91.46
		2	91.88	-0.94	-0.91	91.89
		3	92.32	-0.94	-0.85	92.33
		AVE	91.88	-0.92	-0.91	91.89
		SD	0.44	0.04	0.06	0.43
	500 ppm	1	91.15	-0.87	-0.94	91.16
		2	92.45	-0.94	-0.64	92.46
		3	90.34	-0.94	-0.97	90.35
		AVE	91.31	-0.92	-0.85	91.32
		SD	1.06	0.04	0.18	1.06
	1000 ppm	1	91.21	-0.88	-0.88	91.22
		2	90.24	-0.94	-0.94	90.25
		3	89.89	-0.81	-0.97	89.90
		AVE	90.45	-0.88	-0.93	90.46
		SD	0.68	0.07	0.05	0.68

BIBLIOGRAPHY

Aberle ED, Forrest JC, Gerrard DE and Mills EW. 2001. Principles of meat science. Kendall/Hunt Publishing Company

Ahvenainen R. 2003. Novel food packaging techniques. Woodhead Publishing Ltd.

Aksu MI, Karaoglu M, Esenbuga N, Kaya M and Macit M. 2006. Effect of meat piece. packaging and storage on pH thiobarbituric acid reactive substances and microbial counts in broilers fed diets supplemented with ram horn hydrolysate. Food Science and Technologies International. 12(2): 133-143.

Allen CD, Russell SM, and Fletcher DL. 1998. The relationship of broiler breast color to meat quality and shelf life. Poultry Science. 77: 361-366.

Appendini P and Hotchkiss JH. 1997. Immobilization of lysozyme on food contact polymers as potential antimicrobial films. Packaging Technology and Science. 10(5): 271-279.

Ayres JC. Ogilvy WS and Stewart GF. 1950. Post mortem changes in stored meats. Microorganisms associated with development of slime on eviscerated cut-up poultry. Food Technology. 4: 199-205

Bajard S, Rosso L, Fardel G and Flandrois JP. 1996. The particular behavior of *Listeria monocytogenes* under sub-optimal condition. International Journal of Food Microbiology. 29: 201~211

**Bionewsonline. 2005. What is Salmonella?. Automation in Microbiology and Bioscience.
Available at: http://www.bionewsonline.com/h/what_is_salmonella.htm.**

Bonilauri P, Liuzzo G, Merialdi G and Bentley S. 2004. Growth of *listeria monocytogenes* on vacuum-packaged horsemeat for human consumption. Meat Science. 68: 671-674.

Brody A. 2001. Active packaging for food applications. Technomic Publishing Co.. Inc.. Lancaster. PA.

Brody A. 2005. The application of active packaging. IFT conference meeting. Orlando. USA.

Brody A. 2006. State of the art of active / intelligent food packaging. IFT meeting. Las Vegas. USA.

Buchman GW, Banerjee S and Hansen JN. 1988. Structure, expression and evolution of a gene encoding the precursor of nisin. a small protein antibiotic. Journal of Biological Chemistry. 263: 16260-16266.

Cabo ML, Herrera JR, Sampedro G and Pastoriza L. 2001. Effectiveness of CO₂ and Nisaplin on increasing shelf life of fresh pizza. Food Microbiology. 18: 489-498.

Cagri A, Ustunol Z and Ryser ET. 2002. Inhibition of Three Pathogens on Bologna and Summer Sausage Using Antimicrobial Edible Films. Journal of Food Science. 67(6): 2317-2324.

Center for Disease Control and Prevention (CDC). 1999. Multi-state outbreak of listeriosis. MMWR. 49: 1129-1130.

Center for Disease Control and Prevention (CDC). 1995. Outbreak of Salmonella Serotype Typhimurium Infection Associated with Eating Raw Ground Beef—Wisconsin. Morbidity and Mortality Weekly Report (MMWR). 44(49): 905-909.

Center for Food Safety and Applied Nutrition (CFSAN). 1999. Antimicrobial food additives-guidance. US Food and Drug Administration. Available at: <http://www.cfsan.fda.gov/~dms/opa-antg.html>

Center for Disease Control and Prevention (CDC). 2000. Multistate Outbreak of Listeriosis — United States. 2000. Morbidity and Mortality Weekly Report (MMWR). 49(50):1129-1130.

Center for Disease Control and Prevention (CDC). 2002. Public Health Dispatch: Outbreak of Listeriosis --- Northeastern United States. 2002. Morbidity and Mortality Weekly Report (MMWR). 51(42): 950-951.

Center for Disease Control and Prevention. 2005a. Listeriosis. Division of Bacterial and Mycotic Diseases.

Center for Disease Control and Prevention. 2005b. Food Associated with *Listeria monocytogenes* infections are common at long-term care facilities in eight states. Foodborne Diseases Active Surveillance Network.

Center for Disease Control and Prevention. 2006a. Escherichia coli O157:H7. Division of Bacterial and Mycotic Diseases.

Centers for Disease Control and Prevention. 2006b. Outbreak of multidrug-resistant *Salmonella* enterica serotype Typhimurium definitive type 104 infection linked to commercial ground beef. Northeastern United States. 2003-2004. Clinical Infectious Diseases. 42: 742-752.

Centers for Disease Control and Prevention. 2006c. Salmonellosis-outbreak investigation. October 2006. Division of Bacterial and Mycotic Diseases.

Chickos JS and Acree WE. 2003. Enthalpies of Vaporization of Organic and Organometallic Compounds. Journal of Physical and Chemical Reference Data. 56: 1880-2002.

Cleveland J, Montville TJ, Nes IF and Chikindas ML. 2001. Bacteriocins: safe. natural antimicrobials for food preservation. International Journal of Food Microbiology. 71(1): 1-20.

Clordisys Co.. 2003. What is chlorine dioxide. where is it used. how does it work?. Available: <http://www.clordisys.com/whatiscd.html>

Code of Federal Regulations (CFR). 1999. Synthetic flavoring substance and adjuvant (Title 21. 3. Section 172.515). Washington. Federal Printing House.

Collins-Thompson D and Hwang CA. 2000. Packaging with antimicrobial properties. encyclopedia of food microbiology (edited by Robinson. R. L.. al.). Academic Press. London. 416-420.

Cox LJ, Kleiss T, Cordier C and Cordellana P. 1989. *Listeria* spp. in food processing. non food and domestic environments. *Food Microbiology*. 6: 49-61.

Cox NA, Juven BJ, Thomson JE, Mercuri AJ and Chew V. 1975. Spoilage odors in poultry meat produced by pigmented and non-pigmented *Pseudomonas*. *Poultry Science*. 54: 2001-2006.

Cox NA, Bailey JS and Ryser ET. 1999. Poultry as a source of campylobacter and related organisms. *Journal of Applied Microbiology*. 90: 96-114.

Davidson PM, Sofos JN and Brannen AL. 2005. *Antimicrobials in food*. Taylor & Francis.

David C. Business Manager. Danisco. Copenhagen. Denmark (2004. October). Personal communication via e-mail

Daniels JA, Krishnamurthi, R and Rizvi, SSH. 1985. A review of the effects of carbon dioxide on microbial growth and food quality. *Journal of Food Protection*. 6: 532-537.

Dainty RH. 1996. Chemical/biochemical detection of spoilage. *International Journal of Food Microbiology*. 33: 19-34.

Delaquis PJ and Mazza G. 1995. Antimicrobial properties of Isothiocyanates in Food preservation. *Food Technology*. 43: 73-84.

Delaquis PJ and Sholberg PL. 1997. Antimicrobial activity of gaseous allyl isothiocyanate. *Journal of Food Protection*. 60 (8): 943-947.

Donnelle CW. 1990. Concern of microbial pathogens in associated with dairy foods. *Journal of Dairy Science*. 73: 1656-1661.

Drobnaica L, Kristian P and Augustin J. 1977. The chemistry of the NSC group. in the chemistry of cyanates and their thio derivatives. John Wiley & Sons Inc.. New York. 1003-1221.

Du J, Han Y and Linton RH. 2002. Inactivation by chlorine dioxide gas of *Listeria monocytogenes* spotted onto different apple surfaces. *Food Microbiology*. 19: 481~490.

Dunnick JK, Prejean JD, Haseman J and Thompson RB. 1982. Carcinogenesis bioassay of allylthiocyanate. *Fundamental and Applied Toxicology*. 2: 114-120.

Elliott RP and Michener HD. 1961. microbiological standards and handling codes for chilled and frozen foods- A review. *Applied Microbiology*. 9: 452-468.

Ellis M, Cooksey K, Dawson P, Han I and Vergano P. 2005 Quality of Fresh Chicken Breasts Using a Combination of Modified Atmosphere Packaging and Chlorine Dioxide Sachets. *Journal of Food Protection*. 69(8): 1991–1996.

Environmental Protection Agency (EPA). 1999. Section 4. Chlorine dioxide in Alternative disinfectants and oxidant. EPA Guidance Manual.

Farber JM. 1991. Microbial aspects of modified atmosphere packaging technology-A review. *Journal of Food Protection*. 54(1): 58~70.

Fisker N, Vinding K, Molbak K and Hornstrup MK. 2003. Clinical Review of Nontyphoid *Salmonella* Infections from 1991 to 1999 in a Danish County. *Clinical Infectious Diseases*. 37: 47–52.

Food and Drug Administration (FDA). 1992. Bad bug book. *Salmonella* spp.. Available at: www.dfsam/fda/~mow/chap1.html

Food and Drug Administration (FDA). Center for Food Safety & Applied Nutrition (CSFAN). 1999. antimicrobial food additives-guidance. Available at: <http://www.cfsan.fda.gov/~dms/opa-antg.html>

Food and Drug Administration (FDA). 2001. Agency Response Letter GRAS Notice GRN 000062. Available at: <http://www.cfsan.fda.gov/~rdb/opa-g062.html>

Food and Drug Administration (FDA). 2004. Agency response letter GRAS notice GRN 000144.

Available at: <http://www.cfsan.fda.gov/~rdb/opa-g144.html>

Food and Drug Administration (FDA). 2006. Agency response letter. GRAS Notice GRN 0001333.

Available at: <http://www.cfsan.fda.gov/~rdb/opa-g133.html>

Franke I, Wijma E and Bouma K. 2002. shelf life extension of pre-baked buns by an ACTIVE PACKAGING ethanol emitter. Food Additives and Contaminants. 19(3): 314-322.

Galeano B, Korff E and Nicholson WL. 2003. Inactivation of vegetative cells. but not spores. of Bacillus anthracis. B. cereus. and B. subtilis on stainless steel surfaces coated with an antimicrobial silver and zinc containing zeolite formulation. Applied and Environmental microbiology. 69(7): 4329-4331.

Global Information Inc. 2005. US poultry market research. trend. analysis. Mintel International Group Inc.

Greengrass J. 1993. Films for MAP foods. in principles and application of modified Atmosphere Packaging of foods (ed. Parry R T). Blackie Academic and Professional. 63-100.

Greene J. 1998. U.S. Red Meat & Poultry Markets in a Global Setting. Agricultural Outlook. USDA. 10-12.

Ha JU, Kim YM and Lee DS. 2001. Multilayered antimicrobial polyethylene films applied to the packaged ground beef. Packaging Technology and Science. 15: 55-62.

Han JH and Floros JD. 1997. Casting antimicrobial packaging films and measuring their physical properties and antimicrobial activity. Journal of Plastic Film & Sheeting. 13: 287-298.

Han JH. 2000. Antimicrobial food packaging. Food Technology. 54(3): 56~65.

Han Y, Linton RH, Nielsen SS and Nelson PE. 2001. Reduction of *Listeria monocytogenes* on green peppers by gaseous and aqueous chlorine dioxide and water washing and its growth at 7°C. *Journal of Food Protection*. 64: 1730~1738.

Hara K. 1970. Introduction of polar groups by liquid phase oxidation to polyethylene. *Studies on Adhesion of Polyethylene*. 229(1): 4-7.

Hart CD, Mead GC and Norris AP. 1991. Effects of gaseous environment and temperature on the storage behaviour of *Listeria monocytogenes* on chicken breast meat. *Journal of Applied Bacteriology*. 70: 40-46.

Hatzidimitriu S, Gilbert G and Loukakis G. 1987. Odor Barrier Properties of Multi-Layer Packaging Films at Different Relative Humidities. *Journal of Food Science*. 52(2): 472-474.

Isshiki K, Tokuoka K, Rori R and Chiba S. 1992. Preliminary examination of allyl isothiocyanate vapor for food preservation. *Bioscience, Biotechnology and Biochemistry*. 56:1476-1477.

Jimenez SM, Salsi MS, Tiburzi MC and Rafaghelli RC. 1997. Spoilage microflora in fresh chicken breast stored at 4°C: influence of packaging methods. *Journal of Applied Microbiology*. 83: 613-618.

Jong AR, Boumans H, Slaghek T, Van Veen J and Rijk R. 2005. Active and intelligent packaging for food: is it the future?. *Food Additives and Contaminants*. 22(10): 975-979.

Kaye VS, Murray MB, Harrison D and Beuchat LR. 2005. Evaluation of gaseous chlorine dioxide as a sanitizer for killing salmonella, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and yeasts and molds on fresh and fresh-cut produce. *Journal of Food Protection*. 68(6): 1176-1187.

Kim YS, Ahn ES and Shin DH. 2002. Extension of shelf life by treatment with Allyl isothiocyanate in combination with acetic acid on cooked rice. *Journal of Food Science*. 67(1): 274~279.

Kourai H, Manabe Y and Yamada Y. 1994. Mode of bactericidal action of zirconium phosphate ceramics containing silver ions in the crystal structure. *Journal of Antibacterial and Antifungal Agents*. 22: 595-601.

Knight TD, Murano PS and Murano EA. 2001. Effect of chlorine dioxide releasing packaging film on the objective, microbial, and sensory quality of beef. IFT Annual Meeting. New Orleans. Louisiana.

Kruijf N, Van Beest M, Rijk R, Paseiro P, Sipilainen-Malm T and Meulenaer D. 2002. Active and intelligent packaging: applications and regulatory aspects. *Food Additives and Contaminations*. 19: 144-162.

Lawlis TL and Fuller SL. 1990. Modified atmosphere packaging incorporating an oxygen barrier shrink film. *Food Technology*. 44(6): 124-125.

Labuza TP and Breene WM. 1989. Application of active packaging for improvement of shelf life and nutritional quality of fresh and extended shelf life foods. *Journal of Food Processing and Preservatives*. 13(1): 1-69.

Lawlis TL and Fuller SL. 1990. Modified atmosphere packaging incorporating an oxygen barrier shrink film. *Food Technology*. 44(6): 123-125.

Lawrie RA. 1985. *Meat Science*. Pergamon Press. 200-216.

Lenntech Co. 2006. Chlorine dioxide as a disinfectant.
Available : <http://www.lenntech.com/water-disinfection/disinfectants-chlorine-dioxide.htm#Wat%20zijn%20de%20eigenschappen%20van%20chloordioxide> ?

Levland J. 2001. Bacteriocins: safe, natural antimicrobials for food preservation. *International Journal of Food Microbiology*. 71(1): 1-20.

Lee DS, Hwang YI and Cho SH. 1998. Developing antimicrobial packaging film for curled lettuce and soy bean sprouts. *Food Science and Biotechnology*. 7(2): 117-121.

- Lim LT and Tung MA. 1997. Vapor pressure of Allyl isothiocyanate and its transport in PVDC/PVC copolymer packaging film. *Journal of Food Science*. 62(5): 1061-1066.
- Lin CM, Kim J, Du WX and Wei CL. 2000. Bactericidal activity of isothiocyanate against pathogens on fresh produce. *Journal of Food Protection*. 63(1): 35~30.
- McMeekin TA and Thomas CJ. 1980. Microbiological problems associated with refrigerated poultry. *CSIRO Food Research Quarterly* 40: 141-149.
- Mead GC. 2004. Poultry meat processing and quality. Woodhead Publishing Limited.
- Metiu H. 2004. Physical chemistry-Thermodynamics. Taylor & Francis. 519-546.
- Misko G and Rothschild KL. 2001. The regulation of the use of antimicrobials in food packaging: a new ball game. an article of packaging law magazine. Available at: http://www.packaginglaw.com/index_mf.cfm?id=41
- Montgomery JM. 1985. Water treatment principle and design. Consulting engineers INC.. JMM.
- Muthukumarasamy P, Han JH and Holley RA. 2003. Bactericidal effects of *Lactobacillus reuteri* and allyl isothiocyanate on *Escherichia coli* O157:H7 in refrigerated ground beef. *Journal of Food Protection*. 66 (11): 2038-2044.
- Nathnac. 2005. Salmonella outbreak in Spain. Available at: http://www.nathnac.org/travel/news/salmonella_spain_110805.htm
- Ngoka DA and Froning GW. 1982. Effect of free struggle and pre-slaughter excitement on color of turkey breast muscles. *Poultry Science*. 61: 2291-2293.
- Nielsen PV and Rios R. 2000. Inhibition of fungal growth on bad by volatile components from spices and herbs. and the possible application in active packaging. with special emphasis on mustard essential oil. *International Journal of Food Microbiology*. 60: 219-229.

Occupation safety & health administration (OSHA). 2006. sampling & analytical method.

Available at: <http://www.osha.gov/dts/sltc/methods/partial/t-pv2086-01-8305-ch/t-pv2086-01-8305-ch.html>

Ogilvy WS and Ayres JC. 1951. The effect of atmospheres containing carbon dioxide in prolonging the storage life of cut-up chicken. *Food Technology*. 5: 97.

Ohta Y, Takatani K and Kawakishi S. 1995. Decomposition rate of allyl isothiocyanate in aqueous solution. *Bioscience Biotechnology and Biochemistry*. 59(1): 102-103.

Ouattara B, Simard RE, Piette G and Begin A. 2000. Diffusion of acetic and propionic acids from chitosan-based antimicrobial packaging films. *Journal of Food Science*. 65(5): 768-773.

Ozdemir M, Yurteri CU and Sadikoglu H. 1999. Physical polymer surface modification methods and applications in food packaging polymers. *Food Science and Nutrition*. 39(5): 457-477.

Ozen BF, Mauer LJ and Floros JD. 2002. Effects of ozone exposure on the structural, mechanical and barrier properties of select plastic packaging films. *Packaging Technology and Science*. 15: 301-311.

Perchacek R, Velisek J and Hrabcova H. 1997. Decomposition products of allyl isothiocyanate in aqueous solutions. *Journal of Agricultural Food Chemistry*. 45: 4584-4588.

Phillips C. 1996. Review: Modified atmosphere packaging and its effects on the microbiological quality and safety of produce. *International Journal of Food Science and Technology*. 31: 463-479.

Quintavalla S and Vicini L. 2002. Antimicrobial food packaging in meat industry. *Meat Science*. 62: 373-380.

Quio M, Fletcher DL, Northcutt JK and Smith DP. 2002. The relationship between raw broiler breast meat color and composition. *Poultry Science*. 81: 422-427.

Radomir P, Velisek J and Hrabcova H. 1997. Decomposition products of allyl isothiocyanate in aqueous solutions. *Journal of Food Chemistry*. 45: 4584-4588.

Razumovskii SD. 1983. Degradation and protection of polymeric materials in ozone. *Developments in polymer stabilization*. 6: 239-293.

Rodrigues ET and Han JH. 2000. Antimicrobial whey protein films against spoilage and pathogenic bacteria. proceedings of the IFT annual meeting; Dallas. Tex. Institute of Food Technologists.

Sams AR. 2001. Poultry meat procession. CRC Press. New York.

Sander EH and Soo HM. 1978. Increasing shelf life by carbon dioxide treatment and low temperature storage of bulk pack fresh chickens packaged in nylon Surllyn film. *Journal of Food Science*. 43: 1519-1534.

Sekiyama Y, Mizukamin Y, Takada A and Numata S. 1994. Vapor pressure and stability of allyl isothiocyanate. *Journal of Food Hygiene and Society of Japan*. 35(4): 365-370.

Sekiyama Y, Mizukami Y, Takada A and Oosono M. 1996. Effect of mustard extract vapor on fungi and spore-forming bacteria. *Journal of Antibacteria and Antifungus Agents*. 24(3): 171-178.

Singh N, Singh RK, Bhunia AK and Stroshine RL. 2002. Efficacy of Chlorine Dioxide, Ozone, and Thyme Essential Oil or a Sequential Washing in Killing *Escherichia coli* O157:H7 on Lettuce and Baby Carrots. *Lebensmittel-Wissenschaft und-Technologie*. 35: 720~729.

Siragusa GR, Cutter CN and Willett JL. 1999. Incorporation of bacteriocin in plastic retains activity and inhibits surface growth of bacteria on meat. *Food Microbiology*. 16(3): 229-239.

Smith JP, Oraikul B, Koersen WJ and Van De Voort FR. 1987. Shelf life extension of a bakery product using ethanol vapor. *Food Microbiology*. 4(4): 329-337.

Speronello B. Associate Researcher. Engelhard. Iselin. New Jersey. (2005. Feburary). Personal communication via e-mail.

Suhr and Nielsen. 2003. Antifungal activity of essential oils evaluated by two different application techniques against rye bread spoilage fungi. *Journal of applied microbiology*. 94: 665-674.

Suppakul P, Miltz J, Sonneveld K and Bigger SW. 2003. Active packaging technologies with an emphasis on antimicrobial packaging and its applications. *Journal of food science*. 68(2): 408-420.

Tsobkallo ES, Petrova LN and Khagen V. 1988. Influence of Ozone on the Structure and Mechanical Properties of PE Film. *International Polymer Science and Technology*. 15: 42-44.

U.S. Patent and Trademark Office. 1994. Chlorine dioxide generating polymer packaging films. Patent 5360609.

Vermeiren L, Devlieghere F and Debevere J. 2002. Effectiveness of some recent antimicrobial packaging concepts. *Food Additives and Contaminants*. 19: 163-171.

Ward SM. 1998. Inhibition of spoilage and pathogenic bacteria on agar and pre-cooked roast beef by volatile horseradish distillates. *Food Research International*. 31(1): 19-26.

Wellinghoff ST. 1995. Keeping food fresh longer. *Technology Today Magazine*. Available at: <http://www.swri.edu/3pubs/ttoday/summer95/keepfood.htm>

Weng YM and Hotchkiss JH. 1992. Inhibition of surface molds on cheese by polyethylene film containing the antimycotic imazalil. *Journal of Food Protection*. 55(5): 367-369.

Weng YM, Chen MJ and Chen W. 1999. Antimicrobial food packaging materials from poly(ethylene-co-methacrylic acid). *Lebensmittel-Wissenschaft und-Technologie*. 32(4): 191-195.

Werner SB, Allad J and Ager EA. 1969. Salmonellosis from chickens prepared in commercial rotisseries: report of an outbreak. American Journal of Epidemiology . 90(5): 429-437.

Winther M and Nielsen PV. 2006. Active packaging of cheese with allyl isothiocyanate. an alternative to modified atmosphere packaging. Journal of Food Protection. 69(10): 2430-2435.

Wolfe SK. 1980. Use of CO- and CO₂- enriched atmospheres for meats. fish. and produce. Food Technology. 24: 55-58.

Yang CC and Chen TC. 1993. Effect of refrigerated storage. pH adjustment . and marinade on color of raw and microwave cooked chicken meat. Poultry Science. 72: 355-362.

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