UTILIZATION OF CHLORINE DIOXIDE GAS IN FOOD PACKAGING APPLICATION

By

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ABSTRACT

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By

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Chlorine dioxide (ClO₂) in its gaseous form has been used in numerous studies for vapor-phase decontamination, both in treating produce before packaging, and decontaminating the products inside their packages. Yet, very little is known about its compatibilities with packaging materials or its performance as affected by food packaging systems. The overall goal of this dissertation was to evaluate potential use of ClO₂ gas as an antimicrobial agent for food packaging applications.

In the first study, mass transfer profiles (permeability, solubility and diffusion coefficients) of ClO₂ for 10 types of polymeric packaging materials were determined by an isostatic method using a continuous system for measuring ClO₂ concentration with an electrochemical sensor as a detector. Overall, PET, PLA, BOPP, nylon, and multilayer of EVA/EVOH/EVA had high ClO₂ barrier, while PS, LLDPE, LDPE, HDPE, and PVC provided low barrier to ClO₂. Effects of gaseous ClO₂ on physical, mechanical, chemical, and barrier properties of polymeric packaging materials were then studied by exposing selected materials to ClO₂ gas. After 14 days of exposure, significant changes, such as increases in barriers to O₂ and CO₂ of nylon, changes in permselectivity (P_{CO₂}/P_{O₂}) ratio of up to 46.8% in treated PE, PS, PET, and nylon films, and changes in
FT-IR spectra of PET, PLA, and EVA/EVOH/EVA, indicate possible changes in chemical profiles and performance of the materials.

Study on influences of packaging design on antimicrobial effect of ClO$_2$ gas, on shredded Romaine lettuce, indicated that minimizing the distance between gas releasing location and target surfaces, as well as, maximizing the area of gas release could significantly improve antimicrobial activity of ClO$_2$ gas in particular packaging system. Once the interior of the package was optimized, it was observed that the amount of ClO$_2$ used per package could be reduced to half of its original concentration (from 8 to 4 mgClO$_2$/kg lettuce per day), while still achieving the same level of log$_{10}$ CFU reduction of *Escherichia coli* O157:H7 in packaged shredded lettuce.

When in contact with food, ClO$_2$ gas will decontaminate the surfaces, as well as being absorbed by the product. The latter amount could not be accounted for its antimicrobial capacity. Study on absorption behavior of Romaine lettuce showed that increasing ClO$_2$ level and/or time of exposure increased residual ClO$_2$ and chlorite (ClO$_2^-$) recovered from Romaine lettuce sample. The presence of cuts significantly increased the amount of ClO$_2$ consumed, while exceed water did not increase ClO$_2$ absorption by lettuce.

This research approach could be of great importance when considering antimicrobial packaging with ClO$_2$ gas as a safety measure. Information generated could also be used to generate parameters for computational modeling of packaging systems.
‘WE GROW GREAT by DREAMS’

- Woodrow Wilson –

We grow great by dreams. All big men are dreamers. They see things in the soft haze of a spring day or in the red fire of a long winter's evening. Some of us let these great dreams die, but others nourish and protect them, nurse them through bad days till they bring them to the sunshine and light which come always to those who sincerely hope that their dreams will come true.
ACKNOWLEDGEMENTS

I used to perceive a ‘Dr.’ prefix as something that was easily distinguished from other prefixes. The ‘becoming’ process, to the old me, was somewhat ‘abrupt’. Until 4 years into my Doctoral program at the School of Packaging, Michigan State University that I have come to realize the tremendous effort, time, and ‘heart’ one need to put in, in order to be recognized as ‘Dr.’. Since then my perception has changed; this becoming process is more like a ‘transition’ in which one gradually gather experience and learn knowledge new to them and the earned title is merely an acknowledgement that one had suffered enough research drawbacks and sufficiently academically ‘grown up’ to be able to solve those problems and manage to move on to learn new things. This reminds me of the thermal transition of semi-crystalline polymer. All other people typically recognizing are the change in the polymer properties, before and after processing. They tend to neglect the required time and applied energy needed to make that polymer useful.

I regard this dissertation as something similar to ‘latent heat of fusion’. It is the work people might see as the stepping stone to the next phase of my life-long goals, which has only my name labeled on it. However, this dissertation and what has become of me now would not have been possible without the following individuals; they are the time and energy given to me that tailors me to what I am today.

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CHAPTER 1: INTRODUCTION

1.1. Rationale and significance

Each year, approximately one thousand reports of foodborne disease outbreaks (FBDOs) are collected by the Centers for Disease Control and Prevention (CDC), in the United States, resulting in around 20,000 cases of foodborne illness and 20 deaths (Centers for Disease Control and Prevention, 2010). The cost of foodborne illness in humans caused by bacterial pathogens is between $2.9 to $6.7 billion each year (Buzby, 1996). Among numerous confirmed foodborne disease cases reported, more than half were traced to consumption of raw or minimally processed foods, such as fresh spinach, shredded lettuce, salad, and deli meat (Department of Health and Human Services, 2008; Lynch et al., 2006). Some microorganisms reported to be the causes were Escherichia coli O157:H7, Listeria monocytogenes, Salmonella spp., and Staphylococcus aureus (Centers for Disease Control and Prevention, 2010; Department of Health and Human Services, 2008; FSIS, 2005; Nowak et al., 2006; Phillips, 1996; Sy et al., 2005b).

To improve food safety, additional processing steps, e.g. washing with sanitizing solutions had been added to many food production lines (Huang et al., 2006; Kreske et al., 2006a). The use of packaging systems as an integral part of food processing is another possibility for enhancing food safety and prolonging product shelf-life. This approach is particularly useful when the foods will be consumed without any further processing or with minimal preparation, making contamination of pathogenic microorganisms a very important issue (Appendini and Hotchkiss, 2002; Department of
Health and Human Services, 2008; Ellis et al., 2006; Lynch et al., 2006; Nowak et al., 2006; Phillips, 1996; Sy et al., 2005a; USFDA, 2001), as in the case of ready-to-eat (RTE) food products, e.g. baby spinach leaves, and chicken salad (Appendini and Hotchkiss, 2002; Ellis et al., 2006).

The consumer’s constantly growing demand for ‘near-fresh’ quality and shelf-stable products has encouraged the development of several preservation techniques. Among others, modified atmosphere packaging (MAP) is, currently, one of the most promising and most extensively studied techniques. Modified atmosphere packaging (MAP) is defined as “the packaging of perishable product in an atmosphere which has been modified, so that its composition is other than that of the air” (Hintlian and Hotchkiss, 1986). Once sealed, the dynamic biological, chemical, and physical characteristics of both packages and products will alter the gaseous composition of the pre-set atmosphere inside the package (Hintlian and Hotchkiss, 1986; Ooraikul, 1991; Phillips, 1996). MAP application has found commercial success when applied to agricultural products, e.g. fruits, vegetable, meat, and seafood (Phillips, 1996).

In the past few decades, new packaging strategies, such as including antimicrobial gas in the MAP packaging system, have also become a potential complementary approach to improve the safety of packaged fresh produce (Sy et al., 2005a). The antimicrobial agents can be integrated into the packaging system by means of adding sachets/pads, introducing the compound directly into the package headspace, or coating the antimicrobial compounds on the packaging surfaces (Appendini and Hotchkiss, 2002). Several chemical compounds are commercially used for such application and some show a strong potential for use as antimicrobial agents in the product/packaging...
system, for example, silver substituted zeolite, glucose oxidase, sulfur dioxide, triclosan, allyl isothiocyanate, and essential oils (Appendini and Hotchkiss, 2002). In the past decade, chlorine dioxide (ClO$_2$) is considered by many researchers as one of the most promising choices to be used in vapor-phase decontamination for food products, especially for vegetables and fruits (Gómez-López et al., 2009; Han et al., 1999; Sapers et al., 2003; Sy et al., 2005a; Yuk et al., 2006).

Chlorine dioxide is a strong oxidizing agent and an effective surface disinfectant. Recognized as a disinfectant since the early 1900s, ClO$_2$ was used initially used to treat water, as it causes fewer organoleptic problems than chlorine (Cl$_2$) (USEPA, 1999). It is soluble in water and has equal, if not higher, inactivating capacity than that of Cl$_2$, but is less effective than ozone (O$_3$) (USEPA, 1999). Chlorine dioxide is gaining interest in the food and pharmaceutical industries, due to its broad antimicrobial effects (Sy et al., 2005a; Sy et al., 2005b). Its antimicrobial effect has been reported to be effective against many pathogenic microorganisms, such as Escherichia coli O157:H7, Listeria monocytogenes, and Salmonella spp (Ellis et al., 2006; Huang et al., 2006; Rodgers et al., 2004). In many food applications, ClO$_2$ in solution can be substituted for chlorine (Cl$_2$) solution, as it is known to be more effective and not to produce harmful end-products (Han et al., 1999; Kim et al., 1999; Kreske et al., 2006b; Rico et al., 2007; Ryu and Beuchat, 2005).

The potential use of gaseous ClO$_2$ for vapor-phase decontamination, during post-harvest storage, in the processing line, and inside the product/package system has been
widely explored in the last decade (Ellis et al., 2006; Han et al., 2004; Selby et al., 2005). Adding ClO₂ gas into the packaging system will extend the sanitizing time well beyond the processing line, without any time extension for the production.

Introduction of ClO₂ gas within a food packaging system is often by means of a sachet, either slow- or fast-release (Ellis et al., 2006; Shin, 2007), furthermore, in 2001, FDA approved the use of food packaging films incorporated with ClO₂ to be used as packaging material for meats, poultry and seafood (USFDA, 2001). Most research regarding applications of ClO₂, as a antimicrobial gas in the headspace of packaging systems, focuses on the appropriate dose for particular microorganisms and/or specific types of perishable food (Kaczur and Cawlfield, 1992; Sapers et al., 2003; Sy et al., 2005a). Only rarely, have these studies investigated the gas’s compatibility with polymeric packaging materials for food products (Ozen, 2000; Shin et al., 2006) or the effects of gas distribution on antimicrobial capacity of ClO₂ gas.

Different degrees of change in mechanical and barrier properties of several polymeric materials have been observed after ClO₂ exposure, depending on gas concentration, relative humidity, and testing temperature (Ozen, 2000; Shin et al., 2006). In 2001, Ozen reported permeability (P) coefficients of 100 mg ClO₂/L of gas, at 20°C, for linear low-density poly(ethylene), LLDPE, oriented poly(propylene), OPP, and biaxially oriented nylon (BON). The experiment followed a quasi-isostatic method and used an amperometric titration method as a detection technique. However, the complete mass transfer profiles of the selected materials could not be obtained due to limitations of
the detection method, i.e. the steady state of permeation was reached too fast, and there
was a delayed response in the detection system used, preventing the assessment of the
unsteady region for the calculation of the diffusion coefficient (Ozen, 2000).

If the package is to be considered as a strategy for the delivery and containment of
\( \text{ClO}_2 \) gas, more thorough study on mass transfer behavior of \( \text{ClO}_2 \), as well as its effects
on packaging integrities and performance are critical, as these could affect the polymers’
performance in packaging systems and possibly reduce the food product’s shelf-life.

Also, the acquired knowledge could be used as a general guideline for material
selection and packaging design for product/package systems that will include \( \text{ClO}_2 \) gas as
an antimicrobial agent.

The study of how packaging design could affect gas distribution within the
product/package system is another aspect of the research on antimicrobial packaging
technology that was rarely performed. The uniformity of gas distribution inside the
package is important, because the microbial population increases over time. The more
effective the distribution, the sooner the microorganism will be inactivated (Ellis et al.,
2006). Thus the impacts of packaging design on gas distribution as well as accessibility
of antimicrobial compounds to hard-to-reach places should be explored.
1.2. Objectives and hypothesis

The main goal of this research was to evaluate the potential use of gaseous ClO₂ as an antimicrobial agent for food packaging applications. To achieve the overall goal, specific objectives and hypotheses of this study were set, as follows:

1. Develop a continuous detection method for mass transfer measurement of ClO₂
2. Assess the mass transfer profile of ClO₂ through different polymeric packaging materials

Hypothesis: *There are some differences in the mass transfer behavior of gaseous ClO₂ through different types of polymeric films as indicated by their permeability (P), diffusion (D), and solubility (S) coefficients.* The mass transfer behavior of ClO₂ gas through each polymer is a unique characteristic which is influenced by chemical and physical properties of that particular polymeric material (Schnabel, 1992; Van Krevelen, 1997). The following hypotheses are set to be proved or disproved.

*Null hypothesis (H₀):* There is no difference in the mass transfer behavior of gaseous ClO₂ through different types of polymeric films as indicated by their P, D, and S.

*Alternative hypothesis (H₁):* There are some differences in the mass transfer behavior of gaseous ClO₂ through different types of polymeric films as indicated by their P, D, and S.
3. Determine the impact of ClO$_2$ gas on the chemical, physical, mechanical, and barrier properties of selected polymeric packaging materials

**Hypothesis:** There are some changes in the materials’ integrities and/or performance, after their exposure to ClO$_2$ gas. By exposing the polymeric materials to reactive chemical compounds, like ClO$_2$, several chemical changes, such as main-chain scission, cross-linking, and functional group formation, can occur. These changes can affect the material integrity and the package performance (Ozen, 2000; Schnabel, 1992; Selke et al., 2004). The following hypotheses are set to be proved or disproved.

*Null hypothesis (H$_0$):* There are no changes in the materials’ integrities and performance, after their exposure to ClO$_2$ gas.

*Alternative hypothesis (H$_1$):* There are some changes in the materials’ integrities and/or performance, after their exposure to ClO$_2$ gas.

4. Investigate the antimicrobial effect of ClO$_2$ gas on fresh produce

**Hypothesis:** There are some differences in terms of microbial load of fresh produce packed with air and those packed with ClO$_2$ gas in the package. Since ClO$_2$ demonstrates antimicrobial activity against many important microorganisms (Sy et al., 2005a; USEPA, 1999), adding it into the product/package system should help reduce microbial populations on the product surface (Huang et al., 2006; Sy et al., 2005b; Zhang and Farber, 1996). The following hypotheses are set to be proved or disproved.
Null hypothesis ($H_0$): There is no difference in terms of microbial load of fresh produce packed with air and those packed with ClO$_2$ gas in the headspace, during entire storage duration.

Alternative hypothesis ($H_1$): There are some differences in terms of microbial load of fresh produce packed with air and those packed with ClO$_2$ gas in the headspace, during entire storage duration.

5. Identify the parameters in the packaging design responsible for improved gas distribution within the package, for future packaging development using CFD modeling, by:

5.1. Studying the effects of packaging design on antimicrobial effectiveness of ClO$_2$ gas in product/package systems

**Hypothesis:** There are some differences in terms of microbial load of fresh produce packaged in bags of different designs. Since ClO$_2$ is a very active surface disinfectant, the distance between the point of gas release and the food product surface should be minimized. If the package is designed to ensure fast and thorough distribution of a gas mixture throughout the interior of the packaging system, maximum exposure of the products’ surface can be achieved. This could result in the effective reduction in microbial loads which could lead to shelf life extension and improved safety of food products (Ellis et al., 2006). The following hypotheses are set to be proved or disproved.

Null hypothesis ($H_0$): There is no difference in terms of microbial load of fresh produce packed in bags with different internal designs.
Alternative hypothesis ($H_1$): There are some differences in terms of microbial load of fresh produce packed in bags with different gas distribution pattern, as directed by different internal designs.

5.2. Measuring the absorption of ClO$_2$ gas by lettuce leaf

**Hypothesis:** There is a measurable amount of ClO$_2$ gas being absorbed into lettuce and there is a measurable amount of chlorite (ClO$_2^-$) being generated from the oxidation reactions of ClO$_2$ and organic matters in lettuce. Han *et al* (2004) detected up to 0.52 mg ClO$_2$/kg and 3.03 mg ClO$_2^-$/kg of strawberries after the fruits treatment with 3.0 mg ClO$_2$ gas/L for 10 min (Han *et al*., 2004).

**Null hypothesis ($H_0$):** After ClO$_2$ treatment, there is no ClO$_2$ residual and ClO$_2^-$ detected on the lettuce samples

**Alternative hypothesis ($H_1$):** After ClO$_2$ treatment, there is a measurable amount of ClO$_2$ residual and ClO$_2^-$ found on the lettuce samples
1.3. Content organization

A review of literature related to food safety, the roles of packaging in food applications, chlorine dioxide, product/packaging interactions, and polymer degradations is provided in Chapter 2. The work on mass transfer of ClO₂ gas for polymeric packaging materials is reported in Chapter 3 (objective 1) and 4 (objective 2). Chapter 5 addressed the effect of ClO₂-exposure on properties and performance of packaging materials (objective 3). The impact of packaging design on the antimicrobial effect of ClO₂ gas is reported in Chapter 7 (objective 4 and 5.1) with ClO₂-absorption by the lettuce leaf (objective 2.2) reported in Chapter 6.


and Spores to Chlorine, Chlorine Dioxide, and a Peroxyacetic Acid-Based Sanitizer." Journal of Food Protection 68(12): 2614-2622.


CHAPTER 2: LITERATURE REVIEW

2.1. Food safety of fresh produce

Foodborne disease continues to be a public health threat in the United States (U.S.). The Centers for Disease Control and Prevention (CDC) reported that, in year 2007, 21,244 people was hospitalized, from 1,097 reported foodborne disease outbreaks (FBDOs), resulting in 18 deaths from foodborne illness (Centers for Disease Control and Prevention, 2010). The estimated cost of foodborne illness in terms of mental, financial, and medical expenses ranges from $10-83 billion each year (Department of Health and Human Services, 2008; Lynch et al., 2006; Mead et al., 1999; USFDA, 2004). Major disease carriers include dairy, poultry, beef, and leafy vegetables (Centers for Disease Control and Prevention, 2010; Lynch et al., 2006; USFDA, 2004).

Vegetables and fruits are recognized as an important component of a healthy diet program as a source of fiber, vitamins, minerals, and antioxidants. However, they can also become a microbial carrier as they are: 1) grown in a natural environment and 2) usually consumed raw without any type of preparation (at the consumer level) that would reduce, control, or eliminate microorganisms prior to consumption (Gorny et al., 2006; USFDA, 2004).

Each year (during 1998-2004), around 400 FBDOs are confirmed to be caused by consumption of produce, with around 56% attributed to mixed or unknown food commodities and the rest were related to consumptions of single food commodity, such
as (in the order from highest to lowest numbers of FBDOs) lettuce, sprouts, tomatoes, and berries (Tauxe, 2008).

Microbial contamination onto fresh produce can occur at several points throughout an entire product production line. Figure 2-1 shows the general supply chain flow for lettuce/leafy greens (Gorny et al., 2006), as an example. *Salmonella* spp and *Escherichia coli* O157:H7 are the two pathogenic microorganisms most often associated with produce (Gorny et al., 2006; USFDA, 2004; 2006). Like in the case of any other fresh produce, both pathogens usually contaminate lettuce/leafy greens via fecal-oral route. At merely every step, produce comes in contact with human hands, water, or soil with special attention to control, reduce, or eliminate potential contamination from people and animal (Gorny et al., 2006; USFDA, 2004).

Leafy greens are usually consumed uncooked or raw. These products are generally considered to be ready-to-eat (RTE) since the washing step is used in their production and protective package is employed in their distribution and marketing (Gorny et al., 2006; USFDA, 2006). During production, there are many ‘touch points’, e.g. hand-harvested and hand-sorted which can lead to cross-contamination (Gorny et al., 2006). Other factors include agricultural water quality, the use of manure as fertilizer, and the presence of animals nearby or in packing areas (Gorny et al., 2006; USFDA, 2004).
Figure 2-1. General supply chain flow for lettuce/leafy greens. Adapted from (Gorny et al., 2006).
For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.
2.2. Safety strategies to minimize foodborne outbreaks associated with fresh produce consumption

The safety of food production, distribution, and marketing chain requires an integrated approach, by the manufacturers, to prevent/minimize foodborne illness, along with oversight by the federal food safety agencies (USFDA, 2004).

To minimize the recurring outbreaks associated with fresh and fresh-cut produce, the Food and Drug Administration (FDA) released an action plan, in 2004, to minimize foodborne illness associated with fresh produce consumption (Gorny et al., 2006; USFDA, 2004). The plan suggests that each unit involved in producing, packing, processing, transporting, distributing, or preparing fresh produce (at the consumer level) has a responsibility to reduce, control, or eliminate microbial contamination of the produce (USFDA, 2004). To support the action plan’s goal, FDA developed the safety initiative for each specific commodity, e.g. ‘the Lettuce Safety Initiative’ (USFDA, 2006). The plan includes inspections of the farm and cooling and packing facilities, and focuses on good agricultural practices (GAPs) and good manufacturing practices (GMPs) (Gorny et al., 2006; USFDA, 2006).

Under USDA rules, the programs of safety assurance and preventive process controls like the Hazard Analysis and Critical Control Point (HACCP) system are being implemented by producers and distributors to prevent food safety hazards which are categorized as biological, chemical, or physical hazards. Such systems focus on problem prevention and involve the identification, evaluation, and control of critical control points/steps in the food production and distribution, where failure to take appropriate action is most likely to cause potentially hazardous products to reach the consumer.
HACCP systems are already mandated in particular segments of the food industry, e.g. seafood, juice, and, meat and poultry products (Executive Secretariat, 1998; FSIS, 2006; 2009).

Additional processing steps, e.g. sequential washing, fumigation (with antimicrobial gas), and irradiation are installed in the commercial food production lines and/or being developed at various research institutes, to strengthen end product safety (Han et al., 2004; Huang et al., 2006; Kreske et al., 2006a; Singh et al., 2002).

Hurdle technology is a strategy many researchers recognize to be very efficient in reducing microbial risks and extending shelf-life of minimally processed foods (Allende et al., 2006; Rico et al., 2007). The technology involves the use of a series of preservation techniques, e.g. photochemical process, washing, thermal treatment, and low temperature storage, as well as the use of active packaging to treat the food product. The influences of combined techniques are also reported to be synergistic. In many cases, the food products are found to be of superior quality as compared to those subjected to single preservative technique, since each technique, in the series, is usually applied at a milder condition to achieve a comparable shelf-life/safety level (Huang et al., 2006; Rico et al., 2007; Sothornvit and Kiatchanapaibula, 2009).

2.2.1. Available packaging systems for fresh produce

Using a packaging system as an extension from the production line to improve safety and economically extend shelf-life of fresh produce, is very practical when the food will be consumed without any further processing or with minimal preparation (Appendini and Hotchkiss, 2002; Ellis et al., 2006).
The function of packaging as a preservation tool for food products can be grouped into passive and active protections. When “packaging is independent of the foodstuff preparation and preservation techniques” (Multon, 1996), the package provides passive protection towards mechanical forces and microbial contaminations, as well as, protection against heat and mass transfers. In the case where packaging constitutes as an integral part of food preparation and preservation processes, thus considered as an indispensable element, it may be regarded as having an active role, for example, containers used in aseptic packaging and modified atmosphere packaging (MAP) technique, etc (Multon, 1996).

In MAP technique, respiring produce is packed in polymeric packaging with the gas mixture in the headspace other than air, to reduce the product’s respiration rate, moisture loss, and/or decay, thus extending the product’s shelf life. Once packaged, gas composition in the package headspace is gradually altered as affected by several factors, such as the packaging materials’ permeability to O₂ and CO₂, the product’s biochemical rate, and the storage temperature (Ooraikul and Stiles, 1991; Phillips, 1996; Romig and Mir, 2000). MAP application has been successfully applied to commercial fresh produce and processed food products, e.g. fruits, vegetable, meat, seafood, etc, for decades (Phillips, 1996). The appropriate gas composition can prolong the product’s shelf-life; however, it cannot improve the food’s quality. Thus it is essential to start with raw material with high quality.

Majority of studies in MAP for fresh produce involve 1) development of mathematic modeling, for example, the use of Michaelis-Menton type respiratory model to predict the effects of temperature and O₂ on respiration rate of the product, the use of
Arrhenius equation to predict O₂ partial pressure as a function of temperature, product surface area, and film permeability, and the application of growth model of pathogenic microorganisms under MAP condition and refrigerated temperature (Cameron et al., 1994; Harrison, 2000; Simpson, 2001; Yang, 2002), 2) effects of modified gaseous atmosphere on product’s properties (Cliffe-Byrnes, 2003; Fu, 1992; Lin, 2002), and 3) incorporation of preservation aids, e.g. disinfectants and oxygen scavengers (Cegielska-Radziejewska, 2004; DeEll, 2006). Many innovations and developments have been done on MAP applications regarding improving its package efficiency, and several studies were conducted on the combination of MAP with additional preservation means, to further extend the product’s shelf-life, e.g. the use of blanching, acid treatment, and mild irradiation process to treat fresh food prior to packaging (Bagorogoza et al., 2001; Jimenez et al., 1999; Sawaya et al., 1995).

One of the main concerns regarding using MAP as a packaging technique for food products is: the condition applied may be more effective in killing spoilage microorganisms which provides organoleptic indication of spoilage, giving the possibility for pathogenic microorganisms, e.g. Clostridium botulinum (anaerobe) and L. monocytogenes (psychotropic facultative anaerobe), to outgrow the former, and increase their populations to significant levels or produce toxin (Farber and Dodds, 1995). Thus, the addition of antimicrobial agents, in the form of organic acids, enzymes, spices, etc., into the product/package system has also been widely researched, in the last few decades (Appendini and Hotchkiss, 2002).
2.2.1.1. Antimicrobial packaging

To improve the food products’ safety and prolong their shelf-life, there is the increasing use of antimicrobial packaging for minimally processed and RTE fresh products (Appendini and Hotchkiss, 2002; Ellis et al., 2006). The antimicrobial agents can be integrated into the packaging system by adding the gas providers, such as sachet and pad, coating the agent onto the packaging surface, and embedding the compounds into the polymer matrix (Appendini and Hotchkiss, 2002). The antimicrobial agents, once generated and/or released will reduce, inhibit and/or retard the microbial growth on the packaging interior and on the food product’s surface, where the majority of contamination and spoilage occurs (Appendini and Hotchkiss, 2002; Ooraikul and Stiles, 1991). Examples of antimicrobial agents incorporated into the packaging system, available commercially, are silver substituted zeolite, triclosan, and ethanol vapor (Appendini and Hotchkiss, 2002). Among others, chlorine dioxide (ClO$_2$) is considered by many researchers as one of the promising choice to be used in vapor-phase decontamination within the package of the food products (Appendini and Hotchkiss, 2002; Kaczur and CawlfieId, 1992; Kim et al., 1999; Sapers et al., 2003).
2.3. Chlorine dioxide (ClO₂)

Chlorine dioxide (ClO₂) is an oxidizing agent that is used primarily as a bleaching agent in pulp and paper production. Due to its broad antimicrobial spectrum, ClO₂, both in liquid and gas forms, is also used in a wide range of sanitizing applications (Sy et al., 2005a; USEPA, 1999).

2.3.1. Physical and chemical characteristics

Chlorine dioxide gas phase is greenish yellow in color and has a pungent odor. It is highly soluble in water, particularly in chilled water, and reported to be stable at room temperature, in the absence of light, over a period of days (Mueller and Willner, 1993). Its solubilities in water, at various temperatures, are graphically shown in Figure 2-2. Chlorine dioxide does not chlorinate, but exists as a dissolved gas in the solution (USEPA, 1999).

Photochemical dissociation of ClO₂ is an issue of great concern, as some researches regarded it to be associated with Antarctic O₃ depletion (Solomon, 1999). Photoreactivity of ClO₂ is outlined, in Scheme 2-1, below:

\[
\begin{align*}
    \text{OCIO} + h\nu & \rightarrow \text{ClO} + \text{O} \quad \text{(a)} \\
                  & \rightarrow \text{ClOO} \quad \rightarrow \text{Cl} + \text{O}_2 \quad \text{(b)} \\
                  & \rightarrow \text{Cl} + \text{O}_2 \quad \quad \text{(c)}
\end{align*}
\]

Scheme 2-1. Photochemical dissociation pathways of ClO₂. Adapted from (Vaida and Simon, 1995).
The excitation of OClO, in the near-UV region, lead to; (a) its photochemical dissociation into ClO + O; (b) its photoisomerization to form an unstable ClOO (chlorine superoxide), which will further dissociate into atomic Cl + O₂ (asymmetrical process); or (c) its dissociation into Cl + O₂ (symmetrical process) (Vaida and Simon, 1995). The dissociated ClO molecule is considered by many researchers to be an ‘active form’ of chlorine that can cause ozone depletion through catalytic cycle (Solomon, 1999). Its isomerized form, ClOO, is thermodynamically more stable, but very reactive in the gas phase (Richard and Vaida, 1991).

Photoreactivity of OClO and its partitioning in pathway (a)-(c) depends on phase environments. It reported to be wavelength-dependent if OClO is in a gas phase, while, in condensed-phases, like liquid solutions or solid matrices, it is independent of excitation wavelength (Dunn and Simon, 1992; Mueller and Willner, 1993; Vaida and Simon, 1995).
Figure 2-2. Solubility of ClO₂ in water. Adapted from (Ishi, 1958).
The end-products of ClO₂ treatment and degradation are chloride (Cl⁻), chlorite (ClO₂⁻) and chlorate (ClO₃⁻). The concentrations of remaining ClO₂, chloride, chlorite and chlorate after the reaction depend on the applied dosage, pH, etc (USEPA, 1999). In drinking water, up to 70% of ClO₂ is reduced to ClO₂⁻ and the rest is found in the forms of ClO₃⁻ and Cl⁻, after oxidation reaction (USEPA, 1999). Half reactions of key Redox reactions are as shown:

\[
\begin{align*}
\text{ClO}_2 + e^- & \rightleftharpoons \text{ClO}_2^- & E^0 &= 0.954 \text{V} \\
\text{ClO}_2^- + 2\text{H}_2\text{O} + 4e^- & \rightleftharpoons \text{Cl}^- + 4\text{OH}^- & E^0 &= 0.760 \text{V}
\end{align*}
\]

Examples of ClO₂ reactions through an electron transfer mechanism will be described in section 2.3.3.

### 2.3.2. Measurements of ClO₂ concentration

The reactive nature of ClO₂ gas makes it difficult to find a suitable detection method to accurately measure its concentration (Dunn and Simon, 1992; Vaida and Simon, 1995). Typical analytical methods used to monitor ClO₂ concentration and some important background information is summarized in Table 2-1.

As the main applications of ClO₂ are in pulp production and waste water treatment, most of the detection techniques were developed for measuring ClO₂ in the
solution forms. To determine the concentration of gaseous ClO$_2$, several methods require that the gas be captured by an appropriate solution, e.g. potassium iodide (KI), or distilled water. Most methods also require large volume of gas to be used as a sample, e.g. 7.5 L of air for determination of ClO$_2$ in workplace atmosphere (Agency for Toxic Substances and Disease Registry Division of Toxicology and Environmental Medicine, 2004; Kaczur and Cawlfie, 1992; Occupational Safety & Health Administration, 2010; USEPA, 1999).

### 2.3.3. Microbial activity

ClO$_2$ is recognized as a strong antimicrobial agent against bacterial, viral, and protozoan pathogens. Its inactivating efficiency is generally reported to be equal to or higher than that of Cl$_2$ on a mass-dose basis, but less than that of ozone (USEPA, 1999). Several researches have reported the antimicrobial effects of ClO$_2$ against important microorganisms as listed in Table 2-2 (last updated: August, 2010).

Numbers of research also reported antimicrobial capacities of ClO$_2$ on biofilm network of bacteria, such as *B. cereus*, *L. monocytogenes*, and *E. coli* O157:H7 (Table 2-2) (Annous et al., 2006; Annous et al., 2005; Kreske et al., 2006b; Lindsay et al., 2002; Ölmez and Temur, 2010; Ryu and Beuchat, 2005). A biofilm is an aggregation of microorganisms by a matrix of extracellular polymeric substances (EPS). Biofilm is an important survival mechanism for microorganisms, since inactivation of pathogenic microorganisms by antimicrobial agents is inevitably affected by the accessibility of the
surfaces where the microorganisms resided in. The film creates a protected environment, so that the bacteria living in the biofilm can tolerate harsh environmental conditions better (Ölmez and Temur, 2010; Simões et al., 2010).

Netramai et al studies antimicrobial effects of ClO₂ gas on biofilm of *E. coli* O157:H7 formed on Romaine lettuce leaf by treating inoculated lettuce leaf sample with 0.2 mg ClO₂/L, for 30 and 60 min (see Appendix 1 for more detail). The treatments gave ≤ 1.57 ± 0.05 log CFU/g sample log reduction of pathogen. The comparison on topographic characteristics, between untreated and ClO₂-treated surfaces, using Scanning Electron Microscope (SEM), showed the biofilm of *E. coli* O157:H7 being partially destroyed and/or disappeared, in many area of the surface as shown in Figure 2-3 and Figure 2-4 (Netramai et al., 2010).
<table>
<thead>
<tr>
<th>Method</th>
<th>Principle</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPD Colorimetric method</td>
<td>Colorimetric method, in which, ClO₂ reacts with DPD (N,N-diethyl-p-phenylenediamine) to form colored product. Standard method for chemical analysis of water and wastes.</td>
<td>Interferences = Mn²⁺, Cl₂, and other related oxidants</td>
</tr>
<tr>
<td>(SM-4500-ClO₂·D)¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPD-glycine Colorimetric method</td>
<td>Same as SM-4500-ClO₂·G, but interference from free Cl₂ is solved (up to 6 ppm Cl₂) by adding glycine to the sample. Standard method for chemical analysis of water and wastes.</td>
<td>Slowly reacts with ClO₂⁻ and other oxidants</td>
</tr>
<tr>
<td>(SM-4500-ClO₂·D)¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPD-FAS Titrimetric method</td>
<td>DPD color titration with standard ferrous ammonium sulfate (FAS).</td>
<td>Interferences = Iron and other oxidants</td>
</tr>
<tr>
<td>(SM-4500-ClO₂·D)¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amperometric titration</td>
<td>Iodide (I⁻) oxidation with pH control. Practical method.</td>
<td>Interferences = Free halogens, organic chloramines, and Cu²⁺</td>
</tr>
<tr>
<td>(4500-ClO₂·C)¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-Step Amperometric method</td>
<td>Iodide (I⁻) oxidation with pH control and gas purging step (more detail in Appendix 4).</td>
<td>Skilled analyst is preferred</td>
</tr>
<tr>
<td>(4500-ClO₂·E)¹</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ SM = Standard Method
<table>
<thead>
<tr>
<th>Method</th>
<th>Principle</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiosulfate titration</td>
<td>Colorimetric method, in which, I$_2$ reacts with sodium thiosulfate (more detail in Appendix 2).</td>
<td>Skilled analyst is preferred</td>
</tr>
<tr>
<td>Ion chromatography (EPA Method 300.0 or 300.1)</td>
<td>Ion chromatography with AS9 column and external standard</td>
<td>Chloramines, ClO$_2^-$, OCl$^-$, and HOCl are undetectable</td>
</tr>
<tr>
<td>Toxic gas vapor detector tube</td>
<td>Reduction-oxidation (Redox) reaction</td>
<td>Affected by temperature</td>
</tr>
<tr>
<td>Electrochemical gas sensor</td>
<td>Reduction-oxidation (Redox) reaction</td>
<td>Affected by humidity (condensation)</td>
</tr>
</tbody>
</table>

1 SM = Standard Method
Figure 2-3. Biofilm of *E. coli* O157:H7 on un-treated lettuce surfaces; A – Visible bacteria cells; and B – Continuous layer of biofilm. Adapted from (Netramai *et al.*, 2010).

Figure 2-4. Biofilm of *E. coli* O157:H7 on ClO₂-treated lettuce surfaces; C – Debris observed after ClO₂ treatment; and D – Partially destroyed biofilm. Adapted from (Netramai *et al.*, 2010).
Chlorine dioxide disinfects by oxidation through the one-electron transfer mechanism in which it is usually reduced to ClO$_2^-$ (USEPA, 1999). However, its primary mode of microorganism inactivation is not yet well established, and seems to depend on the type of target microorganism. Several studies reported two possible primary inactivation mechanisms to destroy microorganisms of ClO$_2$ (USEPA, 1999). The first mechanism involves the reaction between ClO$_2$ and, nucleic acid and/or peripheral structures, i.e. inactivates microorganisms primarily through the disruption of protein synthesis (Bernarde et al., 1967). In the second mechanism, microorganisms are inactivated mainly from the alteration of protein (in outer membrane) which alters the permeability of the cell membrane (Aieta and Berg, 1986).

In 2006, Ison et al reported the first-order reaction between ClO$_2$ and cysteine (CSH). Cysteine is one of the amino acid that maintains cellular redox potentials. The reaction rate increased as the pH increases from 2.7 to 9.5 at 25°C. The purposed oxidation mechanism is through an electron transfer from CS$^-$ to ClO$_2$. The reactive intermediates undergo two different pathways depending on the pH of the system. At low pH, the major end-products are cysteic acid (CSO$_3$H) and Cl$^-$, and, at high pH, the major end-products are cystine (CSSC) and chlorite ion (ClO$_2^-$), as shown in Scheme 2-2. Chlorite ion can further reacts with CSH at around neutral pH (6.7), but the rate of reaction is slower than the ClO$_2$/CSH reaction by 6-order of magnitude. Chlorine dioxide can also react with glutathione anion (GS$^-$) with a second-rate constant of 1.40 × 10$^8$ M$^-$
Glutathione is a tripeptide of glycine, cysteine, and glutamic acid. It presents in animals, plants, and many bacteria, and functions in both chemical and enzymatic reactions (Ison et al., 2006). The studies on the reaction of ClO$_2$ with other amino acid, i.e. tyrosine and tryptophan are also reported (Tan et al., 1987).

$$\text{CSH} \quad \Leftrightarrow \quad \text{CS}^- + \text{H}^+ \quad \text{(a)}$$
$$\text{CS}^- + \text{ClO}_2 \quad \rightarrow \quad \text{CS}^- \cdot \text{ClO}_2^- \quad \text{(b)}$$
$$\text{CS}^- \cdot \text{ClO}_2 \quad \rightarrow \quad \text{cysteinyll-ClO}_2 \text{ adduct} \quad \text{(c)}$$

Low pH pathway:
$$\text{cysteinyll-ClO}_2 \text{ adduct} + \text{H}_2\text{O} \quad \rightarrow \quad \text{ClOH} + \text{CSO}_2\text{H} \quad \text{(d)}$$
$$\text{CSO}_2\text{H} + \text{ClOH} \quad \rightarrow \quad \text{Cl}^- + \text{CSO}_3\text{H} \quad \text{(e)}$$

High pH pathway:
$$\text{cysteinyll-ClO}_2 \text{ adduct} + \text{CS}^- \quad \rightarrow \quad \text{ClO}_2^- + \text{CSSC} \quad \text{(f)}$$
$$\text{ClO}_2^- + \text{CSH} \quad \rightarrow \quad \text{CSSC} + \text{Cl}^- + \text{H}_2\text{O} \quad \text{(g)}$$

**Scheme 2-2.** Purposed mechanism for the initial reactions between ClO$_2$ and CSH and subsequent decay of the cysteinyll-ClO$_2$ adduct. Adapted from (Ison et al., 2006).
Table 2-2. Summary of research studies on antimicrobial efficiencies of ClO₂ for food products and other surfaces.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Food Product / Other surface</th>
<th>Treatment $^1$</th>
<th>Result (log$_{10}$ reduction)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alicyclobacillus acidoterrestris (spore)</td>
<td>Stainless steel</td>
<td>50-200 ppm (l) for 1-2 min at 40-90°C</td>
<td>$\leq 2.17$ log CFU/cm$^2$</td>
<td>(Podolak et al., 2009)</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>Apple</td>
<td>10-200 μm/mL (l) for 5 min</td>
<td>$\leq 3.79$ log CFU/fruit</td>
<td>(Kreske et al., 2006a)</td>
</tr>
<tr>
<td></td>
<td>Stainless steel</td>
<td>100-200 μm/mL (l) for 5 min</td>
<td>$\leq 3.71$ log CFU/coupon</td>
<td>(Kreske et al., 2006b)</td>
</tr>
<tr>
<td>Bacillus cereus (spore)</td>
<td>Stainless steel</td>
<td>100-200 μm/mL (l) for 5 min</td>
<td>$\leq 2.55$ log CFU/coupon</td>
<td>(Kreske et al., 2006a)</td>
</tr>
<tr>
<td>Bacillus cereus (cell and spore)</td>
<td>Stainless steel</td>
<td>100-200 μm/mL (l) for 5 min</td>
<td>$\leq 4.48$ log CFU/coupon</td>
<td>(Kreske et al., 2006b)</td>
</tr>
<tr>
<td>Bacillus thuringiensis (spore)</td>
<td>Apple</td>
<td>10-200 μm/mL (l) for 5 min</td>
<td>$\leq 4.25$ log CFU/fruit</td>
<td>(Kreske et al., 2006a)</td>
</tr>
<tr>
<td></td>
<td>Stainless steel</td>
<td>10-200 μm/mL (l) for 5 min</td>
<td>$\leq 2.71$ log CFU/coupon</td>
<td>(Kreske et al., 2006a)</td>
</tr>
<tr>
<td>Cryptosporidium parvum</td>
<td>Basil</td>
<td>4.1 mg/L (g) for 20 min</td>
<td>2.6 log CFU/g</td>
<td>(Ortega et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>Lettuce</td>
<td>4.1 mg/L (g) for 20 min</td>
<td>3.3 log CFU/g</td>
<td>(Ortega et al., 2008)</td>
</tr>
<tr>
<td>Encephalitozoon intestinalis (spore)</td>
<td>Basil</td>
<td>4.1 mg/L (g) for 20 min</td>
<td>3.6 log CFU/g</td>
<td>(Ortega et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>Lettuce</td>
<td>4.1 mg/L (g) for 20 min</td>
<td>3.6 log CFU/g</td>
<td>(Ortega et al., 2008)</td>
</tr>
<tr>
<td>Enterobacter sakazakii</td>
<td>Apple</td>
<td>10-100 μg/mL (l) for 1-5 min</td>
<td>$\geq 5.46$ log CFU/fruit</td>
<td>(Kim et al., 2006)</td>
</tr>
<tr>
<td></td>
<td>Lettuce</td>
<td>10-100 μg/mL (l) for 1-5 min</td>
<td>$\geq 4.81$ log CFU/piece</td>
<td>(Kim et al., 2006)</td>
</tr>
<tr>
<td></td>
<td>Tomato</td>
<td>10-100 μg/mL (l) for 1-5 min</td>
<td>$\geq 5.31$ log CFU/fruit</td>
<td>(Kim et al., 2006)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Apple</td>
<td>0.03-0.30 ppm (g) for 1-20 hours</td>
<td>$\leq 5$ log CFU/g</td>
<td>(Sapers et al., 2003)</td>
</tr>
</tbody>
</table>

$^1$ (g) = ClO₂ in a gas phase; (l) = ClO₂ in a liquid/solution form.
<table>
<thead>
<tr>
<th>Microorganism</th>
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<th>Treatment $^1$</th>
<th>Result (log$_{10}$ reduction)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> O157:H7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alfalfa seed</td>
<td></td>
<td>10-50 mg/L (l) for 3-10 min</td>
<td>$\leq 1.22$ log CFU/g</td>
<td>(Singh, 2003)</td>
</tr>
<tr>
<td>Alfalfa sprout</td>
<td></td>
<td>50 mg/L (l) for 1-10 min</td>
<td>$\leq 2.37$ log CFU/g</td>
<td>(Kim <em>et al.</em>, 2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 mg/L (l) + 0.5 g/100 mL fumaric acid for 1-10 min</td>
<td>$\leq 3.96$ log CFU/g</td>
<td>(Kim <em>et al.</em>, 2009)</td>
</tr>
<tr>
<td>Apple</td>
<td></td>
<td>5-40 ppm (l) for 3-10 min + 170-kHz Ultrasonication</td>
<td>$\leq 3.9$ log CFU/g</td>
<td>(Huang <em>et al.</em>, 2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3-5 ppm (l) for 5 min + storage at $4^\circ$ C</td>
<td>$\leq 5$ log CFU/g</td>
<td>(Rodgers <em>et al.</em>, 2004)</td>
</tr>
<tr>
<td>Apple (calyx, stem cavity and skin)</td>
<td></td>
<td>3-12 mg/L (g) for 10-30 min</td>
<td>$\leq 8$ log CFU/site</td>
<td>(Du <em>et al.</em>, 2003)</td>
</tr>
<tr>
<td>Apple (fresh cut)</td>
<td></td>
<td>3-5 ppm (l) for 5 min + storage at $4^\circ$ C</td>
<td>$\leq 5$ log CFU/g</td>
<td>(Rodgers <em>et al.</em>, 2004)</td>
</tr>
<tr>
<td>Blueberries</td>
<td></td>
<td>4 mg/L (g) for 12 hours</td>
<td>$4.5$ log CFU/g</td>
<td>(Popa <em>et al.</em>, 2007)</td>
</tr>
<tr>
<td>Cabbage (fresh cut)</td>
<td></td>
<td>1.4-4.1 mg/L (g) for 6-21 min</td>
<td>$\leq 3.13$ log CFU/g</td>
<td>(Rodgers <em>et al.</em>, 2004)</td>
</tr>
<tr>
<td>Cantaloupe</td>
<td></td>
<td>3-5 ppm (l) for 5 min + storage at $4^\circ$ C</td>
<td>$\leq 5$ log CFU/g</td>
<td>(Rodgers <em>et al.</em>, 2004)</td>
</tr>
<tr>
<td>Carrot</td>
<td></td>
<td>5-20 mg/L (l) for 1-15 min</td>
<td>$\leq 1.39$ log CFU/g</td>
<td>(Singh <em>et al.</em>, 2002)</td>
</tr>
<tr>
<td>Carrot (fresh cut)</td>
<td></td>
<td>0.5-1.0 mg/L (g) for 5-15 min</td>
<td>$\leq 3.08$ log CFU/g</td>
<td>(Singh <em>et al.</em>, 2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.4-4.1 mg/L (g) for 6-21 min</td>
<td>$\leq 5.62$ log CFU/g</td>
<td>(Sy <em>et al.</em>, 2005b)</td>
</tr>
</tbody>
</table>

$^1$ (g) = ClO$_2$ in a gas phase; (l) = ClO$_2$ in a liquid/solution form.
**Table 2-2. (cont’d)**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Food Product / Other surface</th>
<th>Treatment 1</th>
<th>Result (log(_{10}) reduction)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> O157:H7 (cont’d)</td>
<td>Green pepper</td>
<td>0.15-1.2 mg/L (g) for 30 min</td>
<td>≤ 7.3 log CFU/pepper</td>
<td>(Han <em>et al.</em>, 2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-40 ppm (l) for 3-10 min + 170-kHz Ultrasonication</td>
<td>≤ 2.5 log CFU/g</td>
<td>(Huang <em>et al.</em>, 2006)</td>
</tr>
<tr>
<td></td>
<td>Lettuce</td>
<td>3-5 ppm (l) for 5 min + storage at 4°C</td>
<td>≤ 5 log CFU/g</td>
<td>(Rodgers <em>et al.</em>, 2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.3-8.7 mg/L (g) for 30-180 min</td>
<td>≤ 6.9 log CFU/g</td>
<td>(Lee <em>et al.</em>, 2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-50 ppm (l) for 10 min</td>
<td>≤ 1.44 log CFU/g</td>
<td>(Kim <em>et al.</em>, 2008)</td>
</tr>
<tr>
<td></td>
<td>Lettuce (fresh cut)</td>
<td>3-5 ppm (l) for 5 min + storage at 4°C</td>
<td>≤ 5 log CFU/g</td>
<td>(Rodgers <em>et al.</em>, 2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.4-4.1 mg/L (g) for 6-21 min</td>
<td>≤ 1.57 log CFU/g</td>
<td>(Sy <em>et al.</em>, 2005b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-20 mg/L (l) for 1-15 min</td>
<td>≤ 0.90 log CFU/g</td>
<td>(Singh <em>et al.</em>, 2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5-1.0 mg/L (g) for 5-15 min</td>
<td>≤ 2.31 log CFU/g</td>
<td>(Singh <em>et al.</em>, 2002)</td>
</tr>
<tr>
<td></td>
<td>Potato</td>
<td>9 ppm (l) for 30-300 min with spray washing</td>
<td>≤ 1.4 log CFU/fruit</td>
<td>(Rodgers <em>et al.</em>, 2004)</td>
</tr>
<tr>
<td></td>
<td>Strawberries</td>
<td>3-5 ppm (l) for 5 min + storage at 4°C</td>
<td>≤ 5 log CFU/g</td>
<td>(Park <em>et al.</em>, 2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2-4.0 mg/L (g) for 15-30 min</td>
<td>≤ 5 log CFU/fruit</td>
<td>(Han <em>et al.</em>, 2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6-3.0 mg/L (g) for 10 min</td>
<td>≥ 5 log CFU/fruit</td>
<td>(Han <em>et al.</em>, 2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100-200 ppm (l) for 2 min</td>
<td>≤ 2 log CFU/fruit</td>
<td>(Lukasik <em>et al.</em>, 2003)</td>
</tr>
<tr>
<td></td>
<td>Lactic acid bacteria</td>
<td>Rib eye steak</td>
<td>30-100 ppm (l) for 2 min + vacuum packaging for 4 weeks</td>
<td>≥ 1.0 log CFU/cm(^2)</td>
</tr>
</tbody>
</table>

\(^1\) (g) = ClO\(_2\) in a gas phase; (l) = ClO\(_2\) in a liquid/solution form.
<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Food Product / Other surface</th>
<th>Treatment 1</th>
<th>Result (log₁₀ reduction)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus buchneri</em></td>
<td><em>Stainless steel coated with epoxy</em></td>
<td>2-14 mg/L (g) for 5-120 min</td>
<td>≤ 6 log CFU</td>
<td>(Han et al., 1999)</td>
</tr>
<tr>
<td><em>Leuconostoc mesenteroides</em></td>
<td><em>Stainless steel coated with epoxy</em></td>
<td>8-10 mg/L (g) for 30 min</td>
<td>≤ 6 log CFU</td>
<td>(Han et al., 1999)</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>Alfalfa sprout</td>
<td>50 mg/L (l) for 1-10 min</td>
<td>≤ 2.36 log CFU/g</td>
<td>(Kim et al., 2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 mg/L (l) + 0.5 g/100 mL fumaric acid for 1-10 min</td>
<td>≤ 3.69 log CFU/g</td>
<td>(Kim et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>Apple</td>
<td>3-5 ppm (l) for 5 min + storage at 4°C</td>
<td>≤ 5 log CFU/g</td>
<td>(Rodgers et al., 2004)</td>
</tr>
<tr>
<td></td>
<td>Cantaloupe</td>
<td>3-5 ppm (l) for 5 min + storage at 4°C</td>
<td>≤ 5 log CFU/g</td>
<td>(Rodgers et al., 2004)</td>
</tr>
<tr>
<td></td>
<td>Carrot (fresh cut)</td>
<td>1.4-4.1 mg/L (g) for 10-29 min</td>
<td>≤ 5.88 log CFU/g</td>
<td>(Sy et al., 2005b)</td>
</tr>
<tr>
<td></td>
<td>Lettuce</td>
<td>3-5 ppm (l) for 5 min + storage at 4°C</td>
<td>≤ 5 log CFU/g</td>
<td>(Rodgers et al., 2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.3-8.7 mg/L (g) for 30-180 min</td>
<td>≤ 5.4 log CFU/g</td>
<td>(Lee et al., 2004)</td>
</tr>
<tr>
<td></td>
<td>Lettuce (fresh cut)</td>
<td>3-5 ppm (l) for 5 min + storage at 4°C</td>
<td>≤ 5 log CFU/g</td>
<td>(Rodgers et al., 2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-50 ppm (l) for 10 min</td>
<td>≤ 1.20 log CFU/g</td>
<td>(Kim et al., 2008)</td>
</tr>
</tbody>
</table>

1 (g) = ClO₂ in a gas phase; (l) = ClO₂ in a liquid/solution form.
Table 2-2. (cont’d)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Food Product / Other surface</th>
<th>Treatment¹</th>
<th>Result (log₁₀ reduction)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Listeria monocytogenes</em> (cont’d)</td>
<td>Lettuce (fresh cut) (cont’d)</td>
<td>1.4-4.1 mg/L (g) for 10-29 min</td>
<td>≤ 1.53 log CFU/g</td>
<td>(Sy et al., 2005b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3-5 ppm (l) for 5 min + storage at 4°C</td>
<td>≤ 5 log CFU/g</td>
<td>(Rodgers et al., 2004)</td>
</tr>
<tr>
<td></td>
<td>Mungbean sprout</td>
<td>100 ppm (l) for 5 min + MAP in 7 days</td>
<td>≤ 2.08 log CFU/g</td>
<td>(Jin and Lee, 2007)</td>
</tr>
<tr>
<td></td>
<td>Strawberries</td>
<td>0.2-4.0 mg/L (g) for 15-30 min or 0.6-3.0 mg/L for 10 min</td>
<td>≥ 5 log CFU/g</td>
<td>(Han et al., 2004)</td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td><em>Stainless steel</em></td>
<td>100-200 μm/mL (l) for 5 min</td>
<td>≥ 6.15 log CFU/coupon</td>
<td>(Kreske et al., 2006b)</td>
</tr>
<tr>
<td><em>Salmonella spp.</em></td>
<td>Apple</td>
<td>5-40 ppm (l) for 3-10 min + Ultrasonication</td>
<td>≤ 4.3 log CFU/g</td>
<td>(Huang et al., 2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.4-4.1 mg/L (g) for 6-25 min</td>
<td>≤ 4.21 log CFU/g</td>
<td>(Sy et al., 2005b)</td>
</tr>
<tr>
<td></td>
<td>Bell pepper</td>
<td>100 mg (g) for 1 hour</td>
<td>≤ 5.97 log CFU/fruit</td>
<td>(Yuk et al., 2006)</td>
</tr>
<tr>
<td></td>
<td>Blueberries</td>
<td>4-8 mg/L (g) for 30-120 min</td>
<td>≤ 3.67 log CFU/g</td>
<td>(Sy et al., 2005a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 mg/L (g) for 12 hours</td>
<td>3.8 log CFU/g</td>
<td>(Popa et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Cabbage (fresh cut)</td>
<td>1.4-4.1 mg/L (g) for 10-31 min</td>
<td>≤ 4.42 log CFU/g</td>
<td>(Sy et al., 2005b)</td>
</tr>
<tr>
<td></td>
<td>Carrot (fresh cut)</td>
<td>1.4-4.1 mg/L (g) for 10-31 min</td>
<td>≤ 5.15 log CFU/g</td>
<td>(Sy et al., 2005b)</td>
</tr>
<tr>
<td></td>
<td>Lettuce (fresh cut)</td>
<td>5-40 ppm (l) for 3-10 min + 170-kHz Ultrasonication</td>
<td>up to 3.5 log CFU/g</td>
<td>(Huang et al., 2006)</td>
</tr>
<tr>
<td></td>
<td>Lettuce (fresh cut)</td>
<td>1.4-4.1 mg/L (g) for 10-31 min</td>
<td>up to 1.58 log CFU/g</td>
<td>(Sy et al., 2005b)</td>
</tr>
<tr>
<td></td>
<td>Onion</td>
<td>1.4-4.1 mg/L (g) for 5-20 min</td>
<td>up to 1.94 log CFU/g</td>
<td>(Sy et al., 2005b)</td>
</tr>
</tbody>
</table>

¹ (g) = ClO₂ in a gas phase; (l) = ClO₂ in a liquid/solution form.
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</thead>
<tbody>
<tr>
<td><em>Salmonella spp.</em> (cont’d)</td>
<td>Peach</td>
<td>1.4-4.1 mg/L (g) for 5-20 min</td>
<td>up to 3.23 log CFU/g</td>
<td>(Sy et al., 2005b)</td>
</tr>
<tr>
<td></td>
<td>Raspberries</td>
<td>4-8 mg/L (g) for 30-120 min</td>
<td>up to 1.54 log CFU/g</td>
<td>(Sy et al., 2005a)</td>
</tr>
<tr>
<td></td>
<td>Strawberries</td>
<td>4-8 mg/L (g) for 30-120 min</td>
<td>up to 3.76 log CFU/g</td>
<td>(Sy et al., 2005a)</td>
</tr>
<tr>
<td></td>
<td>Tomato</td>
<td>1.4-4.1 mg/L (g) for 5-20 min</td>
<td>up to 4.33 log CFU/g</td>
<td>(Sy et al., 2005b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 ppm (l) + spray washing for 10-60 sec</td>
<td>up to 5.6 log CFU/cm$^2$</td>
<td>(Pao et al., 2009)</td>
</tr>
<tr>
<td></td>
<td><em>Salmonella Montevideo</em></td>
<td>Strawberries</td>
<td>100-200 ppm (l) for 2 min</td>
<td>less than 2 log CFU/fruit</td>
</tr>
<tr>
<td><em>Salmonella Typhimurium</em></td>
<td>Alfalfa sprout</td>
<td>50 mg/L (l) for 1-10 min</td>
<td>≤ 2.23 log CFU/g</td>
<td>(Kim et al., 2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 mg/L (l) + 0.5 g/100 mL fumaric acid for 1-10 min</td>
<td>≤ 3.57 log CFU/g</td>
<td>(Kim et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>Chicken</td>
<td>2.25 mg (g) in 22 days and 6.6 mg (g) in 26 h.</td>
<td>1 log CFU/breast less than package without ClO$_2$ after 15 days of storage</td>
<td>(Ellis et al., 2006)</td>
</tr>
<tr>
<td></td>
<td>Lettuce</td>
<td>4.3-8.7 mg/L (g) for 30-180 min</td>
<td>≤ 5.4 log CFU/g</td>
<td>(Lee et al., 2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-50 ppm (l) for 10 min</td>
<td>≤ 1.95 log CFU/g</td>
<td>(Kim et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>Mungbean sprout</td>
<td>100 ppm (l) for 5 min + MAP in 7 days</td>
<td>≤ 2.76 log CFU/g</td>
<td>(Jin and Lee, 2007)</td>
</tr>
<tr>
<td></td>
<td>Potato</td>
<td>9 ppm (l) for 30-300 min with spray washing</td>
<td>≤ 1.9 log CFU/fruit</td>
<td>(Park et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>Tomato</td>
<td>Up to 1 mg/kg fruit (g) for 2 hours</td>
<td>≤ 7 log CFU/fruit</td>
<td>(Mahovic et al., 2009)</td>
</tr>
</tbody>
</table>

1 (g) = ClO$_2$ in a gas phase; (l) = ClO$_2$ in a liquid/solution form.
Table 2-2. (cont’d)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Food Product / Other surface</th>
<th>Treatment 1</th>
<th>Result (log$_{10}$ reduction)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total aerobic plate count</td>
<td>Crawfish</td>
<td>10-40 mg/L (l) for 2 min</td>
<td>≤ 4 log CFU</td>
<td>(Andrews et al., 2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>high-pressure water spray at 600 psi for 30 sec + 10-40 mg/L (l) for 2 min</td>
<td>≤ 4.5 log CFU</td>
<td>(Andrews et al., 2002)</td>
</tr>
<tr>
<td></td>
<td>Mungbean sprout</td>
<td>100 ppm (l) for 5 min + MAP in 7 days</td>
<td>≤ 1.35 log CFU/g</td>
<td>(Jin and Lee, 2007)</td>
</tr>
<tr>
<td></td>
<td>Potato</td>
<td>9 ppm (l) for 30-300 min with spray washing</td>
<td>≤ 1.1 log CFU/fruit</td>
<td>(Park et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>Rib eye steak</td>
<td>30-100 ppm (l) for 2 min + vacuum packaging for 4 weeks</td>
<td>≥ 1.0 log CFU/cm$^{2}$</td>
<td>(Unda et al., 2006)</td>
</tr>
<tr>
<td></td>
<td>Shrimp</td>
<td>high-pressure water spray at 600 psi for 30 sec + 10-40 mg/L (l) for 2 min</td>
<td>≤ 4 log CFU</td>
<td>(Andrews et al., 2002)</td>
</tr>
<tr>
<td>Molds</td>
<td>Blueberries</td>
<td>4 mg/L (g) for 12 hours</td>
<td>3.0 log CFU/g</td>
<td>(Popa et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Potato</td>
<td>9 ppm (l) for 30-300 min with spray washing</td>
<td>≤ 0.9 log CFU/fruit</td>
<td>(Park et al., 2008)</td>
</tr>
<tr>
<td></td>
<td><strong>Stainless steel coated with epoxy</strong></td>
<td>8-10 mg/L (g) for 10-30 min</td>
<td>≤ 4 log CFU</td>
<td>(Han et al., 1999)</td>
</tr>
<tr>
<td>Yeasts</td>
<td>Blueberries</td>
<td>4 mg/L (g) for 12 hours</td>
<td>3.2 log CFU/g</td>
<td>(Popa et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Potato</td>
<td>9 ppm (l) for 30-300 min with spray washing</td>
<td>≤ 1.1 log CFU/fruit</td>
<td>(Park et al., 2008)</td>
</tr>
</tbody>
</table>

1 (g) = ClO$_{2}$ in a gas phase; (l) = ClO$_{2}$ in a liquid/solution form.
Table 2-2. (cont’d)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Food Product / Other surface</th>
<th>Treatment 1</th>
<th>Result (log_{10} reduction)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeasts (cont’d)</td>
<td><em>Stainless steel coated with epoxy</em></td>
<td>8-10 mg/L (g) for 10-30 min</td>
<td>≤ 4 log CFU</td>
<td>(Han et al., 1999)</td>
</tr>
<tr>
<td>Yeasts and Molds</td>
<td>Apple</td>
<td>1.4-4.1 mg/L (g) for 6-25 min</td>
<td>≤ 1.68 log CFU/g</td>
<td>(Sy et al., 2005b)</td>
</tr>
<tr>
<td></td>
<td>Blueberries (calyx, stem cavity and skin)</td>
<td>4-8 mg/L (g) for 30-120 min</td>
<td>≤ 2.78 log CFU/g</td>
<td>(Sy et al., 2005a)</td>
</tr>
<tr>
<td></td>
<td>Onion</td>
<td>1.4-4.1 mg/L (g) for 5-20 min</td>
<td>≤ 0.22 log CFU/g</td>
<td>(Sy et al., 2005b)</td>
</tr>
<tr>
<td></td>
<td>Peach</td>
<td>1.4-4.1 mg/L (g) for 5-20 min</td>
<td>≤ 2.65 log CFU/g</td>
<td>(Sy et al., 2005b)</td>
</tr>
<tr>
<td></td>
<td>Raspberries (calyx, stem cavity and skin)</td>
<td>4-8 mg/L (g) for 30-120 min</td>
<td>≤ 3.18 log CFU/g</td>
<td>(Sy et al., 2005a)</td>
</tr>
<tr>
<td></td>
<td>Strawberries (calyx, stem cavity and skin)</td>
<td>4-8 mg/L (g) for 30-120 min</td>
<td>≤ 4.16 log CFU/g</td>
<td>(Sy et al., 2005a)</td>
</tr>
<tr>
<td></td>
<td>Tomato</td>
<td>1.4-4.1 mg/L (g) for 6-25 min</td>
<td>≤ 1.16 log CFU/g</td>
<td>(Sy et al., 2005b)</td>
</tr>
<tr>
<td><em>Penicillium expansum</em></td>
<td><em>(Suspension)</em></td>
<td>3 ppm (l) for 30-300 sec</td>
<td>≤ 4 log CFU/mL</td>
<td>(Okull et al., 2006)</td>
</tr>
<tr>
<td>Poliovirus 1</td>
<td>Strawberries</td>
<td>100-200 ppm (l) for 2 min</td>
<td>≤ 2 log CFU/fruit</td>
<td>(Lukasik et al., 2003)</td>
</tr>
</tbody>
</table>

1 (g) = ClO₂ in a gas phase; (l) = ClO₂ in a liquid/solution form.
Inactivation efficiency of ClO₂ is not significantly affected by change of pH (USEPA, 1999), however, its antimicrobial capacities are influenced by change in temperature. Benarde et al investigated the effects of temperature on bactericidal activities of ClO₂ by exposing *E. coli* suspension to 0.25-0.75 mgClO₂/L solutions, for 5-300 seconds, at 5-32° C. They reported increases in killing rates as ClO₂ concentrations and/or testing temperatures increased, for example, to achieve 2 log₁₀ CFU reduction (99% kill) at concentration of 0.25 mg/L required 110 sec at 5° C, 74 sec at 10° C, 41 sec at 20° C, or 16 sec at 30° C, while, to obtain the same inactivation level, at 0.75 mg/L, required 60 sec at 5° C or 14 sec at 30° C (Benarde et al., 1967).

### 2.3.4. Applications

Chlorine dioxide is used as a bleaching agent in many applications, especially for the paper, pulp, and textile industries (Department of the Environment Water Heritage and the Arts, 2005; Kaczur and Cawlfield, 1992). As registered bactericide, fungicide and algaecide biocide, ClO₂ is approved by the U.S. Environmental Protection Agency (EPA) in both liquid and gas forms to be used as a sanitizing agent for food processing equipment, and pharmaceutical and factory tools, as well as, in potable- and waste water treatment (Department of Environment Food and Rural Affairs (DEFRA), 2003; Kaczur and Cawlfield, 1992; Kim et al., 1999; USEPA, 1999). One of the major uses of ClO₂ is as a disinfectant for public water system as it causes less organoleptic effect and less
toxic by-products than those caused by Cl2 (Department of Environment Food and Rural Affairs (DEFRA), 2003; USEPA, 1999).

Specifically, in food applications, ClO2 is reported to have antimicrobial effects against many important spoilage and pathogenic microorganisms (Table 2-2), such as E. coli O157:H7, Listeria monocytogenes, Salmonella spp., yeasts and molds, that reside on the surface of food products such as fresh and fresh-cut fruits and vegetables, as well as on fresh meats (Sy et al., 2005b; Taylor et al., 1999; Yuk et al., 2005).

While ClO2 is often used as a sanitizing solution for meat, fruits, and vegetables, (Kim et al., 1999; Yuk et al., 2005), its gaseous form is gaining interest as an antimicrobial agent, for vapor-phase decontamination, in cleaning food-contact surface (Han et al., 1999), treating produce, such as green peppers, blueberries, and apples, before packaging them (Sapers et al., 2003; Sy et al., 2005a; Vermeiren et al., 1999), and sanitizing products, like chicken, directly inside their packages (Ellis et al., 2006).

The introduction of gaseous ClO2 within the food packaging systems is often by means of sachet, either slow- or fast-release (Ellis et al., 2006; Shin, 2007); however, in 2001, the FDA approved the incorporation of ClO2 precursors within food packaging materials to be used as packaging material for meats, poultry, and seafood (USFDA, 2001).

Most of the studies regarding food packaging applications of ClO2 gas focus on its sanitizing effects on particular microorganism and/or specific type of perishable food (Kaczur and Cawlfiefield, 1992; Sapers et al., 2003; Sy et al., 2005a). Only a few studies
consider its effects on the properties of the food packaging systems, as well (Ozen, 2000; Shin et al., 2006).

2.4. **Effects of gaseous oxidizing agents on packaging materials**

Protection and containment are two of the most important roles of the package. The ability to protect and contain the product that the packaging provides depends on the stability of the material to the environmental factors, such as temperature, humidity, and exposure to chemical compounds.

In the case where packaging is going to be considered as a strategy for the release and application of ClO₂ gas, the interaction and compatibility between packaging materials and gaseous ClO₂, such as the mass transfer properties of ClO₂ and its effects on packaging integrities and performance are needed to be determined since these will impact the selection of materials that compatible with such gas, for particular application. The possible effects of exposing polymeric packaging materials to oxidizing agents will be discussed further in the next two sections.

2.4.1. **Mass transfer of packaging materials**

Plastic packaging materials, unlike glass, metals, etc., are relatively permeable to small molecules such as permanent gases, water vapor, organic vapors, and liquids. Polymeric packaging materials provide a broad range of mass transfer characteristics for particular gas or vapor, from high to low barrier values. The specific barrier requirements of the package system will depend upon the product’s characteristics and the intended end-use application (Taub and Singh, 1998).
Permeation includes the transfer through the package of molecules from the product to the external storage environment or from the environment to the product. The mass transfer process provides the basis for further molecular activities within the package system, which can result in changes in the product, as well as physical damage to the package, or both (Selke et al., 2004; Van Krevelen, 1997).

The permeation process starts with the sorption of the penetrant onto the polymer surface. Then, the penetrant travels through the polymer matrix by diffusion with the difference in chemical potential as a driving force, and desorp from the polymer at the other side of the film. Thus, permeation behavior of the polymer depends on its solubility and diffusion properties (Selke et al., 2004; Van Krevelen, 1997).

The mass transfer phenomenon of ‘simple gases’, through a polymer film or sheet is a measure of the steady-state transfer rate of the permeant, and is normally expressed as the permeability coefficient (P). The relationship between P, solubility coefficient (S) and diffusion coefficient (D) usually follows Equation 1.

\[ P = D \cdot S \]  

(1)

The permeability coefficient defines as the amount of gas traveling through the polymeric material with particular thickness, per unit area, per second and at specific pressure gradient.

The diffusion coefficient indicates how fast the permeant can move through the polymer matrix. It reported as the amount of gas, per unit area, traveling through a
material per second, at a particular concentration gradient as expressed by Fick’s first law:

\[
\frac{dm}{dt} = D \cdot A \left( \frac{dc}{dx} \right) \tag{2}
\]

Diffusion of simple gases usually depends only on temperature, not on concentration or time of exposure. This behavior is called Fickian (Selke et al., 2004; Van Krevelen, 1997).

Solubility coefficient defines as the amount of gas per unit volume of the polymer at equilibrium for particular partial pressure according to Henry’s law:

\[
c = S \cdot p \tag{3}
\]

Several techniques are available for P, D, and S determinations. P and D are usually measured either by isostatic or quasi-isostatic methods (Selke et al., 2004; Van Krevelen, 1997).

In isostatic procedure, both values are obtained from a continuous monitoring of the mass transfer process of the permeant through the material. While, in quasi-isostatic method, the accumulated amount of permeant permeated through the material is quantified at predetermined time intervals (Selke et al., 2004). S and D can be determined by gravimetric method, i.e. monitoring the weight change of the film during a sorption experiment. Generally, S values of simple gases in polymeric materials are low, thus it not easy to accurately determine (Matteucci et al., 2006).
The mass transfer of simple gases, especially at low concentration of permeant where there are no strong penetrant/polymer interaction, normally show this ideal permeation behavior, i.e. the solubility follows Henry’s law and diffusion follows Fick’s first law (Van Krevelen, 1997).

However, if the significant interactions between penetrant and polymeric material, as in the cases of organic vapor, and/or between the penetrant themselves exist, the sorption behavior might be affected, i.e. divert from Henry’s law (Selke et al., 2004; Van Krevelen, 1997).

2.4.1.1. Isostatic permeation technique

In this study, P, D, and S values of ClO₂ for polymeric packaging materials were determined using a permeation approach that follows an isostatic method. The diffusion process of gaseous ClO₂ through the materials was assumed to be independent of the permeant’s concentration and the polymer relaxation, which is known as Fickian diffusion (Matteucci et al., 2006; Selke et al., 2004). The diffusion coefficient was calculated from the mass transfer profile in the transient region that leads to the steady-state of the mass transfer, where the concentration gradient of the permeant across the film remains constant.

The quantity of permeated ClO₂ gas (kg) at time t (s) was then plotted to obtain the permeation curve (Figure 2-5). The P value was calculated from the flow rate of the permeated ClO₂ gas at steady-state, $F_{ss}$ (kg/s).
The ratio of permeant flow as a function of time can be described as followed:

\[
\frac{F_t}{F_\infty} = \left(\frac{4}{\sqrt{\pi}}\right)\left(\frac{\ell^2}{4Dt}\right) \sum_{n=1,3,5} \exp\left(-\frac{n^2 \ell^2}{4Dt}\right)
\]

(4)

where \( F_t \) is a permeated gas flow rate at transient time \( t \) (kg/s), \( F_\infty \) is a permeated gas flow rate at steady state (kg/s), \( \ell \) is the film thickness (m), and \( D \) is diffusion.

Equation (4) can be simplified to:

\[
\phi = \frac{F_t}{F_\infty} = \left(\frac{4}{\sqrt{\pi}}\right)X^{1/2} \exp(-x)
\]

(5)

\[
X = \frac{\ell^2}{4Dt}
\]

(6)
At steady state, $\phi = 1$ and D is assumed constant, the P can be calculated from:

$$P = F_{ss} \times \frac{\ell}{A \cdot \Delta p} \quad (7)$$

$$\Delta p = p_2 - p_1 \quad (8)$$

The diffusion coefficient, D, was obtained from the transient state according to the following calculations:

$$D = \frac{\ell^2}{7.2 t_{1/2}} \quad (9)$$

where $A$ is the surface area of the film sample ($m^2$), and $\Delta p$ is the partial pressure gradient of $ClO_2$ between the lower half ($p_2$) and upper half ($p_1$) of the permeability cell. Since there is a constant removal of permeated $ClO_2$ from the upper chamber, $p_1$ is equal to 0.00 Pa. The partial pressure of gaseous $ClO_2$ over the solution placed in the lower chamber was calculated from Henry’s solubility coefficient mentioned earlier and is equal to $3.65 \times 10^2$ Pa (Ishi, 1958; USEPA, 1999). The $t_{1/2}$ is the time taken to reach a flow rate equivalent to half of $F_{ss}$.

The $S$ values can be calculated using the “solution-diffusion” model, i.e. Equation 1 (Matteucci et al., 2006).
2.4.1.2. Factors affecting permeation

The variables affecting mass transfer can be grouped into compositional variables and environmental and geometric variables. The examples of compositional variables are chemical composition of the packaging material and penetrant, morphology of the polymer, concentration of the penetrant, and presence of co-permeant. Important environmental and geometric variables are temperature, relative humidity (RH) and packaging geometry (Selke et al., 2004; Van Krevelen, 1997).

\[ P, D, \text{and } S \text{ are temperature dependent and can be described by a Van't Hoff-Arrhenius equation.} \]

\[
P(T) = P_0 \exp \frac{E_P}{RT} \quad (10)
\]

\[
D(T) = D_0 \exp \frac{E_D}{RT} \quad (11)
\]

\[
S(T) = S_0 \exp \frac{-\Delta H_S}{RT} \quad (12)
\]

From Equation 1 and 10 –12:

\[
P(T) = D(T) \times S(T) = S_0 \times D_0 \times \exp \frac{-\Delta H_S}{E_D} \quad (13)
\]

where \( \Delta H_S \) is molar heat of sorption, \( E_D \) is activation energy of diffusion, and \( E_P \) is apparent activation energy of permeation (Van Krevelen, 1997).
\[ P_0, D_0, S_0, \Delta H_S, E_D \text{ and } E_P \text{ can be estimated from the measurement of } \]
\[ P(T), D(T), \text{ and } S(T) \text{ at different testing temperature (Van Krevelen, 1997).} \]

Raising the testing temperature gives the studied polymer the energy and enables the segmental mobility within the polymer structure. This facilitates the diffusion of the penetrant molecule, thus increases the mass transfer of the system (Selke et al., 2004; Van Krevelen, 1997).

Barrier properties of polymeric materials are strongly influenced by their chemical and physical characteristics, such as crystallinity, polarity, free volume, orientation, and cohesive energy density (Yang and Li, 1997). Polymer’s physical properties like crystallinity affect both diffusion and solubility. Since crystallization decrease amorphous area of the material, less volume is available for the gas to move through. Crystalline also impede the movement of the gas molecules by creating the torturous path they will have to travel (Schnabel, 1992; Selke et al., 2004; Van Krevelen, 1997).

As diffusion coefficients are particularly affected by the polymer’s glass transition temperature (\( T_g \)) and free volume properties (Matteucci et al., 2006). The free volume (FV), the unoccupied spaces between molecules in the polymers’ structures (Selke et al., 2004), in the matrix of the semi-crystalline polymer, at particular temperature, can be calculated from its fractional free volume (FFV):

\[
\text{FFV} = \frac{V - V_0}{V} = \frac{1}{\rho} - 1.3V_w \rho
\]

\[ (14) \]
where \( V \) is the specific volume \((\text{m}^3/\text{kg})\) of the amorphous polymer at the testing temperature and \( V_0 \) is a specific volume \((\text{m}^3/\text{kg})\) at 0 K. The FFV can also be estimated from polymer’s density, \( \rho \) \((\text{m}^3/\text{kg})\) and its Van der Waals volume, \( V_w \) \((\text{m}^3/\text{mol})\) as shown in Equation 14. \( V_w \) values quoted from literature (Van Krevelen, 1997), is the space occupied by the polymer molecule, hence, a barrier for the penetrant (Recio et al., 2008; Van Krevelen, 1997).

The crystalline region serves as the impermeable volume for the diffusion of the permeant, creates the tortuous path for the molecules, and somewhat restricts the polymer chain mobility of the structure in the amorphous region, impeding the mass transfer process. Such regions are also excluded from the sorption process. Since there is a negligible amount of unoccupied space in the crystalline region of the material (Van Krevelen, 1997), the FV values of the semi-crystalline polymeric material can be corrected by:

\[
\text{FV} = \left(1 - \frac{\%\text{Crystallinity}}{100}\right) \times \text{FFV}
\]

Even though free volume property is normally regarded as an independent factor, it can also be considered that a formation/existence of free volume will be affected by the intermolecular forces within the polymer molecules described as cohesive energy density (CED). The material with high polarity has high CED (Alentiev and Yampolskii, 2002; Shimazu et al., 1999; Yang and Li, 1997). CED can be estimated based on cohesive energy \((E_{coh})\) and molar volume \((V_m)\) data as followed:
CED = \frac{E_{coh}}{V_m} \quad (16)

where the \( E_{coh} \) (kJ/mol) and \( V_m \) (m\(^3\)/mol) were estimated by mean of group contribution method (Van Krevelen, 1997).

No confirmed relation was reported between CED and solubility coefficient (S), though the polymers with high FV values usually have high gas solubilities. This could be due to the fact that S values of a particular gas in different polymeric materials vary in a narrower range as compared to their D values (Alentiev and Yampolskii, 2002; Van Krevelen, 1997). Polymers with high CED and low FV tend to have higher barrier to a particular compound as compared to polymers with low CED and high FV, as they both influence molecular packing properties of the materials (Alentiev and Yampolskii, 2002; Van Krevelen, 1997).

2.4.1.3. Mass transfer of gaseous ClO\(_2\)

Ozen (2000) studied mass transfer of gaseous ClO\(_2\) through polymeric materials, using a quasi-isostatic method, by exposing 100 mg/L ClO\(_2\) to the film sample in a three-compartment permeability cell, and utilizing an amperometric titration method as a detection technique. The reported P values of ClO\(_2\) gas, at 20\(^\circ\)C, for linear low-density poly(ethylene), LLDPE, oriented poly(propylene), OPP, and biaxially oriented nylon (BON) were 7.62 x 10\(^{-16}\), 6.21 x 10\(^{-17}\), and 2.34 x 10\(^{-17}\) KgClO\(_2\)\(\cdot\)m\(^2\)\(\cdot\)s\(^{-1}\)\(\cdot\)Pa\(^{-1}\), respectively. Since i) the steady state was reached too fast and ii) the delay response of
the detection system used, prevented the assessment of the unsteady region for the calculation of the diffusion coefficient (Ozen, 2000).

2.4.2. Polymer degradation and its effects on packaging performance

Polymer degradation can be initiated by several factors; thermal, radiation chemical, mechanical, photochemical, biological, and chemical degradations. The process causes the alterations in the materials’ functionality. More than one factor can simultaneously trigger the changes, for example, thermoplastic polymers are subjected to oxidative degradation during their processing due to high heat, applied forces and atmospheric oxygen (Schnabel, 1992).

Chemical reactions normally found during the degradation process of macromolecules are categorized as 1) single step reaction in which the reaction rate is directly proportional to the initiation rate, and 2) chain reaction in which the reaction, once initiated, is self-propagating (Schnabel, 1992). Example of single step reactions are Norrish Type II reaction in ketone polymers initiated by photochemical process and enzymatic attack of glycosidic linkage caused by biological factor. This type of process can result in significant changes in physical and mechanical properties of the linear polymers as their properties are influenced by average molecular weight (MW) (Schnabel, 1992).

In chain reaction process, the products from single initiation step can start several propagation reactions with other molecules, resulting in manipulation of deterioration process (Schnabel, 1992). Among others, autoxidation reactions initiated by heat, light, forces, or chemical exposure are quite common in polymers. The initiation steps result in
the formation of free radicals, which, in the presence of O₂, further promote the chain reaction. Scheme 2-3 shows the free radical mechanism of autooxidation in linear polymers:

**Scheme 2-3.** Free radical mechanism of autooxidation in linear polymers; R• is free radical generated by deterioration process of the polymer; and PH is the polymer.

### 2.4.2.1. Chemical degradation

Chemical degradation defines as “processes which are induced under the influence of chemicals (e.g. acids, bases, solvents, reactive gases etc.) brought into contact with polymers” (Schnabel, 1992). A significant change usually occurs at elevated temperature due to the rather high activation energy of such processes. The degree of changes also depends on the polymers’ stabilities against particular chemical and the polymers’ characteristics, e.g. the presence of crystalline or unsaturated bonds in the structure (Schnabel, 1992).
For the polymers that contain hetero (non-carbon) atoms, e.g. O, N, or halogen, in their structure, the rupture of C-X (C-hetero) bonds (Scheme 2-4), especially in the main-chain, can be of great concern as it leads to a main-chain destruction. Common chemicals caused solvolysis are water, alcohols, ammonia, etc (Schnabel, 1992).

\[ \text{Scheme 2-4. Solvolysis reaction of C-hetero bond in the polymers’ main-chain; YZ is a solvolysis agents} \]

In the case where a reactive C=C bond exists in the structure of the polymer, it is subjected to chemical reactions like metathesis where the degradation process is catalyzed by transition metal and ozonolysis where the reaction with ozone (O\textsubscript{3}) causes the scission through the formation of five-membered cyclic intermediates, which eventually will decompose into free radicals (Scheme 2-5). As mentioned previously, that ClO\textsubscript{2} gas also demonstrates strong oxidizing capacity, so the exposure of polymeric materials to ClO\textsubscript{2} might cause same type of chemical changes as those with O\textsubscript{3} (Ozen, 2000; USEPA, 1999).
Scheme 2-5. Five-membered cyclic intermediates I and II yielded from the reaction of O₃ with olefinic double bonds; I decomposes into carbonyl and biradical compounds; and II decomposes into free radicals.

Ozone can also slowly react with saturated hydrocarbon, at ambient temperature, to create free radicals (Scheme 2-6). The free radicals from the reaction between O₃ with either unsaturated or saturated hydrocarbon can extract H atoms from the polymer structure, and, in the presence of O₂, initiate the autoxidation reaction (Scheme 2-3 and Scheme 2-6).

Ozonation of cellulose was studied by Lemeune et al. (2004). The significant reduction in the cellulose’s degree of polymerization (DPᵥ) was observed, and the degradation increased as the ozone charge increased. The changes in chemical composition of the exposed cellulose sample confirmed that the primary reaction of ozone with cellulose fibers was glycosidic bond cleavage and that the oxidation mechanism followed a three-step process: 1) formation of the carbonyl groups, 2) oxidation to carboxyl groups, and 3) decarboxylation resulting in glycosidic bond cleavage (Lemeune et al., 2004).
When polymeric materials are exposed to strong oxidizing agents, such as O₃ and ClO₂, oxidative degradation can take place, as described previously. Such degradations are usually ‘selective’, either by reacting specifically with certain functional groups, or, taking place exclusively in the amorphous region of the semi-crystalline polymer that is more readily accessible (Kulshreshtha, 1992; Schnabel, 1992; Walzak et al., 1995). In most cases, the maximum interactions and changes occur on the surface of the materials. The typical changes caused by oxidative degradation are main chain scission,
depolymerization, cross-linking, and the changes in functional groups, such as formations of conjugated double bonds, carbonyl groups, etc. (Rivaton and Gardette, 1998; Rodriguez et al., 2003), and consequently, leading to the changes in polymer characteristics which may result in changes in mechanical properties, embrittlement, lack of transparency, as well as, the loss of additives, and the formation of toxic compounds.

Bond scission in the polymer backbone causes the reduction in the polymer’s molecular weight. On the other hand, intermolecular cross-linking, which is the formation of the new chemical bonds between different polymer chains, results in the increase in molecular size (Kulshreshtha, 1992).

The development of color in the oxidized polymers is a consequence of several possible degradation reactions, such as the formation of conjugated double bond, the oxidation of additives, etc. This change in color had been previously reported for some polymeric materials when exposed to oxidizing agents (Buchalla et al., 1993; Rodriguez et al., 2003; Walzak et al., 1995).

Since the chemical changes caused by oxidative degradation could result in the alterations of mechanical, physical, and barrier properties, which eventually, lead to the changes in the polymers’ performance in packaging systems which could affect the product’s shelf-life or. It is critical to assess how ClO₂ may impact the polymeric materials, especially when one potential application of ClO₂ gas is to be used in combination with other gases, such as O₂ and CO₂ in a MAP system, the impact of ClO₂ gas on the barrier to O₂, CO₂, and moisture of the different polymeric material is of great concern.
Barrier property of the polymeric materials is one of the most important performance of packaging system for food application, especially for fresh produce where respiration is still taking place during postharvest period (Martinez-Romero et al., 2003). The concentrations of O₂ and CO₂ accumulated in the package headspace affect the deterioration rate of fresh produce. Once the material is selected for a particular commodity, it is crucial that its permselectivity (P_{CO2}/P_{O2}) ratio be maintained through the shelf-life of the product, as in the case of MAP where the stable gaseous ratio in the package headspace is necessary (Martinez-Romero et al., 2003; Selke et al., 2004).

The study on exposure of nylon to O₃, which is considered to be a stronger oxidizing agent as compared to ClO₂, showed an increase in tensile properties of the exposed polymeric materials, regardless of the applied conditions, but the barrier to oxygen of the material decreased as the time of exposure increased (Ozen, 2000; Ozen et al., 2002). The same trend on oxygen barrier was observed when exposed polyethylene (PE) film to O₃. However, the changes PE film’s mechanical properties varied depending on the treatment conditions. IR spectra of the exposed nylon sample indicated the increase in –C-N- stretching, while, the exposure of polyethylene film to O₃ caused the formation of oxygen-containing groups in the polymer main chain (Ozen et al., 2002).
2.4.3. Effects of gaseous ClO$_2$ on packaging materials

The exposure of polymeric material to ClO$_2$ under different applied conditions could lead to changes in the overall performance of the polymeric materials (Ozen et al., 2002). Shin et al. (Shin et al., 2006) reported the changes in mechanical properties of PS, nylon, and LDPE after exposed to ClO$_2$ gas at 20,000 ppmV or higher.

The decreases in tensile strength and elongation (Kulshreshtha, 1992), a slight increase in oxygen permeability, of LDPE, LLDPE, oriented polypropylene (OPP), and biaxially oriented nylon (BON), after exposure of ClO$_2$ gas, was reported by Ozen (Ozen, 2000). The degree of change varied depending on gas concentration (0.1–1.0 mg/L), relative humidity (45–85% RH), and testing temperature (5-35$^\circ$C).


CHAPTER 3: CONTINUOUS DETECTION SYSTEM FOR MASS TRANSFER MEASUREMENT OF ClO₂

3.1. Introduction

Study on mass transfer of ClO₂ gas through polymeric packaging materials, as well as understanding the effects of ClO₂ gas on important properties of the materials are beneficial when designing a packaging system that will be used in conjunction with gaseous chlorine dioxide (ClO₂), since presence of ClO₂ gas within the food packaging system, at the correct concentration, is a crucial point of sufficient sanitization of the food product within the package.

Due to the reactive nature of ClO₂ gas, it is difficult to find a suitable detection method to accurately measure gaseous ClO₂ concentration (Kaczur and Cawlfield, 1992; USEPA, 1999). Most of the devices and techniques currently available are designed to determine the concentration of ClO₂ in liquid form as its most common applications are in waste- and portable water treatment, and pulp production (Kaczur and Cawlfield, 1992). Selecting a suitable detection technique for detecting ClO₂ in the gaseous state is a very important step in the mass transfer study of ClO₂ gas through polymeric films. The detection technique should have high sensitivity, repeatability, and accuracy, and, at the same time, be robust and economically feasible.
Ozen (2000) studied the mass transfer of gaseous ClO$_2$ through polymeric materials. In the study, two film samples, each with an area of 45 cm$^2$, were installed in a three-compartment permeation cell. The upper and lower chambers were flushed with 100 mg/L ClO$_2$ for 5 min, while the middle compartment was filled with nitrogen. At predetermined time intervals, the specific volume of gas was sampled from the middle chamber and injected into distilled water. The concentration of permeated ClO$_2$ in water was then determined using amperometric titration (Greenberg et al., 1992), and the permeability coefficient was calculated according to the quasi-isostatic technique. Since the steady state was reached too fast for the detection technique used, only the linear portion of the plot of cumulative amount of permeated ClO$_2$ versus time was obtained from the experiment. Thus the diffusion coefficient which can be calculated using the data in the transient state could not be determined (Ozen, 2000). To obtain a complete mass transfer profile of ClO$_2$ through packaging membranes, it is critical to develop a continuous detection technique that can provide information of the unsteady-state region.

The objective of this study was to develop a continuous detection method for mass transfer measurement of gaseous ClO$_2$ to be able to obtain its complete permeation profile, i.e. both the transient and steady state.
3.2. Materials and Methods

The flow diagram and set up of the permeation system developed is shown in Figure 3-1. The system consisted of a nitrogen supply, a flow meter/controller, a permeability cell, and an electrochemical (EC) detector. Nitrogen gas from the generator picks up gaseous ClO$_2$ that permeates through the film sample installed in the permeability cell, and carries ClO$_2$ gas to the EC detector. The detector records the output of concentration of permeated ClO$_2$ (in ppm or ppmV) every 30 seconds.

The permeability cell (Figure 3-2) is made of grade 316 stainless-steel, which has excellent overall corrosion resistance properties (AK Steel Corporation, 2007). The two half-cells are sealed together using a Viton® O-ring made from fluoroelastomers which did not interact with ClO$_2$ gas at the testing concentrations (Anchor Rubber Products, 2007).
Figure 3-1. Flow diagram of mass transfer study. Adapted from (Netramai et al., 2009).
3.2.1. Permeability cell

The film sample is placed between the upper and lower chambers that have volumes of $8.7 \times 10^{-5}$ m$^3$ and $20.3 \times 10^{-5}$ m$^3$, respectively. The permeated ClO$_2$ collects in the upper chamber. A temperature control device, which consists of six heating wires fitted in the metal wall (two wires for the upper chamber, and four wires for the lower chamber), heats the permeability cell to the desired temperature. Temperature is monitored by two thermocouples located at the top and bottom of the unit, and data is transferred to the temperature controller. The temperature fluctuation inside the chamber varied $\pm 0.50^\circ$C from the testing condition. A relative humidity (RH) sensor installed in the upper and lower chambers monitored %RH of the chamber throughout the experiment.
**Figure 3-2.** Schematic of permeation of ClO$_2$ through the polymer film. Adapted from (Netramai *et al.*, 2009).
3.2.2. Electrochemical detector

The detector used in this study was a ToxiPro® electrochemical (EC) detector (Biosystems, Plano, TX) for ClO₂. The detector is able to determine the concentration of a particular gas by generating an electrical signal that correlates the reaction of the electrode with the target gas. A typical EC sensor (two-electrode type), the main component of an EC detector, consists of the following components: (a) a hydrophobic membrane that protects and controls the amount of gas reaching the electrode; (b) a sensing (or working) electrode which the diffused gas reacts with; (c) a counter electrode that supplies or receives electrons for the sensing electrode; and, (d) an electrolyte that facilitates the reaction and enables ionic flow. The system produces an electric current proportional to the gas concentration. A three-electrode type EC sensor also has a third electrode, a reference electrode, in order to stabilize the sensing electrode (Emond and Kearney, 2007)

The reaction that takes place in the sensor for ClO₂ is (Emond and Kearney, 2007):

$$\text{ClO}_2 + 4\text{H}^+ + 5e^- \rightarrow \text{Cl}^- + 2\text{H}_2\text{O} \quad (1)$$

For the ToxiPro® detector, the operating temperature and humidity ranges are –20 to 50°C, and up to 95% RH, respectively, with no condensation (according to the manufacturer’s specifications) (Emond and Kearney, 2007).
The unit displayed on the detector is in ppm (or ppmV), i.e., μl of ClO₂ gas per one liter of sampled gas mixture. The concentration in ppmV of ClO₂ can also be converted to mg of ClO₂/L of gas (mg/L), which is one of the common units used to describe the concentration of gaseous ClO₂ (ICA TriNova LLC, 2006; Ishi, 1958).

3.2.2.1. Calibration

The EC detector was calibrated every two weeks by exposing the detector to air (zero calibration, 0.00 ppmV of ClO₂) and 1.00 ppmV of ClO₂ gas generated by an EC gas generator (Cal 2000, ACD, Tucson, AZ). A bump test to monitor the response of the detector, i.e., exposing the detector to a known concentration of ClO₂ at 0.00, 1.00, 2.50, 3.00, and 4.00 ppmV, was also performed every month by the local Office of Radiation, Chemical & Biological Safety (ORCBS) (East Lansing, MI). The detector gave a linear response with a correlation factor ($R^2$) of 0.9995.

3.2.2.2. Determination of noise and signal to noise ratio

The background noise of the EC detector was determined by collecting mass transfer data in the absence of ClO₂ solution in the lower chamber of the permeability cell over 24 hours for all temperature conditions used in this study. The test was repeated four times at each temperature. The data obtained were then used to calculate the amplitude of noise as 3s, where s is the square root of the mean squared error (MSE) (Freund and Wilson, 2003). The calculated amplitude of noise and the response of the
detector obtained from the bump test (mentioned previously) were then used to calculate the signal to noise (S/N) ratio of the EC detector at various concentrations of gaseous ClO₂.

### 3.2.3. Preparation of ClO₂ solutions

A stock solution of 1000 mg ClO₂ per liter was prepared by submerging a sachet containing the chemical precursors, i.e., sufficient sulfuric acid (H₂SO₄) and sodium chlorite (NaClO₂) to generate approximately 2 × 10⁻³ kg of ClO₂ (z-series, ICA TriNova, Newnan, GA), in 2 liters of deionized, distilled water for 48 hours. The solution was titrated to determine the actual concentration using the titration procedure outlined by ICA TriNova (ICA TriNova LLC, 2006). The stock solution was diluted, as required, right before use. The final concentration was determined by titration and, according to Henry’s constant of gaseous ClO₂ as reported in the literature (Ishi, 1958; Kaczur and Cawlfield, 1992), gave a concentration of 10 mg ClO₂/L of gas (approximately 3600 ppmV) in the headspace. The selected concentration is considered to provide a high dose range of gaseous ClO₂ for food applications (Kaczur and Cawlfield, 1992; Sy et al., 2005).

### 3.2.4. Statistical analysis

The data obtained from four replicates of each sample were statistically analyzed by analysis of variance (ANOVA) using the Statistical Analysis System (SAS) software
(SAS Institute Inc., Cary, NC) at the confidence level of 95% ($\alpha = 0.05$) with Tukey’s adjustment for comparison of means.

### 3.3. Results and Discussion

The amplitudes of the background noise of the permeation system at 23, 30, and 40°C, as shown in Table 3-1, increased with increasing temperature. At 23°C, the S/N ratio of the system improved significantly as the concentration of ClO$_2$ increased. In this study, the concentration of permeated ClO$_2$ at the steady state varied, depending on the type of polymeric material being studied, and ranged from approximately 0.10 to 3.00 ppmV. The noise level was very low as compared to the signal; e.g., noise was only 1.29% at the signal of 0.10 ppmV. The permeation system developed in this study could be considered reliable and consistent for the measurements. This continuous system is also easy to set up and operate, as well as being space- and cost-effective.
Table 3-1. Permeation system mean square error (MSE) and amplitude of noise at 0.00 ppmV, and signal to noise (S/N) ratio at various concentrations of ClO₂

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>ClO₂ ppmV</th>
<th>MSE × 10⁻⁷</th>
<th>Amplitude × 10⁻³</th>
<th>S/N × 10²</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td></td>
<td>1.95 ± 1.09ᵃ,¹</td>
<td>1.29 ± 0.36ᵃ</td>
<td>-</td>
</tr>
<tr>
<td>1.00</td>
<td></td>
<td>-</td>
<td>-</td>
<td>7.82 ± 0.17ᵃ</td>
</tr>
<tr>
<td>23</td>
<td>2.50</td>
<td>-</td>
<td>-</td>
<td>19.7 ± 0.31ᵇ</td>
</tr>
<tr>
<td>3.00</td>
<td></td>
<td>-</td>
<td>-</td>
<td>23.4 ± 0.22ᶜ</td>
</tr>
<tr>
<td>4.00</td>
<td></td>
<td>-</td>
<td>-</td>
<td>30.7 ± 0.24ᵈ</td>
</tr>
<tr>
<td>30</td>
<td>0.00</td>
<td>3.30 ± 0.74ᵃ</td>
<td>1.71 ± 0.19ᵃ</td>
<td>-</td>
</tr>
<tr>
<td>40</td>
<td>0.00</td>
<td>5.06 ± 0.35ᵇ</td>
<td>2.13 ± 0.07ᵇ</td>
<td>-</td>
</tr>
</tbody>
</table>

¹ Within columns, means sharing the same superscript letter are not significantly different (p > 0.05; n = 4).


4.1. Introduction

Due to its bactericidal effects, ClO$_2$ gas is gaining significant interest in the food and pharmaceutical industries (Sy et al., 2005a; Sy et al., 2005b). One of the most recent applications of ClO$_2$ is as a headspace gas in packaging systems where it acts in vapor-phase decontamination to extend the shelf-life of perishable food products (Kaczur and Cawfield, 1992; Sapers et al., 2003; Sy et al., 2005a). Understanding the interaction of gaseous ClO$_2$ with polymeric materials is critical once ClO$_2$ is included in the package headspace. The obtained data could lead to an improved packaging system that is able to deliver and maintain certain amounts of ClO$_2$ to sanitize the product, without compromising package integrity.

If a package is to be considered as a device for the release and application of ClO$_2$ gas, then the mass transfer properties of ClO$_2$ must be determined since this will impact the selection of material(s) with an appropriate gas barrier. The reported permeability (P) values of ClO$_2$ gas, at 20°C, for linear low-density polyethylene (LLDPE), oriented poly(propylene) (OPP), and biaxially oriented nylon (BON), by Ozen (2000), were 7.62 × 10^{-16}, 6.21 × 10^{-17}, and 2.34 × 10^{-17} kgClO$_2$·m·m$^{-2}$·s$^{-1}$·Pa$^{-1}$, respectively (Ozen, 2000).
However, the diffusion and solubility coefficients could not be determined due to the reasons discussed previously.

The objectives of this study were to assess the mass transfer of ClO₂ through various polymeric packaging materials by determining their permeability, diffusion and solubility coefficients (P, D, and S, respectively), using the developed continuous detection system described in Chapter 3.

4.2. Materials and Methods

4.2.1. Polymeric packaging materials

The polymeric materials selected for this study were low-density polyethylene (LDPE) and linear low-density polyethylene (LLDPE) (Flexopack S.A., Attiki, Greece), high-density polyethylene (HDPE) (James River Corp. Flexible Packaging Group, Richmond, VA), biaxially-oriented poly(propylene) (BOPP) (Cryovac, Duncan, SC), polystyrene (PS) (TRYCITE™ 8001, Dow Chemical Company, Midland, MI), poly(ethylene terephthalate) (PET) (Mylar® A, DuPont, Wilmington, DE), poly(vinyl chloride) (PVC) (BEMIS, Shirley, MA), nylon 66 (Dartek F-101, DuPont, Wilmington, DE), poly(lactic acid) (PLA) (EVLON®, BI-AX International Inc., Wingham, Ontario, Canada), and a multilayer structure of ethylene vinyl acetate (EVA) and ethylene vinyl alcohol (EVOH) (EVA/EVOH/EVA; Cryovac, Duncan, SC). These materials are normally used in packaging systems for perishable food products and various non-perishable and pharmaceutical goods. Samples of each film were taken from the same lot
and were conditioned at 23°C and 50% RH for at least 24 hours before starting any experiment.

4.2.2. Polymer characterization

4.2.2.1. Determination of the film’s density

The density, \( \rho \), in kg/m\(^3\) of the polymer film samples was measured using the flotation method at 23°C. A mixture of methanol and water, or a solution of calcium nitrate (Ca(NO\(_3\))\(_2\)), was used as the flotation media for the films with \( \rho < 1 \) or \( \rho > 1 \), respectively (Kaczur and Cawlfield, 1992; Selby et al., 2005).

4.2.2.2. Determination of physical properties

The glass transition temperature, \( T_g (°C) \), and enthalpy of fusion, \( \Delta H_m (kJ/kg) \), of the polymeric films were determined using a Q-100 differential scanning calorimeter (DSC) (TA Instruments, New Castle, DE) according to the ASTM D3418-03 method (ASTM, 2003). At least \( 5 \times 10^{-6} \) kg of sample was used in each run, which consisted of two cycles (heat/cool/heat) with heating and cooling rates of 10°C/min. The experiment was repeated five times for each type of material. The \( \Delta H_m \) and \( T_g \) data were collected from the first and second heating, respectively, and data analyses were done using Universal Analysis Software (UAS Version 3.9A, TA Instruments, New Castle, DE). The crystallinity of the film samples was calculated by:
\[
\% \text{ Crystallinity} = \frac{\Delta H_m}{\Delta H_m^0} \times 100 \tag{1}
\]

where \( \Delta H_m \) is the heat of fusion of the sample obtained by DSC, and \( \Delta H_m^0 \) is the heat of melting of 100% crystalline material. Values used were 288 kJ/kg (PE), 79 kJ/kg (BOPP), 138 kJ/kg (PET), 93.6 kJ/kg (PLA), 301 kJ/kg (nylon), 293 kJ/kg (EVA), and 163 kJ/kg (EVOH) (Auras et al., 2003; Rodriguez et al., 2003).

### 4.2.2.3. Determination of free volume

The free volume (FV) of the semi-crystalline polymer, at a particular temperature, can be calculated from its fractional free volume (FFV) (Equation 2) and corrected as shown below:

\[
\text{FFV} = \frac{V-V_0}{V} = \frac{1}{\rho} - 1.3V_w \tag{2}
\]

\[
\text{FV} = \left(1 - \frac{\% \text{Crystallinity}}{100}\right) \times \text{FFV} \tag{3}
\]

where \( V \) is the specific volume (m\(^3\)/kg) of the amorphous polymer at the testing temperature (23\(^\circ\)C), and \( V_0 \) is a specific volume (m\(^3\)/kg) at 0 K. The FFV can also be estimated from a polymer’s density, \( \rho \), and its van der Waals volume, \( V_w \) (m\(^3\)/mol), as shown in Equation 2. \( V_w \) values correspond to the space occupied by the polymer molecule that is a barrier for the permeant (Recio et al., 2008; Van Krevelen, 1997).
4.2.2.4. Determination of cohesive energy density

The cohesive energy density (CED) can be estimated based on cohesive energy \( E_{coh} \) and molar volume \( V_m \) data as follows:

\[
CED = \frac{E_{coh}}{V_m}
\]  

(4)

where the \( E_{coh} \) (kJ/mol) and \( V_m \) (m\(^3\)/mol) were estimated from the means obtained by group contribution method (Van Krevelen, 1997).

4.2.3. Preparation of ClO\(_2\) solution

The same methodology as described in chapter 3 was used to prepare a final solution from a stock solution of 1,000 mgClO\(_2\)/L.

To assess of ClO\(_2\) stability in the permeability cell, the solution was placed in the lower chamber, with aluminum foil installed, instead of a film sample. Amounts of ClO\(_2\) (mg) in the solution were monitored, after which the ClO\(_2\) concentration (ppmV) was calculated in the gas phase, above the solution, which is assumed to be in equilibrium with the ClO\(_2\) solution. The experiments were repeated 4 times. The degradation profiles of ClO\(_2\) in the gas phase, above ClO\(_2\) solution, at 23, 30, and 40\(^\circ\)C are reported in Figure 4-1 and Table 4-1.
4.2.4. Determination of ClO₂ mass transfer parameters

The permeation experiment for each material was repeated four times. The amount of ClO₂ gas permeated per second was calculated as follows:

Permeated ClO₂ measured at time t (kg/s)

\[
= \frac{\text{permeated ClO}_2 (\mu L/L) \times 67.5 \text{ g/mol} \times N_2 \text{ gas flow rate (L/s)}}{10^6 \mu L/L \times 1 \text{ mol of ideal gas at particular temperature (L/mol)} \times 10^3 \text{ g/kg}}
\]

where 67.5 g/mol is the molecular weight of ClO₂. The N₂ gas flow rate is equal to 4.81 × 10⁻³ L/s and represents the volume of N₂ gas that flows through the upper chamber of the permeability cell in one second and carries the permeated ClO₂ gas to the detector. The volumes of 1 mol of ideal gas at 23, 30 and 40°C are 24.3, 24.9, and 25.7 L/mol, respectively.
Figure 4-1. Calculated ClO₂ concentrations in the gas phase above ClO₂ solution (ppmV) at 23, 30, and 40°C without the presence of film sample.
<table>
<thead>
<tr>
<th>Time (Hour)</th>
<th>23°C</th>
<th>30°C</th>
<th>40°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>nd(^1)</td>
<td>-(^3)</td>
<td>-(^3,4)</td>
</tr>
<tr>
<td>8</td>
<td>-(^3)</td>
<td>-</td>
<td>1.54 ± 1.32(^a)</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>-(^4)</td>
<td>2.01 ± 1.12(^{a,b})</td>
</tr>
<tr>
<td>16</td>
<td>-</td>
<td>2.75 ± 0.73(^a)</td>
<td>5.32 ± 1.22(^b)</td>
</tr>
<tr>
<td>20</td>
<td>-</td>
<td>3.34 ± 1.15(^{a,b})</td>
<td>nd</td>
</tr>
<tr>
<td>24</td>
<td>2.49 ± 1.34(^{a,4})</td>
<td>4.83 ± 0.42(^b)</td>
<td>nd</td>
</tr>
<tr>
<td>28</td>
<td>5.35 ± 1.31(^b)</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

1. Not determined.
2. Within columns, means sharing the same superscript letter are not significantly different (\(p > 0.05\); \(n = 4\)).
3. Time for the last polymer to reach steady state.
4. Time to terminate the experiment for the last polymer.

Table 4-1. Degradation profile of calculated ClO₂ in the gas phase above ClO₂ solution (%) at 23, 30, and 40°C
Following an isostatic method, the quantity of permeated ClO$_2$ gas (kg) at time t (s) was then plotted to obtain the permeation curve. The P value was calculated from the flow rate of the permeated ClO$_2$ gas at steady-state, $F_{ss}$ (kg/s). In this study, the steady-state was reached when the concentration of the permeated ClO$_2$ detected did not increase over time and did not fluctuate more than ± 0.01 ppmV for the remainder of the experiment. The experiment was successfully terminated when steady-state was reached and held for four-fold the lag time of the particular material.

The diffusion coefficient, D, was obtained by the following calculations:

$$P = F_{ss} \times \frac{\ell}{A \cdot \Delta p}$$  \hspace{1cm} (6)

$$\Delta p = p_2 - p_1$$  \hspace{1cm} (7)

$$D = \frac{\ell^2}{7.2r_{1/2}}$$  \hspace{1cm} (8)

where $\ell$ is the film thickness (m), A is the surface area of the film sample (m$^2$), and $\Delta p$ is the partial pressure gradient of ClO$_2$ between the lower half ($p_2$) and upper half ($p_1$) of the permeability cell. Since there is a constant removal of permeated ClO$_2$ from the upper chamber, $p_1$ is equal to 0.00 Pa. The partial pressure of gaseous ClO$_2$ over the solution placed in the lower chamber was calculated from Henry’s solubility coefficient (obtained from the literature), as mentioned previously in Chapter 2, and is equal to $3.65 \times 10^2$ Pa.
(Ishi, 1958; Kaczur and Cawlfield, 1992). The $t_{1/2}$ is the time taken to reach a flow rate equivalent to half of $F_{ss}$.

4.2.5. **Determination of activation energy for permeation**

The temperature-dependence of the mass transfer phenomenon commonly follows an Arrhenius relationship. The activation energy of permeation ($E_p$) can be calculated by:

$$P = P_0 e^{-\frac{E_p}{RT}}$$

(9)

where $P_0$ is the proportionality constant (pre-exponential term), $R$ is the gas constant $\left(8.314472 \times 10^{-3} \text{kJ} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}\right)$, and $T$ is temperature in Kelvin (K).

The $E_p$ (kJ/mol) of ClO$_2$ for PET and PLA were determined by running permeation tests at 23, 30, and 40$^\circ$C and plotting the $P$ data obtained on a graph of $\ln P$ versus $T^{-1}$. $E_p$ values were calculated from the slope which is equal to $-\frac{E_p}{R}$ (Auras et al., 2003; Matteucci et al., 2006; Selke et al., 2004).

4.2.6. **Statistical analysis**

The data obtained from four replicates of each sample were statistically analyzed by analysis of variance (ANOVA) using the Statistical Analysis System (SAS) software (SAS Institute Inc., Cary, NC) at the confidence level of 95% ($\alpha = 0.05$) with Tukey’s adjustment for comparison of means.
4.3. Results and Discussion

4.3.1. Mass transfer of ClO₂ in polymeric films

The mass transfer profiles of 10 mgClO₂/L of gas (3600 ppmV) through various polymeric materials are shown in Figure 4-2. These permeation profiles represent the cumulative permeated gaseous ClO₂ (kg) of each material versus time (s) (Rubino et al., 2001). Consistency tests were performed on obtained data and all values were reported in Appendix 3. The experiments assumed to follow the isostatic method (Gavara and Hernandez, 1993). The corresponding P, D, and S values of the materials as determined at 23°C are listed in Table 4-2.

The P value of gaseous ClO₂ for LLDPE was $96.8 \times 10^{-17}$ kgClO₂·m·m⁻²·s⁻¹·Pa⁻¹, and is comparable to the value previously reported by Ozen (Ozen, 2000). Permeability coefficients of BOPP and nylon determined in this study are also comparable to those of OPP and BON reported. Since the response of the particular EC detector (ToxiPro®) is typically within 90 seconds (Emond and Kearney, 2007), the lag time of the mass transfer phenomenon could be recorded, thus the D and S values were determined in this work. For an isostatic technique, such an immediate response of the detector also leads to the more precise identification of the time to reach steady state flow rate, $F_{ss}$.

Permeabilities of gaseous ClO₂ through PET, nylon, BOPP, PLA and multilayer EVA/EVOH/EVA were at least one order of magnitude lower than those through PE, PVC, and PS, indicating that the former materials are better barriers for ClO₂ gas.
Figure 4-2. Mass transfer of 10 mgClO$_2$/L ClO$_2$ gas (3600 ppmV) through polymeric material. Adapted from (Netramai et al., 2009).
Table 4-2. Permeability (P), diffusion (D), and solubility (S) coefficients of 10 mgClO₂/L (3600 ppmV) for selected polymers at 23°C

<table>
<thead>
<tr>
<th>Polymer</th>
<th>P × 10⁻¹⁷ (\frac{KgClO₂ \cdot m}{m² \cdot s \cdot Pa})</th>
<th>D × 10⁻¹⁴ (\frac{m²}{s})</th>
<th>S × 10⁻³ (\frac{Kg}{m³ \cdot Pa})</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDPE</td>
<td>24.1 ± 0.42ᵃ,ᵇ</td>
<td>3.14 ± 0.06ᵃ</td>
<td>7.68 ± 0.27ᵃ</td>
</tr>
<tr>
<td>LDPE</td>
<td>66.0 ± 1.09ᵇ</td>
<td>26.2 ± 5.11ᵇ</td>
<td>2.59 ± 0.46ᵇ,ᵈ</td>
</tr>
<tr>
<td>LLDPE</td>
<td>96.8 ± 1.34ᶜ</td>
<td>40.4 ± 2.40ᶜ</td>
<td>2.40 ± 0.17ᵇ</td>
</tr>
<tr>
<td>BOPP</td>
<td>3.04 ± 0.16ᵈ</td>
<td>0.39 ± 0.02ᵈ</td>
<td>7.84 ± 0.17ᵃ</td>
</tr>
<tr>
<td>PS</td>
<td>41.8 ± 0.82ᵉ</td>
<td>9.15 ± 0.47ᵉ</td>
<td>4.57 ± 0.02ᶜ</td>
</tr>
<tr>
<td>PVC</td>
<td>23.5 ± 0.46ᵃ</td>
<td>7.65 ± 0.96ᵉ</td>
<td>3.11 ± 0.38ᵈ</td>
</tr>
<tr>
<td>PET</td>
<td>1.26 ± 0.03ᶠ</td>
<td>0.32 ± 0.02ᵈ</td>
<td>3.92 ± 0.19ᵉ</td>
</tr>
<tr>
<td>PLA</td>
<td>5.40 ± 0.13ᵍ</td>
<td>2.86 ± 0.18ᵃ</td>
<td>1.90 ± 0.15ᶠ</td>
</tr>
<tr>
<td>Nylon</td>
<td>1.80 ± 0.07ʰ</td>
<td>0.53 ± 0.02ᶠ</td>
<td>3.43 ± 0.18ᵈ</td>
</tr>
<tr>
<td>EVA/EVOH/EVA</td>
<td>Permeability is less than 0.07 (\frac{KgClO₂ \cdot m}{m² \cdot s \cdot Pa}) (24 hour of exposure)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Within columns, means (± S.D.) sharing the same superscript letter are not significantly different \((p > 0.05; n = 4)\). Adapted from (Netramai et al., 2009).
No permeability (at detection limit of $7.32 \times 10^{-19} \text{ kgClO}_2 \cdot \text{m}^2 \cdot \text{s}^{-1} \cdot \text{Pa}^{-1}$) was observed in EVA/EVOH/EVA multilayer film samples exposed to ClO$_2$ for 24 h. Among all the selected materials under consideration, this material seemed to have the highest barrier against gaseous ClO$_2$; the strong intermolecular forces from polar groups and hydrogen bonds may lower the chain mobility of the polymer and limit the available free volume as the molecules are held close together (Matteucci et al., 2006).

The diffusion mechanism plays an important role in the mass transfer of ClO$_2$ in PE films (Table 4-2). While the S values of PE films are in the same range, the D values of LDPE and LLDPE are approximately 8 and 13 times higher, respectively, than that of HDPE. As diffusion coefficients are particularly affected by the polymer’s $T_g$ and free volume properties, (Matteucci et al., 2006) the differences in mass transfer profiles of ClO$_2$ through different types of PE were probably due mainly to the differences in their free volumes, i.e., the unoccupied spaces between molecules in the polymers’ structures (Selke et al., 2004).

The obtained FFV, FV, CED, crystallinity, and $T_g$ of each material are listed in Table 4-3. The estimated FV of PE films have the following relationship: LLDPE > LDPE > HDPE. In HDPE, the polymer chains are closely packed, resulting in a denser structure and, consequently, less free volume and less active area for permeation, as compared to those of LDPE and LLDPE, which are branched and partial-branched structures (Rodriguez et al., 2003; Selke et al., 2004). For the selected PE samples, ClO$_2$ permeability of the films increased, as their estimated FV increased.
Materials considered to be a good barrier usually have low FV and high CED (small D and S) (Alentiev and Yampolskii, 2002). Even though the material’s free volume is regarded as an independent property, it is implied that the free volume will be more abundant in material with weak intermolecular interactions, i.e., low CED (Alentiev and Yampolskii, 2002). Numbers of researches also reported positive empirical correlations between the FV/CED ratio and mass transfer properties of materials for many permeant/polymer pairs (Alentiev and Yampolskii, 2002; Li et al., 1996; Lopez-Rubio et al.). In this work, the plot of the FV/CED ratio against D and P values of ClO₂ for various polymeric materials at 23°C (Figure 4-3 and Figure 4-4, respectively) showed a positive correlation between FV/CED and mass transfer coefficients.

FV and CED were then separately plotted against D values to further pinpoint the major factor affecting mass transfer of ClO₂ gas, in Figure 4-5 and Figure 4-6, respectively. Positive correlation between FV and D values was observed, while there was no significant correlation between CED and D values. Also, from the plots in Figure 4-5, Figure 4-3, and Figure 4-4), a polymer tested in the glassy stage with a high barrier to ClO₂ has a low FV/CED ratio resulting from low FV and/or high CED values.

Thus, the glassy or rubbery stage (T₉) of a material and the FV has an important effect on the permeability of ClO₂.

Even though the FV of the PE films were less than those of most of the remaining materials, the D values of PE films were very high, resulting in high P values. The PE films were tested under rubbery stages (the testing temperature is above the T₉), which
enables the polymer’s chain mobility and facilitates diffusion of the permeant (Van Krevelen, 1997).

BOPP was also tested above its $T_g$, but its low $P$ value could be due to the decreased mobility of the oriented material (Dias et al., 2008) and the highly crystalline nature of isotactic PP, which reduces the availability of free volume in its structure (Selke et al., 2004). The tightly packed polymer chains create a tortuous path for the permeant to move through, thus limiting the diffusion of ClO$_2$ gas and resulting in its low permeability.

PS had a high permeability value due to high $D$ and fairly high $S$ values. This result, similar to the case of PE films, was due to the abundant free volume created by the bulkiness of the benzene ring side group and the chain stiffness of the PS molecule (Matteucci et al., 2006). Also, the PS commonly used for packaging purposes is atactic, having a noncrystalline structure (Selke et al., 2004).

For PVC, its high $D$ value resulted in a rather high permeability of ClO$_2$. Since most PVC available for packaging purposes is usually highly plasticized to improve processability (Selke et al., 2004), its poor barrier to ClO$_2$ could be due to the presence of plasticizers that act as lubricants, reducing the intermolecular forces between polymer molecules, increasing the chain mobility, and facilitating the movement of ClO$_2$ molecules through the polymer’s matrix (Matteucci et al., 2006).

The high CED and low FV values that lead to the low $S$ and $D$ values likely accounted for the low permeability of ClO$_2$ in nylon and PET. The incompatibility of
both materials to ClO$_2$ was possibly due to their high intermolecular forces, generating a chemically rigid compact structure to ClO$_2$. For PLA film, the lower CED value and higher FV make the polymer a much poorer barrier to ClO$_2$ as compared to PET, even though the barrier properties to gases and vapors of the two polymers are normally comparable (Auras et al., 2003; Matteucci et al., 2006).

### 4.3.2. Activation energy of ClO$_2$

The $E_p$ data of ClO$_2$ for PET and PLA films obtained from plotting $P$ data obtained at various temperatures ($\ln P$ versus $T^{-1}$) are shown in Table 4-4. The $E_p$ value for PET is significantly lower than that for PLA indicating that the permeation of ClO$_2$ through PLA is less temperature dependent, which could be considered beneficial in a packaging system that is occasionally subjected to temperature abuse during transportation or distribution.
Table 4-3. Polymer film characteristics including density ($\rho$), fractional free volume (FFV), free volume (FV), and cohesive energy density (CED)

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Thickness $\times 10^{-5}$ (m)</th>
<th>$\rho \times 10^3$ (kg/m$^3$)</th>
<th>Crystallinity (%)</th>
<th>$T_g$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDPE</td>
<td>5.59</td>
<td>0.931</td>
<td>38.63 ± 3.11</td>
<td>nd$^1$</td>
</tr>
<tr>
<td>LDPE</td>
<td>2.79</td>
<td>0.924</td>
<td>23.04 ± 3.31</td>
<td>nd</td>
</tr>
<tr>
<td>LLDPE</td>
<td>4.57</td>
<td>0.917</td>
<td>24.13 ± 1.52</td>
<td>nd</td>
</tr>
<tr>
<td>BOPP</td>
<td>1.78</td>
<td>0.917</td>
<td>33.08 ± 2.01</td>
<td>3.00 ± 1.36</td>
</tr>
<tr>
<td>PS</td>
<td>2.54</td>
<td>1.03</td>
<td>na$^4$</td>
<td>92.65 ± 0.20</td>
</tr>
<tr>
<td>PVC</td>
<td>2.79</td>
<td>1.30</td>
<td>na</td>
<td>57.96 ± 0.49</td>
</tr>
<tr>
<td>PET</td>
<td>1.27</td>
<td>1.39</td>
<td>22.38 ± 1.36</td>
<td>81.66 ± 0.50</td>
</tr>
<tr>
<td>PLA</td>
<td>3.81</td>
<td>1.21</td>
<td>31.44 ± 0.55</td>
<td>69.07 ± 0.27</td>
</tr>
<tr>
<td>Nylon</td>
<td>2.03</td>
<td>1.13</td>
<td>24.35 ± 0.46</td>
<td>nd</td>
</tr>
<tr>
<td>EVA/EVOH/EVA:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EVA</td>
<td>1.78</td>
<td>na</td>
<td>17.25 ± 3.66</td>
<td>nd</td>
</tr>
<tr>
<td>EVOH</td>
<td>1.78</td>
<td>na</td>
<td>2.25 ± 0.20</td>
<td></td>
</tr>
</tbody>
</table>

$^1$ FV and FFV calculations were based on data at 23°C.

$^2$ CED values were calculated according to the group contribution method at 25°C (Van Krevelen, 1997).

$^3$ Not determined due to equipment limitations.

$^4$ Not available due absence of a particular attribute, or test not performed.
Table 4-3. (cont’d)

<table>
<thead>
<tr>
<th>Polymer</th>
<th>FFV $\times 10^{-2}$</th>
<th>FV $\times 10^{-2}$</th>
<th>CED $\times 10^5$</th>
<th>FV/CED $\times 10^{-7}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDPE</td>
<td>11.7</td>
<td>7.21</td>
<td>3.00</td>
<td>2.40</td>
</tr>
<tr>
<td>LDPE</td>
<td>12.5</td>
<td>9.58</td>
<td>3.00</td>
<td>3.19</td>
</tr>
<tr>
<td>LLDPE</td>
<td>13.1</td>
<td>9.93</td>
<td>3.00</td>
<td>3.31</td>
</tr>
<tr>
<td>BOPP</td>
<td>13.1</td>
<td>8.78</td>
<td>2.66</td>
<td>3.29</td>
</tr>
<tr>
<td>PS</td>
<td>19.3</td>
<td>19.3</td>
<td>4.11</td>
<td>4.69</td>
</tr>
<tr>
<td>PVC</td>
<td>21.0</td>
<td>21.0</td>
<td>4.41</td>
<td>4.76</td>
</tr>
<tr>
<td>PET</td>
<td>11.2</td>
<td>8.67</td>
<td>5.43</td>
<td>1.60</td>
</tr>
<tr>
<td>PLA</td>
<td>21.9</td>
<td>15.0</td>
<td>5.18</td>
<td>2.89</td>
</tr>
<tr>
<td>Nylon</td>
<td>15.5</td>
<td>11.7</td>
<td>6.47</td>
<td>1.81</td>
</tr>
<tr>
<td><strong>EVA/EVOH/EVA:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EVA</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>EVOH</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
</tbody>
</table>

1. FV and FFV calculations were based on data at 23°C.
2. CED values were calculated according to the group contribution method at 25°C (Van Krevelen, 1997).
3. Not determined due to equipment limitations.
4. Not available due absence of a particular attribute, or test not performed.
Figure 4-3. Correlation between the free volume and cohesive energy density ratio (FV/CED) and the diffusion coefficient (D) of ClO$_2$ gas
Figure 4-4. Correlation between the free volume and cohesive energy density ratio (FV/CED) and the permeability coefficient (P) of ClO$_2$ gas
**Figure 4-5.** Correlation between the free volume (FV) and the diffusion coefficient (D) of ClO₂ gas
Figure 4-6. Correlation between the free volume (FV) and the diffusion coefficient (D) of ClO₂ gas.
Table 4-4. Permeability coefficients (P) at various temperatures and activation energy for permeation (EP) of ClO₂ for PET and PLA films

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>PET</th>
<th>PLA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P × 10⁻¹⁷</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(\left(\frac{\text{KgClO}_2 \cdot \text{m}}{\text{m}^2 \cdot \text{s} \cdot \text{Pa}}\right))</td>
<td>(\left(\frac{\text{KgClO}_2 \cdot \text{m}}{\text{m}^2 \cdot \text{s} \cdot \text{Pa}}\right))</td>
</tr>
<tr>
<td>23</td>
<td>1.26 ± 0.00ᵃ,¹</td>
<td>5.40 ± 0.13ᵃ</td>
</tr>
<tr>
<td>30</td>
<td>2.67 ± 0.04ᵇ</td>
<td>94.4 ± 0.52ᶜ</td>
</tr>
<tr>
<td>40</td>
<td>3.98 ± 0.05ᶜ</td>
<td>94.4 ± 0.52ᶜ</td>
</tr>
</tbody>
</table>

¹ Within columns, means (± S.D.) sharing the same superscript letter are not significantly different \((p > 0.05; n = 4)\). Adapted from (Netramai et al., 2009).
4.4. Conclusion

A continuous system for measuring mass transfer of ClO₂ through different polymeric materials was developed utilizing an electrochemical detector (details in Chapter 3). The permeability, diffusion, and solubility coefficients (P, D and S, respectively) of 10 mg ClO₂/L of gas (3600 ppmV) for 10 types of selected polymeric materials were determined by an isostatic method. The results ranged from a permeability value below $0.07 \times 10^{-17}$ kgClO₂·m·m⁻²·s⁻¹·Pa⁻¹ for multilayer EVA/EVOH/EVA to $96.8 \times 10^{-17}$ kgClO₂·m·m⁻²·s⁻¹·Pa⁻¹ for LLDPE. BOPP, PET, PLA, nylon, and multilayer EVA/EVOH/EVA are good barriers for gaseous ClO₂ as compared to PEs, PVC, and PS, which have higher permeability coefficients than the former group. Calculated cohesive energy density and free volume values can play an important role in predicting the mass transfer behavior of ClO₂ through the tested film samples. The activation energy of permeation, in the temperature range of 23 to 40°C, for ClO₂ gas for PET and PLA were found to be 51.05 and 129.03 kJ/mol, respectively.


CHAPTER 5: EFFECTS OF GASEOUS ClO₂ EXPOSURE ON IMPORTANT PROPERTIES OF POLYMERIC FILMS

5.1. Introduction

New packaging strategies, such as including antimicrobial gas in the headspace have become a potential complementary approach to improve the safety of packaged fresh produce (Sy et al., 2005). Chlorine dioxide (ClO₂), with its high oxidizing capacity and broad disinfecting property, is considered by many researchers as a promising choice for such applications (Kaczur and Cawlfieid, 1992; Kim et al., 1999; Sapers et al., 2003; Sy et al., 2005), however, when exposing polymeric materials to oxidizing agents, chemical changes can occur. These changes can impact the integrity and performance of the packaging material, consequently, resulting in a reduction of the product shelf-life.

Thus, the specific objectives of this work were to determine and evaluate the effects of exposure of gaseous ClO₂ on a) chemical, b) physical, c) mechanical, and d) barrier properties of different polymeric materials.
5.2. Materials and Methods

5.2.1. Experimental design

Film samples were randomly placed in glass jars (2 films, with random polymer type, per jar) and exposed to ClO$_2$ gas generated by a ClO$_2$ solution in the jar bottom, which released 10 mgClO$_2$/L (approximately, 3,600 ppmV) of ClO$_2$ gas into the headspace. Film samples were periodically removed from the ClO$_2$ treatment in order to characterize their chemical, physical, mechanical, and barrier properties as outlined in Figure 5-1.

The samples were conditioned at 23$^\circ$C, 50% RH for at least 24 hours, before ClO$_2$ exposure. The films were then exposed to gaseous ClO$_2$ for 1, 7, and 14 days (‘Day 1’, ‘Day 7’, and ‘Day 14’ samples, respectively). After removing the sample at the specific time, the treated films were conditioned by storing at 23$^\circ$C, 50% RH for at least 24 hours. The unexposed films or ‘control’ (Day 0) were also similarly conditioned.

The chemical, physical and mechanical properties of the conditioned control (Day 0) and treated (Day 1, Day 7, and Day 14) polymeric materials were then evaluated.
Figure 5-1. Flow diagram of ClO$_2$ treatment of polymeric materials. Adapted from (Netramai et al., 2010).
5.2.2. Polymeric packaging materials

The same polymeric materials used in a previous study (Netramai et al., 2009) were considered for this study, i.e. LDPE (Flexopack S.A., Attiki, Greece), LLDPE (Flexopack S.A., Attiki, Greece), HDPE (James River Corp. Flexible Packaging Group, Richmond, VA), BOPP (Cryovac, Duncan, SC), PS (TRYCITE™ 8001, Dow Chemical Company, Midland, MI), PET (Mylar® A, DuPont, Wilmington, DE), PVC (BEMIS, Shirley, MA), nylon (Dartek F-101, DuPont, Wilmington, DE), PLA (EVLO®N, BI-AX International Inc. Wingham, ON, Canada), and multilayer structure EVA/EVOH/EVA (Cryovac, Duncan, SC).

5.2.3. Preparation of ClO₂ solution

The same methodology as described in chapter 3 was used to prepare a final solution from a stock solution of 1,000 mg/L ClO₂.

To monitor ClO₂ concentration over time, ClO₂ solution in 8 random glass jars containing film samples were periodically sampling (another set of 8 glass jars for control). Figure 5-2 shows mg of ClO₂ in the solution after 1, 7, and 14 days of film exposure. The differences between ClO₂ remaining in control glass jars and those in jars with samples (Table 5-1) were assumed to be the amount of ClO₂ that reacted with film samples.
Figure 5-2. Remaining amount of ClO$_2$ (mg) in 200 mL solution for blank and film treatment

Table 5-1. Amount of ClO$_2$ consumed by film samples (mg)

<table>
<thead>
<tr>
<th>Time of Exposure (Day)</th>
<th>Amount of ClO$_2$ consumed by film samples (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.56 ± 0.26</td>
</tr>
<tr>
<td>7</td>
<td>4.50 ± 0.30</td>
</tr>
<tr>
<td>14</td>
<td>8.03 ± 0.07</td>
</tr>
</tbody>
</table>
5.2.4. Evaluation of chemical structure of polymeric material

Fourier transform infrared (FT-IR) spectroscopy was used to evaluate the chemical changes of the polymer after ClO₂ exposure using a FT-IR spectrophotometer (Shimadzu IR Prestige-21, Shimadzu Scientific Instruments, Columbia, MD). In addition to comparing the IR spectrum of the control, exposed, and conditioned films, IR spectra of exposed samples without conditioning were also collected, in order to evaluate if the changes were transient or permanent.

5.2.5. Evaluation of physical properties

Glass transition (T_g) and melting (T_m) temperatures (°C), and enthalpy of fusion, \( \Delta H_m \) (J/g) of the control and exposed polymeric films were determined using a differential scanning calorimeter (DSC Q-100, TA Instruments, New Castle, DE) according to ASTM D3418-03 (ASTM, 2003b). The analyses were done using Universal Analysis Software (UAS Version 3.9A, TA Instruments, New Castle, DE).

5.2.6. Evaluation of mechanical properties

Tensile strength, TS (N⋅m⁻²) and modulus of elasticity, MoE (N⋅m⁻²), in both the machine direction (MD) and transverse direction (TD) of the control and exposed polymeric films, were measured using a universal tensile tester machine (Instron 5565, Instron Inc., Canton, MA), according to ASTM D 882-97 (ASTM, 2002). Each film sample was run by five replicates. The predetermined parameters for measuring TS and MoE of the samples were as follows:
• Initial grip separation of $1.25 \times 10^{-1}$ m with a rate of grip separation of $2.08 \times 10^{-4}$ m/s were used for PS and PLA.

• Initial grip separation of $1.00 \times 10^{-1}$ m with a rate of grip separation of $8.33 \times 10^{-4}$ m/s were used for BOPP, PVC, PET, and multilayer structure of EVA/EVOH/EVA.

• Initial grip separation of $5.00 \times 10^{-2}$ m with a rate of grip separation of $8.33 \times 10^{-3}$ m/s were used for PEs and nylon.

5.2.7. Evaluation of barrier properties

Barrier characteristics of the control and exposed polymeric films were determined in accordance to ASTM D1434-82 (2003) (ASTM, 2003a). Water vapor transmission rates (WVTR) were evaluated using a water vapor permeability analyzer 100, (Permatran-W® Model 3/11, Mocon, Minneapolis, MN). Carbon dioxide permeation rates (CO2TR) were evaluated using a carbon dioxide permeability analyzer (Permatran-C™ Model 4/41, Mocon, Minneapolis, MN). Oxygen transmission rates (O2TR) were measured using an oxygen permeability analyzer (O2TR 8001, Illinois Instruments, Johnsburg, IL). The experimental conditions for each of the measurement were as follows:

• Water vapor transmission rate (kg•m$^{-2}$•s$^{-1}$): relative humidity difference across the film 100%, temperature 23°C.
• Carbon dioxide transmission rate (kg·m⁻²·s⁻¹): permeant 101325 Pa of CO₂, carrier gas N₂, temperature 23°C, and 0% relative humidity.

• Oxygen transmission rate (kg·m⁻²·s⁻¹): permeant 21278.25 Pa of O₂, carrier gas N₂, temperature 23°C, and 0% relative humidity.

The tested film areas were between 7.90 x 10⁻⁵ to 5.00 x 10⁻³ m², depending on the barrier characteristic of each sample. The obtained WVTR, CO₂TR, and O₂TR were then used to calculate $P_{H_2O}$, $P_{CO_2}$, and $P_{O_2}$, respectively, of the film samples by multiplying the thickness of the samples and dividing by the partial pressure gradients. The final values were expressed as Kg·m⁻¹·s⁻¹·Pa⁻¹.

### 5.2.8. Color measurement of polymeric material

Surface color of the films was measured from two random locations per piece of film, four films for each sample type, for L*, a* and b* values using a reflectometer (Integrating Reflectometer JY 9800, TMI Testing Machines INC, Ronkonkoma, NY). The overall color difference ($\Delta E$) was calculated using:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$  \hspace{1cm} (1)

where $\Delta L$, $\Delta a$, and $\Delta b$ are the differences between the L, a, and b values, respectively, of the control sample, and those of the exposed sample.
5.2.9. Statistical analysis

With the exception of mechanical testing (5 replicates), all other tests were based on 4 replicates. The data obtained were statistically analyzed by analysis of variance (ANOVA) using the Statistical Analysis System (SAS) software (SAS Institute Inc., Cary, NC) at a confidence level of 95% (α = 0.05) with Tukey’s adjustment for comparison of means.

5.3. Results and Discussion

To monitor the changes in the materials’ integrities and performance, the film samples were exposed to gaseous ClO₂ at a concentration of 3,600 ppmV, for up to 14 days, which is near the upper range of ClO₂ gas used in treating food produce. The exposure times were selected according to the shelf-life of fresh produce (Shin et al., 2006).

A one day exposure was considered short-term exposure, at which the mass transfer of ClO₂ at this particular concentration is at steady state in all of the testing polymeric materials, with the exception of multilayer EVA/EVOH/EVA (Netramai et al., 2009). The 7 and 14 days exposures were regarded as long-term exposure which is referred to as ‘persistent gas exposure’ (PGE). A PGE could be encountered when a sustained release device is included in the packaging system. According to this scenario, ClO₂ gas could be present at a specific concentration within the package atmosphere up to the end of the product shelf-life. The chemical, physical and mechanical properties were evaluated in function of the short and long-term exposure.
5.3.1. Chemical structure of polymeric material

Changes in the IR spectra were attributed to possible interactions between ClO$_2$ gas and the polymeric film samples (Figure 2-5). In general, the types of changes observed from persistent ClO$_2$ exposure agreed with those for short-term. As the time of exposure increased, the degree of change also increased.

In order to determine if the chemical changes were transient or permanent, IR spectra of the unconditioned exposed samples were also collected. Generally, the changes in the absorbance intensities of most of the short-term exposed samples seemed to be somewhat temporary, as the absorbance of the exposed samples, after conditioning was equivalent to those of the controls. In the long-term exposure, however, most of the changes tended to be permanent, i.e. after conditioning, the absorbance of the exposed samples differed from the control.

The most notable result was that of the nylon (Figure 5-3). In this case, an additional IR spectrum was also obtained at 10 hours of exposure, in which the mass transfer of ClO$_2$ in the nylon film was at steady state (Netramai et al., 2009). Changes in the IR spectrum at exposure time of 10 hours was equivalent to those seen at 1 and 7 days when compared to the control (Day 0) sample.
Figure 5-3. FT-IR spectra of nylon; (---) day 0; (-----) 10 hours; (- - -) day 1; (· · ·) day 7; and (———) day 14. Adapted from (Netramai et al., 2010).
Generally, for film samples affected by ClO\textsubscript{2}, the changes in peak intensity of the 14 days had been the most dramatic, followed by the 7 days and those of the short-term exposure. However, the peak intensities changed at a slower rate during longer exposure. This could be attributed to the availability of functional groups that react with ClO\textsubscript{2}, as oxidative degradation is normally a surface phenomenon (Rivaton and Gardette, 1998; Rodriguez \textit{et al.}, 2003).

The IR spectra for HDPE, LDPE, and LLDPE (Figure 5-4) showed minor changes in the intensity of the peaks in the 2700-3000 cm\textsuperscript{-1} region, which indicates possible changes in the C-H bond in the methyl or methylene group. The IR spectra of PVC, and BOPP materials (not shown) showed only slight changes in their absorbance intensity. The shifts of the peaks in the fingerprint area, i.e. 750-1400 cm\textsuperscript{-1} region, to the higher wavenumbers in the exposed PEs and PS samples, indicate the possible presence of a C-Cl bond in the exposed samples (Robinson \textit{et al.}, 2005).

The increased absorbance intensities in the fingerprint area of the exposed nylon (Figure 5-3) and EVA/EVOH/EVA films (Figure 5-6) also suggest partial chlorination (Robinson \textit{et al.}, 2005) in the exposed samples. If the degree of chlorination is significantly high, this alteration should increase the polymers’ polarity, leading to a film with improved barrier properties to gases and other organic compounds, (Kharitonov, 2000; Selke \textit{et al.}, 2004).
Figure 5-4. FT-IR spectra of LLDPE; (—) day 0; (⋯⋯) day 1; (- - -) day 7; and (- - -) day 14. Adapted from (Netrnamai et al., 2010).
Increased absorbance intensities of the exposed PLA, and nylon (Figure 5-3) samples in the 3300-3700 cm$^{-1}$ region coincided with changes in the hydroxyl group, and N-H bond, respectively. The increase in absorbance intensities in the 1100-1200 cm$^{-1}$ region for the exposed nylon could indicate changes in the C-N bond (Ozen et al., 2002; Robinson et al., 2005).

In the spectra shown in Figure 5-5, slight changes can be seen in the peak intensities in 2800-3100 cm$^{-1}$ region for the exposed PET films that might represent changes in the C-H bond in its methylene group and/or benzene ring. Such changes were not observed in exposed PS samples where the absorbance signals, at that particular region, were at the noise level. The surface oxidations for PET are reported to be complex, and can lead to the formation of many functional groups, e.g. carboxylic acid, terminal vinyl groups, and phenols (Walzak et al., 1995), however, there seemed to be no significant formation of such groups in the ClO$_2$-exposed samples, under these testing conditions.

The spectrum of the exposed multilayer EVA/EVOH/EVA (Figure 5-6) showed an increase in the intensity of the peaks within the 3000-3700 cm$^{-1}$ region, which indicates a change in the hydroxyl group. The minor increase in absorbance of the peak in the 1600-1700 cm$^{-1}$ region could be due to the formation of a carbonyl group.
Figure 5-5. FT-IR spectra of PET; (——) day 0; (- - -) day 1; (---) day 7; and (——) day 14. Adapted from (Netramai et al., 2010).
Figure 5-6. FT-IR spectra of EVA/EVOH/EVA; (—) day 0; (····) day 1; (- - -) day 7; and (- · · ) day 14. Adapted from (Netramai et al., 2010).
5.3.2. **Physical properties**

$T_g$ of PS and PET, and the $T_m$ of nylon and EVOH (Table 5-2), were significantly lower than those of the control samples. An increase in the heat of fusion of exposed nylon was also observed, indicating an increase in crystallinity after exposure. The physical properties of other ClO$_2$-exposed samples remained.

The shift in $T_m$ and the increase in crystallinity of ClO$_2$-exposed nylon could result from an increase in molecular ordering, frequently observed when exposing nylon to strong oxidizing compounds, which, in turn, can increase crystallinity (Ozen et al., 2002). The small decrease in $T_g$ for ClO$_2$-exposed PS and PET, together with changes in the C-H bond in the methyl group observed through IR spectra mentioned previously, suggests that the main chain scission reaction could have taken place, promoting slight degradation of the exposed materials which increased polymer chain mobility (Buchalla et al., 1993a; Ozen et al., 2002; Walzak et al., 1995).

In light of these changes in chemical and physical properties after ClO$_2$-exposure, a change in the material’s performance as a packaging film could reasonably be expected (Buchalla et al., 1993a; b; Ozen et al., 2002). Thus, the barrier, and mechanical properties of the exposed samples were characterized.
Table 5-2. Physical properties of selected polymeric packaging materials exposed to gaseous ClO₂. Adapted from (Netramai et al., 2010).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time of Exposure (day)</th>
<th>Tg (°C)</th>
<th>Tm (°C)</th>
<th>Heat of Fusion (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDPE</td>
<td>0</td>
<td>na</td>
<td>133.55 ± 0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>111.27 ± 8.96&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>na</td>
<td>133.86 ± 0.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>111.10 ± 10.41&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDPE</td>
<td>0</td>
<td>na</td>
<td>112.44 ± 0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>66.35 ± 9.53&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>na</td>
<td>112.31 ± 0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.97 ± 2.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LLDPE</td>
<td>0</td>
<td>na</td>
<td>122.52 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.48 ± 4.37&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>na</td>
<td>122.27 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.85 ± 5.90&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PVC</td>
<td>0</td>
<td>57.96 ± 0.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>58.15 ± 0.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>PS</td>
<td>0</td>
<td>92.65 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>91.64 ± 0.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>PP</td>
<td>0</td>
<td>57.40 ± 2.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>161.51 ± 0.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.14 ± 1.59&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>59.55 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>160.35 ± 0.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.99 ± 3.77&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1 Within columns, means (± S.D.) sharing the same superscript letter are not significantly different (p > 0.05; n = 5).
2 Not available due absence of a particular attribute, or test not performed.
Table 5-2. (cont’d)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time of Exposure (day)</th>
<th>Tg (°C)</th>
<th>Tm (°C)</th>
<th>Heat of Fusion (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>PLA</td>
<td></td>
<td>69.07 ± 0.27(^a)</td>
<td>69.20 ± 1.21(^a)</td>
<td>29.24 ± 0.51(^a)</td>
</tr>
<tr>
<td>PET</td>
<td></td>
<td>81.66 ± 0.50(^a)</td>
<td>80.37 ± 0.22(^b)</td>
<td>31.33 ± 1.91(^a)</td>
</tr>
<tr>
<td>Nylon</td>
<td></td>
<td>na</td>
<td>261.35 ± 0.15(^a)</td>
<td>56.01 ± 1.06(^a)</td>
</tr>
<tr>
<td>EVA/EVOH/EVA:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EVA</td>
<td>0</td>
<td>na</td>
<td>123.74 ± 1.22(^a)</td>
<td>50.55 ± 10.73(^a)</td>
</tr>
<tr>
<td>EVOH</td>
<td>0</td>
<td>na</td>
<td>159.39 ± 0.46(^a)</td>
<td>54.35 ± 11.54(^a)</td>
</tr>
<tr>
<td>EVA</td>
<td>14</td>
<td>na</td>
<td>122.82 ± 0.68(^a)</td>
<td>52.54 ± 7.14(^a)</td>
</tr>
<tr>
<td>EVOH</td>
<td>14</td>
<td>na</td>
<td>147.81 ± 1.14(^b)</td>
<td>56.49 ± 7.68(^a)</td>
</tr>
</tbody>
</table>

1. Within columns, means (± S.D.) sharing the same superscript letter are not significantly different (p > 0.05; n = 5).
2. Not available due absence of a particular attribute, or test not performed.
5.3.3. Mechanical properties

Tensile strength (TS) and/or modulus of elasticity (MoE) of the exposed PE films (Table 5-3) decreased significantly due to oxidative degradation of the polymer chains which was confirmed by the increase in methyl groups as seen in the IR spectra (Figure 5-4) (Ozen et al., 2002). Such degradation can reduce film structure’s rigidity, and consequently alter the tensile characteristics of the oxidized polymer (Selke et al., 2004).

Mechanical performance of the control and other ClO2-exposed films remained statistically unchanged, even though the formations of polar groups were observed in most of the exposed samples. Theoretically, increased polarity should improve the intermolecular forces between the chains, leading to an increase in tensile strength and a decrease in elongation at break, as it limits the mobility of the polymer chain (Selke et al., 2004).
Table 5-3. Tensile properties of selected polymeric packaging materials exposed to gaseous ClO₂. Adapted from (Netramai et al., 2010).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time of Exposure (day)</th>
<th>Tensile strength $\times 10^7$ (N/m$^2$)</th>
<th>Modulus (secant) $\times 10^8$ (N/m$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MD</td>
<td>TD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MD</td>
<td>TD</td>
</tr>
<tr>
<td>HDPE</td>
<td>0</td>
<td>4.15 ± 0.12$^{a,b}$</td>
<td>2.71 ± 0.06$^a$</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>3.45 ± 0.38$^b$</td>
<td>2.24 ± 0.52$^a$</td>
</tr>
<tr>
<td>LDPE</td>
<td>0</td>
<td>3.08 ± 0.12$^a$</td>
<td>1.99 ± 0.03$^a$</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>2.85 ± 0.12$^b$</td>
<td>1.88 ± 0.10$^a$</td>
</tr>
<tr>
<td>PS</td>
<td>0</td>
<td>8.48 ± 0.44$^a$</td>
<td>7.98 ± 0.32$^a$</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>8.65 ± 0.23$^a$</td>
<td>7.56 ± 0.13$^a$</td>
</tr>
<tr>
<td>PET</td>
<td>0</td>
<td>19.2 ± 1.36$^a$</td>
<td>21.5 ± 0.41$^a$</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>18.8 ± 0.95$^a$</td>
<td>21.4 ± 0.68$^a$</td>
</tr>
<tr>
<td>PVC</td>
<td>0</td>
<td>9.13 ± 0.26$^a$</td>
<td>9.87 ± 0.62$^a$</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>9.17 ± 0.38$^a$</td>
<td>9.34 ± 0.55$^a$</td>
</tr>
<tr>
<td>Nylon</td>
<td>0</td>
<td>6.86 ± 0.38$^a$</td>
<td>6.80 ± 0.53$^a$</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>6.42 ± 0.52$^a$</td>
<td>5.83 ± 1.06$^a$</td>
</tr>
<tr>
<td>EVA/EVOH/EVA</td>
<td>0</td>
<td>11.2 ± 0.34$^a$</td>
<td>9.53 ± 0.39$^a$</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>10.0 ± 2.31$^a$</td>
<td>8.59 ± 0.69$^a$</td>
</tr>
</tbody>
</table>

1 Within columns, means (± S.D.) sharing the same superscript letter are not significantly different ($p > 0.05$; n = 5).
5.3.4. Barrier properties

One potential application of ClO₂ gas is to be used in combination with other gases, such as O₂ and CO₂ in a modified atmosphere packaging (MAP) system, so the impact of ClO₂ gas on the barrier properties to O₂, CO₂ and moisture of different polymeric materials is of concern. Barrier properties of these polymeric materials are one of the most important performance concerns in food packaging systems, especially for fresh produce that is respiring after harvest (Martinez-Romero et al., 2003). The concentrations of O₂ and CO₂ that accumulate in the package headspace affect the deterioration rate of fresh produce. Once the material is selected for a particular commodity, it is crucial that its permselectivity (P_CO₂/P_O₂) ratio be maintained through the shelf-life of the product, as in the case of MAP where a stable gaseous ratio in the package headspace is necessary (Exama et al., 1993; Martinez-Romero et al., 2003; Selke et al., 2004).

By exposing polymeric materials to reactive chemical compounds such as ClO₂, their barrier properties might be altered (Ozen, 2000). The comparisons of P_H₂O, P_CO₂, and P_O₂, between the control and the exposed samples, indicated significant changes in barrier characteristics of some polymeric materials commonly used in packaging fresh produce.
5.3.4.1. Barrier to moisture

After ClO₂ exposure, the moisture barrier of PVC and PET significantly decreased, while \( P_{H_2O} \) of the other materials remained statistically unchanged (Table 5-4).

It is important to note the shift to lower \( T_g \) in the exposed PET samples (Table 5-2), which implies oxidative degradation of the material after ClO₂ exposure, could increase polymer chain mobility and decrease intermolecular forces. This plays an important role in the significant increase in \( P_{H_2O} \) of the material, since it will accelerate diffusion process (Kharitonov, 2000).

The IR spectra for ClO₂-exposed PLA sample also showed an increase in hydroxyl groups, possibly indicating an increase in film polarity, which could increase intermolecular forces between the polymeric chains, and promote rigidity in the structure. However, the hydroxyl group also will favor the interaction between polymer and moisture (Ozen et al., 2002; Walzak et al., 1995). Thus, the barrier properties of PLA may remain unchanged after exposure as a result of these antagonistic interactions.
Table 5-4. Barrier properties of selected polymeric packaging materials exposed to gaseous ClO₂. Adapted from (Netramai et al., 2010).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time of Exposure (day)</th>
<th>$P_{H_2O} \times 10^{-18}$ ($Kg \cdot m \div m^2 \cdot s \cdot Pa$)</th>
<th>$P_{CO_2} \times 10^{-18}$ ($Kg \cdot m \div m^2 \cdot s \cdot Pa$)</th>
<th>$P_{O_2} \times 10^{-18}$ ($Kg \cdot m \div m^2 \cdot s \cdot Pa$)</th>
<th>$P_{CO_2} / P_{O_2}$</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDPE</td>
<td>0</td>
<td>$0.49 \pm 0.00^{a,1}$</td>
<td>$58.3 \pm 8.08^a$</td>
<td>$9.26 \pm 1.24^a$</td>
<td>$6.30 \pm 0.32^a$</td>
<td>+ 46.8</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>$0.48 \pm 0.00^b$</td>
<td>$77.6 \pm 0.40^b$</td>
<td>$8.40 \pm 0.29^a$</td>
<td>$9.25 \pm 0.28^b$</td>
<td></td>
</tr>
<tr>
<td>PS</td>
<td>0</td>
<td>$10.5 \pm 0.74^a$</td>
<td>$111 \pm 6.63^a$</td>
<td>$16.8 \pm 0.57^a$</td>
<td>$6.58 \pm 0.17^a$</td>
<td>+ 18.0</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>$9.84 \pm 1.06^a$</td>
<td>$123 \pm 6.37^b$</td>
<td>$15.8 \pm 0.64^a$</td>
<td>$7.77 \pm 0.19^b$</td>
<td></td>
</tr>
<tr>
<td>BOPP</td>
<td>0</td>
<td>$0.24 \pm 0.00^a$</td>
<td>$5.30 \pm 0.27^a$</td>
<td>$3.86 \pm 0.20^a$</td>
<td>$4.75 \pm 0.15^a$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>$0.25 \pm 0.01^a$</td>
<td>$5.38 \pm 0.25^a$</td>
<td>$3.91 \pm 0.18^a$</td>
<td>$4.91 \pm 0.25^a$</td>
<td>-</td>
</tr>
<tr>
<td>PLA</td>
<td>0</td>
<td>$28.2 \pm 1.18^a$</td>
<td>$37.3 \pm 17.2^a$</td>
<td>$21.2 \pm 3.25^a$</td>
<td>$1.73 \pm 0.64^a$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>$26.9 \pm 1.56^a$</td>
<td>$43.4 \pm 13.9^a$</td>
<td>$30.8 \pm 12.3^a$</td>
<td>$1.41 \pm 0.05^a$</td>
<td>-</td>
</tr>
<tr>
<td>PET</td>
<td>0</td>
<td>$1.64 \pm 0.01^a$</td>
<td>$1.54 \pm 0.04^a$</td>
<td>$0.22 \pm 0.01^a$</td>
<td>$6.88 \pm 0.13^a$</td>
<td>- 14.9</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>$1.94 \pm 0.03^b$</td>
<td>$1.53 \pm 0.04^a$</td>
<td>$0.26 \pm 0.04^a$</td>
<td>$5.85 \pm 0.65^b$</td>
<td></td>
</tr>
<tr>
<td>Nylon</td>
<td>0</td>
<td>na</td>
<td>$1.23 \pm 0.01^a$</td>
<td>$0.24 \pm 0.01^a$</td>
<td>$5.08 \pm 0.08^a$</td>
<td>+ 10.9</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>na</td>
<td>$1.21 \pm 0.03^a$</td>
<td>$0.22 \pm 0.00^b$</td>
<td>$5.63 \pm 0.06^b$</td>
<td></td>
</tr>
<tr>
<td>EVA/EVOH/EVA</td>
<td>0</td>
<td>$0.40 \pm 0.01^a$</td>
<td>$0.35 \pm 0.04^a$</td>
<td>$0.10 \pm 0.01^a$</td>
<td>$3.86 \pm 0.04^a$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>$0.41 \pm 0.01^a$</td>
<td>$0.71 \pm 0.04^b$</td>
<td>$0.13 \pm 0.01^b$</td>
<td>$5.30 \pm 0.24^b$</td>
<td>+ 37.2</td>
</tr>
</tbody>
</table>

1 Within columns, means (± S.D.) sharing the same superscript letter are not significantly different ($p > 0.05; n = 4$).
5.3.4.2. **Barrier to oxygen and carbon dioxide**

Based on the results of $P_O_2$ listed in Table 5-4, only the nylon films showed an increase in barrier to O$_2$, after the exposure to ClO$_2$. This could be due to an increase in crystallinity (Table 5-2), since the exposed material had a lower available amorphous region in which permeation can take place (Kulshreshtha, 1992; Schnabel, 1992). This coincides with a study on the effects of ozone treatment on biaxial oriented nylon mentioned previously (Ozen et al., 2002).

An increase of the O$_2$ barrier in ClO$_2$-exposed materials was speculated to be due to a possible partial chlorination which promotes polarity within the polymer matrix, however, barrier to O$_2$ of LDPE (data not shown) and multilayer EVA/EVOH/EVA films decreased after being exposed to ClO$_2$ gas. These results suggest that the main chain scission as shown by FT-IR spectroscopy results of PEs samples could be the dominant degradation reaction, due to ClO$_2$ exposure, in both materials, as this would increase the chain mobility and facilitate the transfer of gas throughout the polymer structure (Table 5-4). The same explanation can reasonably be given for the increase in $P_{CO_2}$ of the treated PEs, PS and EVA/EVOH/EVA films.

The $P_{CO_2}$ of polymeric material is usually higher than the $P_O_2$ of the same material. Given that both gases are of the same penetrant type, i.e. non-reactive gas (Selke et al., 2004), a similar trend would be expected for ClO$_2$-exposed polymeric films. However, the degree of change varied, depending on the particular polymer/penetrant pair (Matteucci et al., 2006). This led to the alteration of the $P_{CO_2}/P_{O_2}$ ratio of the
materials after they have been exposed to ClO₂. With the exception of 7-day exposed HDPE film sample, a comparison of the modified $P_{CO2}/P_{O2}$ ratio to the original value showed no significant change for the other materials, after 1 and 7 days of treatment. However, after 14 days of persistent exposure, the $P_{CO2}/P_{O2}$ ratios of many materials have changed ranging from 10.9% to 46.8%. The most notable change occurred in the exposed HDPE sample. This is critical information when designing MAP systems for fresh produce where the ratio of CO₂ and O₂ within the packaging atmosphere will impact the shelf-life of the product.

5.3.5. Color measurement

Overall lightness ($L^*$) of the exposed LLDPE, PVC, PS, PET and nylon increased as compared to the unexposed samples (Table 5-5). The changes in $b^*$ values of the day 1 and day 7 samples of exposed PEs, PVC, PS, PET, and nylon films indicated that the films became more yellow in color, however, after 2 weeks of exposure, the color shifted toward being more bluish. Significant overall color differences ($\Delta E$) were found in exposed PVC and PS films.

From visual observations on discoloration of the exposed samples, in general, the film samples changed from opaque white or transparent to yellowish dull and became darker in color at the end of exposure.
Table 5-5. Color properties of selected polymeric packaging materials exposed to gaseous ClO₂. Adapted from (Netramai et al., 2010).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time of Exposure (day)</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>ΔE*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>38.34 ± 0.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-2.69 ± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-6.06 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>39.24 ± 0.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-2.67 ± 0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-5.91 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.91 ± 0.85&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>39.02 ± 1.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-3.09 ± 0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-5.68 ± 0.18&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>0.88 ± 0.80&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>39.44 ± 0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-2.75 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-6.53 ± 0.20&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>1.20 ± 0.69&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDPE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>28.27 ± 0.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-2.82 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-2.10 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>29.28 ± 1.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-2.37 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-2.48 ± 0.51&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>1.17 ± 1.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>28.95 ± 1.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-2.53 ± 0.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-2.03 ± 0.35&lt;sup&gt;a,d&lt;/sup&gt;</td>
<td>0.83 ± 0.81&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>32.87 ± 0.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-2.37 ± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-3.79 ± 0.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.92 ± 0.53&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>PVC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>25.96 ± 0.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-2.29 ± 0.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-1.90 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>34.55 ± 1.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-3.14 ± 0.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-1.06 ± 0.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.67 ± 1.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>35.74 ± 1.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-3.28 ± 0.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-1.01 ± 0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.87 ± 1.40&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>38.05 ± 2.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-3.59 ± 0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-2.16 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.2 ± 1.64&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>PS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Within columns, means (± S.D.) sharing the same superscript letter are not significantly different ($p > 0.05; n = 4$).
<table>
<thead>
<tr>
<th>Sample</th>
<th>Time of Exposure (day)</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>ΔE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PET</td>
<td>0</td>
<td>34.91 ± 1.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-3.13 ± 0.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-3.27 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>33.12 ± 1.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-2.96 ± 0.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-2.95 ± 0.31&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.83 ± 1.42&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>33.71 ± 0.81&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>-2.96 ± 0.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-2.93 ± 0.22&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.26 ± 1.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>36.38 ±0.97&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>-2.59 ± 0.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-3.98 ± 0.28&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>1.72 ± 1.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nylon</td>
<td>0</td>
<td>33.68 ± 0.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-2.82 ± 0.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-6.06 ± 0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>38.01 ± 2.77&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>-2.67 ± 0.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-4.97 ± 0.87&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.47 ± 1.98&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>37.50 ± 0.77&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>-2.72 ± 0.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-4.91 ± 0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.99 ± 0.75&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>37.42 ± 2.25&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>-2.61 ± 0.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-5.75 ± 0.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.76 ± 1.68&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1 Within columns, means (± S.D.) sharing the same superscript letter are not significantly different (p > 0.05; n = 4).
5.4. Conclusion

The effects of ClO₂ gas on properties and performance of 10 types of polymeric packaging materials, including HDPE, LDPE, LLDPE, BOPP, PS, PVC, PET, PLA, nylon, and a multilayer structure of EVA/EVOH/EVA were studied by assessing their IR spectrum, physical, mechanical, barrier, and color properties. The samples were exposed to 3,600 ppmV ClO₂ gas, at 23°C for 1, 7, and 14 days. The IR spectra of the ClO₂ treated samples indicated many possible changes in their chemical characteristics, such as formation of polar groups in polyolefins, changes in functional groups, and main chain scission degradation, as well as, possible chlorination of several sample types. The ClO₂ treated PE samples showed a decrease in tensile properties as compared to the untreated control films. Decreases in barrier to moisture, oxygen, and/or carbon dioxide were found in treated HDPE, PVC, PET, PLA, and multilayer EVA/EVOH/EVA. A significant increase in barrier to O₂ was observed in ClO₂ treated nylon film, which could be the result of molecular re-ordering, as implied through the increase in the crystallinity of the material. After 14 days of exposure, the permselectivity of several polymer films was altered, up to 46.8%.


6.1. Introduction

The absorption behavior of gaseous ClO₂ by shredded lettuce was investigated in this chapter. The study assessed 1) residual ClO₂; and 2) chlorite (ClO₂⁻) in ClO₂-exposed lettuce pieces. The amount of ClO₂ absorbed by the lettuce, as well as, identifying the factors affecting this behavior, will help determine the optimal dose of ClO₂ gas to be included in specific product/package system.

When absorbed into produce, ClO₂ can react with organic matter, such as plant cells and pigments (Richardson et al., 1998; USEPA, 1999), and also with normal microflora residing on the plant’s surface. These reactions typically generate chlorite (ClO₂⁻) as a major by-product, along with numerous other compounds produced in trace amounts (Han et al., 2004). Han et al. (2004) reported measurable levels of residual ClO₂ and ClO₂⁻ on strawberries after ClO₂-treatment (3.0 mg/L for 10 min), with small amounts of ClO₂⁻ present even after one week of storage at 4°C (Han et al., 2004). The later form of ClO₂ absorbed by produce does not possess antimicrobial activity, and, if
determined to be significantly high; should be taken into consideration when determining the appropriate ClO$_2$ dose for the target food product.

Other factors that must be considered when studying ClO$_2$-absorption behavior are those associated with processing steps in the food production chain, i.e. the presence of cuts and/or bruises, and excess water on the produce surface. Fresh-cut and ready-to-eat products are typically subjected to a wide range of food processing steps, however processing of these products is typically regarded as ‘less severe’ than other preparation and preservation techniques used for other product types.

After sorting, trimming, cutting, washing and drying, the plant cells become damaged which, in turn, accelerates physiological deterioration, biochemical changes, and microbial degradation (Allende et al., 2006; Rico et al., 2007). In addition, the intracellular plant compounds released during processing will react with ClO$_2$. Chlorine dioxide which has high solubility (Ishi, 1958; USEPA, 1999) could also attracted to intracellular liquid in the damaged cells, and/or the presence of water on the target surfaces, resulting in more ClO$_2$ being absorbed.

6.2. Materials and Methods

6.2.1. Development of experimental setup for absorption study

To be able to effectively study ClO$_2$-absorption in lettuce, the experimental set up was designed to provide stable testing environment with constant concentration of ClO$_2$ gas in the head space. The experimental setup used (Figure 6-1) consisted of 1) a 740 in$^3$
(approximately 12 L) glass chamber (custom made at Glassblowing Facility, Department of Chemistry, MSU, East Lansing, MI) with a removable glass lid providing access to lettuce samples; 2) low-density poly(ethylene) (LDPE) screen for supporting the lettuce sample; (3) Viton® O-ring (Anchor Rubber Products, 2007); and (4) metal clamp ring (together with the O-ring) for hermetic sealing of the glass chamber. A chlorine dioxide sampling port was installed at the bottom of the glass chamber.

Chlorine dioxide solution with known concentration was placed in the bottom of the chamber to provide 3.0 and 6.0 mg/L of ClO₂ in the gas phase (details on selected ClO₂ level in section 6.2.4). Even though the glass chamber, O-ring, and metal clamp ring provided air-tight conditions and minimize the loss of ClO₂ gas to the environment, small amount of ClO₂ gas was lost through reactions with the system, photo-degradation (Vaida and Simon, 1995) and sorption into the LDPE screen (Netramai et al., 2009). The chlorine dioxide degradation profiles for particular system were determined by monitoring amount of ClO₂ in solution (Appendix 2), without lettuce sample as described in Appendix 5.
Figure 6-1. Experimental setup for absorption study
6.2.2. Preparation of lettuce samples

Romaine lettuce (*Lactuca sativa* L. var. *longifolia*) was purchased from a local supermarket in East Lansing, MI, during August – November, 2010, and stored at 4°C, until 1 h before use. To avoid variations of the samples’ textures, each batch of lettuce was purchased and used within 5 days, and then discarded if they were not used in the experiments.

For each experiment, 3 lettuce leaves with no visible or minor bruises, were selected from the inner, middle, and outer layers of the head, and cut to measure 1.25 × 1.5 in². Cut pieces included the midrib and leaf areas, but excluded the midrib connected to the stem. Twenty-five pieces of shredded lettuce weighting between 40-50 g were then used in each experiment.

6.2.2.1. Preparation of whole leaf and washed shredded lettuce samples

To study the factors that could affect ClO₂ absorption, i.e. cuts and moisture on the leaf surface, the following samples were prepared:

(1) Three whole lettuce leaves, from the inner, middle, and outer layers of the lettuce head weighting between 40-50 g; and

(2) 25 pieces of shredded lettuce (with initial weight comparable to the shredded sample prepared in section 6.2.2) that were subjected to a 5 min submersion in water and spin-dried using a kitchen type salad spinner (5 pumps).
6.2.3. Preparation of ClO₂ solution

The same methodology as described in chapter 3 was used to prepare the final ClO₂ solution from a stock solution containing 1,000 mgClO₂/L.

6.2.4. Concentration range to be used in absorption study

Preliminary experiments were conducted to determine the concentration range of ClO₂ gas to be used in absorption study. Twenty-five lettuce leaf pieces were placed upright in a glass container, along with ClO₂ gas generated from ClO₂ solution (1 L) as shown in Figure 6-1. The concentrations of gaseous ClO₂ used were between 2.0 – 10.0 mg/L (with 2.0 mg/L increment) with exposure times of 15, 30, 60, 45, 90, and 120 min.

Visual inspection of the exposed lettuce pieces showed that exposure to greater than 6.0 mgClO₂/L, for less than 15 min, discolored the lettuce with the leaves changing from green to brownish white. Exposing shredded lettuce more than 60 min, even at the lowest ClO₂ level, i.e. 2.0 mg/L also bleached the samples. Furthermore, the degree and area of discoloration increased as the concentration and/or time increased.

Based on preliminary tests, selected ClO₂ levels to be used in absorption work were 3.0 and 6.0 mg/L with predetermined exposure times of 7.5, 15, 30, 45, 60, and 90 min. A treatment time of 90 min was selected to observe the absorption behavior of the bleached lettuce pieces.
6.2.5. Quantification of residual ClO$_2$ and ClO$_2^-$ on shredded lettuce

Residual ClO$_2$ and ClO$_2^-$ on the lettuce leaf were quantified using a modification of the standard titration procedure for the examination of water and wastewater (Greenberg et al., 1992). Briefly, 25 pieces of shredded lettuce were exposed to ClO$_2$ gas released from solution in the closed glass chamber (Figure 6-1) for a predetermined time period.

To measure residual ClO$_2$ and ClO$_2^-$, the ClO$_2$-exposed lettuce sample was washed with 300 ml of distilled and deionized water for 15 min (Han et al., 2004), after which the washing solution was titrated for residual ClO$_2$ and ClO$_2^-$, using an amperometric titration method (Greenberg et al., 1992). This specific amperometric titration (4500-ClO$_2$ C) involves four titration steps with phenylarsine oxide (C$_6$H$_5$AsO), in which free chlorine (Cl$_2$), chloramines, ClO$_2^-$, and ClO$_2$ can be determined separately. The titration procedure was described, in detail, in Appendix 4.

For each experiment, the amount of ClO$_2$ in solution was determined at the beginning and end of the treatment, using the thiosulfate titration method outlined in Appendix 2, to determine the recovery rate of the titration procedure for residual ClO$_2$ and ClO$_2^-$ (see Appendix 5).
6.2.5.1. Evaluation of factors affecting chlorine dioxide absorption

To determine the impact of cuts and excess water on the leaf, whole leaves and washed shredded lettuce samples prepared as described in section 6.2.2.1 was subjected to the same ClO₂-treatments as described earlier, after which residual ClO₂ and ClO₂⁻ were determined.

6.2.6. Statistical analysis

Each ClO₂ treatment was repeated five times. The results obtained were statistically analyzed by analysis of variance (ANOVA) using the Statistical Analysis System (SAS) software (SAS Institute Inc., Cary, NC) at a confidence level of 95% (α = 0.05) with Tukey’s adjustment for comparison of means.

6.3. Results and discussion

An experimental system that provided a constant ClO₂ level of 3.0 and 6.0 mg/L in the gas phase over a certain period of time (≤ 90 min) was developed and used to study ClO₂-absorption behavior by lettuce leaves. The absorption study was carried out by exposing different lettuce samples, i.e. shredded, whole, and washed shredded lettuces to ClO₂ gas for different exposure times, after which the levels of residual ClO₂ and ClO₂⁻ were quantified in the treated samples.
6.3.1. Absorption of ClO$_2$ into shredded lettuce

The residual ClO$_2$ and ClO$_2^-$ values are showed in Figure 6-2. The levels of ClO$_2^-$ recovered indicate the amount of absorbed ClO$_2$ gas that reacted with organic matter and/or normal flora in lettuce leaf (Han et al., 2004). At any given treatment time, increasing the ClO$_2$ level increased the amount of ClO$_2^-$ recovered; and at any given ClO$_2$ level, longer treatment times gave the same effect. Based on visual observation, bleached area was noticeable initially at treatment of 3.0 mg/L for 45 min and 6.0 mg/L for 30 min; and over half of lettuce samples were bleached after treatment of 3.0 mg/L for 90 min or 6.0 mg/L for 45 min. At equivalent treatments, such as 3.0 mg/L for 30 min or 6.0 mg/L for 15 min, the amounts of ClO$_2^-$ recovered were comparable.

Differences in the amount of ClO$_2^-$ recovered at different treatment conditions (Table 6-1) reflected different activity levels between ClO$_2$ gas and organic matter and/or normal flora of the lettuce leaf.

Microbial enumerations were not performed on any of these samples (samples were washed and discarded after recovering the residuals). However, in separate experiments using continuous exposure to ClO$_2$ gas (details in Appendix 1, specifically in Table A-1) indicated that increasing the ClO$_2$ levels and/or time of exposure, led to greater reductions of microbial background flora. Since the primary end-product from disinfecting mechanism of ClO$_2$ is chlorite (USEPA, 1999), the differences in residual
ClO$_2^-$ recovered depended, at least in part, on the degree of disinfection occurring at each treatment condition.

In regards to residual ClO$_2$ recovered from lettuce, since only small amounts of ClO$_2$ were absorbed without reacting (i.e. < 0.73 mgClO$_2$/kg lettuce for every testing condition that did not cause noticeable discoloration), this portion of ClO$_2$ could be reasonably neglected when calculating a gas treatment for lettuce, within the typical range used in food applications.

Since the levels of residual ClO$_2$ recovered from all lettuce samples were close to the detection limit of this particular titration procedure, which was calculated to be < 0.32 mgClO$_2$/kg lettuce, the variations of residual ClO$_2$ determined were quite high, as can be observed from error bars in Figure 6-2.
Figure 6-2. Absorption of ClO₂ gas on shredded lettuce
<table>
<thead>
<tr>
<th>Treatment</th>
<th>ClO₂⁻ mgClO₂/kg lettuce</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.0 mgClO₂/L¹</td>
</tr>
<tr>
<td>7.5 min</td>
<td>nd²</td>
</tr>
<tr>
<td>15 min</td>
<td>≤ 7.17 ± 0.47ᵃ</td>
</tr>
<tr>
<td>30 min</td>
<td>≤ 9.37 ± 1.71ᵇ</td>
</tr>
<tr>
<td>45 min</td>
<td>≤ 10.82 ± 1.03ᵇ</td>
</tr>
<tr>
<td>60 min</td>
<td>16.94 ± 1.19ᶜ</td>
</tr>
<tr>
<td>90 min</td>
<td>20.36 ± 0.97ᵈ,ᵇ</td>
</tr>
</tbody>
</table>

¹ For the solutions to release 3.0 and 6.0 mg/L of ClO₂ gas into the chamber headspace, at 23°C, the actual amounts of ClO₂ in 1 L of solution are approximately 87 and 175 mg.
² Not determined.
³ Within columns, means sharing the same superscript letter are not significantly different (p > 0.05; n = 5).
⁴ ≥ 50% of sample showed discoloration.
6.3.2. Factors affecting absorption behavior of ClO₂ into lettuce

Whole leaf and washed shredded lettuce samples were used to investigate if the presence of cuts or excess water could affect ClO₂ absorption. The determined ClO₂⁻ values for both sample types are listed in Table 6-2 and Table 6-3 and graphically shown, in comparison to those for shredded lettuce (original data reported in Table 6-1), in Figure 6-3.

The levels of residual ClO₂ recovered from whole leaf samples were comparable to those found in shredded samples i.e. < 0.51 mgClO₂/kg lettuce, regardless of the treatment condition.

However, ClO₂⁻ levels were vastly different for whole leaf samples. The ClO₂⁻ levels were only comparable to those found in shredded lettuce at 3.0 mg/L for 60 min and 6.0 mg/L for 30 min, where the leaf samples bleached severely (Figure 6-4). For ClO₂-treatments that did not cause noticeable bleaching (i.e. 3.0 mg/L for < 45 min and 6.0 mg/L for 15 min), the ClO₂⁻ levels found were approximately 10 times lower than those for shredded lettuces.

Increase in ClO₂ uptake in shredded lettuce is likely due to the presence of cuts and/or bruises. Cutting damages cells and releases intracellular compounds which accelerate chemical and microbial degradations of lettuce by giving potential reactants and microorganism access to the plant tissue (Allende et al., 2006; Rico et al., 2007). Chlorine dioxide can also penetrate cuts or bruises and react more readily with organic matter in plant exudate.
Washing increased the initial weight of the shredded lettuce by 6.85 ± 0.71 %. Based on Figure 6-3, chlorite levels followed the same trend, and were comparable in the amount recovered. Levels of residual ClO₂ recovered from washed shredded lettuce were also < 0.74 mgClO₂/kg lettuce for all treatment that did not lead to discoloration. Thus, water uptake (at the level mentioned earlier) during washing did not significantly affect ClO₂ absorption. However, visual observations (Figure 6-4) showed that discoloration first appeared on washed samples faster than those occurred on shredded samples. The increased moisture on the surface may also cause ClO₂ gas to dissolve more easily (Ishi, 1958), with this reaction occurring more readily on specific areas where water droplet are located. Apart from excess moisture, washing and spin-drying introduced additional damage to shredded lettuce, which accelerated the biochemical reactions even further.

Due to concerns surrounding residual ClO₂ and ClO₂⁻ on ClO₂-treated food products (Couri et al., 1982; Qingdong et al., 2006), treated shredded lettuce (treated with 6.0 mgClO₂, for 15 min) was placed on a clean surface at room temperature for 15 and 60 min; and after which the amount of residual ClO₂ and ClO₂⁻ were determined. These values were reported below (Table 6-4).
Table 6-2. ClO$_2^-$ recovered from whole leaf lettuce after treatment with 3.0 and 6.0 mg/L ClO$_2$ gas

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ClO$_2^-$ mgClO$_2$/kg lettuce</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0 mgClO$_2$/L$^1$</td>
<td>6.0 mgClO$_2$/L$^1$</td>
</tr>
<tr>
<td>15 min</td>
<td>≤ 0.56 ± 0.31$^{a,2}$</td>
</tr>
<tr>
<td>30 min</td>
<td>≤ 0.92 ± 0.40$^a$</td>
</tr>
<tr>
<td>45 min</td>
<td>≤ 1.80 ± 0.36$^b$</td>
</tr>
<tr>
<td>60 min</td>
<td>≤ 15.71 ± 0.86$^{c,3}$</td>
</tr>
<tr>
<td>90 min</td>
<td>Nd</td>
</tr>
</tbody>
</table>

1 For the solutions to release 3.0 and 6.0 mg/L of ClO$_2$ gas into the chamber headspace, at 23$^0$C, the actual amounts of ClO$_2$ in 1 L of solution are approximately 87 and 175 mg.
2 Within columns, means sharing the same superscript letter are not significantly different ($p > 0.05; n = 5$).
3 ≥ 50% of sample showed discoloration
4 Not determined
Table 6-3. ClO$_2^-$ recovered from washed shredded lettuce after treatment with 3.0 and 6.0 mg/L ClO$_2$ gas

<table>
<thead>
<tr>
<th>Treatment</th>
<th>3.0 mgClO$_2$/L$^1$</th>
<th>6.0 mgClO$_2$/L$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5 min</td>
<td>nd$^2$</td>
<td>$\leq 7.64 \pm 1.40^{a,3}$</td>
</tr>
<tr>
<td>15 min</td>
<td>$\leq 6.96 \pm 0.27^{a}$</td>
<td>$\leq 9.75 \pm 1.35^{a}$</td>
</tr>
<tr>
<td>30 min</td>
<td>$\leq 9.36 \pm 1.07^{b}$</td>
<td>$\leq 14.87 \pm 1.24^{b}$</td>
</tr>
<tr>
<td>45 min</td>
<td>$\leq 11.61 \pm 0.92^{c}$</td>
<td>$\leq 21.98 \pm 1.17^{c,4}$</td>
</tr>
<tr>
<td>60 min</td>
<td>$\leq 15.48 \pm 1.60^{d}$</td>
<td>nd</td>
</tr>
<tr>
<td>90 min</td>
<td>$\leq 21.62 \pm 1.63^{e,4}$</td>
<td>nd</td>
</tr>
</tbody>
</table>

$^1$ For the solutions to release 3.0 and 6.0 mg/L of ClO$_2$ gas into the chamber headspace, at 23°C, the actual amounts of ClO$_2$ in 1 L of solution are approximately 87 and 175 mg.

$^2$ Not determined

$^3$ Within columns, means sharing the same superscript letter are not significantly different ($p > 0.05$; n = 5).

$^4$ $\geq 50\%$ of sample showed discoloration
Figure 6-3. Residual $\text{ClO}_2^-$ recovered from whole leaf and washed shredded lettuce samples as compared to those of unwashed shredded lettuce
These findings indicate that; given enough time after ClO₂ exposure, residual ClO₂ and ClO₂⁻ will decrease to undetectable levels. Thus, if packaging systems were designed to deliver ClO₂ gas in a manner that releases the gas for a predetermined time and allows sufficient additional storage time (with the absence of ClO₂ gas) for the residuals to degrade, any concerns regarding the presence of unwanted residuals could be reasonably solved. However, degradation patterns for residual ClO₂ and ClO₂⁻ are also affected by commodity type. For example, about 6% of ClO₂⁻ still remained in strawberry samples after 1 week of storage at 4°C (Han et al., 2004).

Table 6-4. Residual ClO₂ and ClO₂⁻ in shredded lettuce after treatment with 6.0 mg/L ClO₂ gas, for 15 min, and left at room temperature for 15 and 60 min

<table>
<thead>
<tr>
<th>Time left at room temperature (min)</th>
<th>ClO₂ mgClO₂/kg lettuce</th>
<th>ClO₂⁻ mgClO₂⁻/kg lettuce</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>&lt; 0.32&lt;sup&gt;1&lt;/sup&gt;</td>
<td>≤ 4.90 ± 0.46&lt;sup&gt;a,2&lt;/sup&gt;</td>
</tr>
<tr>
<td>60</td>
<td>&lt; 0.32&lt;sup&gt;1&lt;/sup&gt;</td>
<td>≤ 0.90 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> Reached detection limit of the equipment.
<sup>2</sup> Within columns, means sharing the same superscript letter are not significantly different ($p > 0.05$; n = 5).
Whole lettuce leaf:

Shredded lettuce:

Washed shredded lettuce:

Figure 6-4. Color profiles; Noted extensive discolorations on whole lettuce leaf and washed lettuce at 6.0 mgClO₂/L treatment for 30 min.
6.4. Conclusion

Residual levels of ClO₂ and ClO₂⁻ were successfully quantified on ClO₂-treated lettuce leaves. The results showed that Romaine lettuce absorbed more ClO₂ as the treatment time lengthened and/or the ClO₂ level increased. Amount of ClO₂ recovered from the lettuce were < 0.73 mgClO₂/kg lettuce for every applicable treatment and could be dismissed when determining a ClO₂ treatment for shredded lettuce. After treatment, amounts of both compounds decreased to undetectable levels if left to expose to air for sufficient time.

The presence of cuts/bruises significantly increased ClO₂ absorption in lettuce, while excess moisture did not significantly affect absorption. Excess moisture and more severe treatments increased changes in appearance, i.e. bleaching; of lettuce.


CHAPTER 7: EFFECTS OF PACKAGING DESIGN ON GAS DISTRIBUTION WITHIN THE PACKAGE

7.1. Introduction

Given a proper packaging design, maximum surface exposure of food products to antimicrobial gases can be achieved. Several studies indicated that direct-contact between antimicrobial gas and the product surface is crucial for effective inactivation of microorganisms (Du et al., 2002; 2003; Ellis et al., 2006; Han et al., 2000; Lee et al., 2006; Lindsay et al., 2002; Shin, 2007).

Packages contain many hard to reach areas where headspace gases have limited or no access to, including the center and the bottom of the package (Ellis et al., 2006; Gómez-López et al., 2009). In an earlier work by Shin (2007), ClO₂ gas was used to sanitize chicken breasts packaged in sealed trays. When chlorine dioxide sachet was placed at one end of the tray and the meat at the other end, the meat’s surface near the sachet turned brownish black (Shin, 2007). In terms of antimicrobial efficacy of ClO₂ gas, the results indicated that uniform gas distribution is required in order to sufficiently reduce the number of microorganisms, as microbial growth of the pathogen on the bottom of the meat was significantly higher than on the top (Shin, 2007). Ellis et al. (2006) investigated antimicrobial effect of ClO₂ gas, generated from sachets containing ClO₂ precursors, on packed chicken breasts. Higher microbial loads were found at the bottom of the meat where the area was less accessible to ClO₂ gas generated by the sachet, as
compared to the top surface. Significant discoloration of the meat surface caused by the gas was observed at the area closer to ClO₂ sachet, indicating that ClO₂ was distributed unevenly within the package and react mainly with the surface adjacent to the sachet (Ellis et al., 2006). Furthermore, Du et al. (2002 and 2003) reported differences log₁₀ CFU/site reduction for *E. coli* O157:H7 and *Listeria monocytogenes*, from different areas of apples, after ClO₂ treatment (Du et al., 2002; 2003).

This study explored the effect of packaging design on antimicrobial ability of ClO₂ gas which should be considered when a packaging system is to be used as an additional microbial reduction step. This research was conducted in preparation for a collaborative project between Eastern Regional Research Center (ERRC), USDA, Rutgers University, and the School of Packaging at Michigan State University (MSU). The overall objective of the project was to evaluate the integration of 1) post-harvest strategies of produce sanitizing and 2) exposure to an antimicrobial through a packaging system; to improve microbial safety of fresh produce, by using packaging design to improve gas distribution in flexible and rigid container. The aim of this hurdle strategy is to achieve a microbial reduction of ≥ 5 log₁₀ CFU/g for 2 target pathogenic microorganisms, i.e. *Escherichia coli* O157:H7 and *Salmonella* spp., inoculated on shredded Romaine lettuce and cherry tomatoes, respectively.

The food product selected for this study portion was ready-to-eat (RTE) shredded lettuce which has been responsible for several foodborne outbreaks, in the past few years. RTE vegetables product is a minimally processed fresh produce, which is gaining popularity as it reduce the preparation time and waste generated (Zhou et al., 2004).
Shredded lettuce is one of the most important RTE products. As any other RTE leafy green product, the shelf life of shredded lettuce is limited due to discoloration (browning) and microbiological deterioration. Such processes are accelerated as the shredding process destroys the protective epidermal layer of the leaf and ruptures the cells, resulting in biochemical reactions. The cut surfaces also allow microorganisms the access to nutrients (Diaz and Hotchkiss, 1996; Zhou et al., 2004).

Normally, minimally processed products are consumed without any further cooking, making contamination by pathogenic microorganism a very important issue. In this study, the selected pathogenic microorganism was *Escherichia coli* O157:H7 which has been reported as main microorganism among others, responsible of foodborne disease cases (Department of Health and Human Services, 2008; FSIS, 2005; Nowak et al., 2006; Phillips, 1996; Sy et al., 2005). This pathogen has been identified in food carrier such as raw or minimally processed foods, such as fresh spinach, shredded lettuce, and salads (Department of Health and Human Services, 2008; Lynch et al., 2006).

This project focuses on the use of ClO$_2$ gas in decontaminating shredded lettuce, by using a developed packaging system as an additional/extended step of food production. Once optimized, the new packaging system could effectively minimize the recurring problem of foodborne outbreaks related to retail and food services. Specific objectives of this section are to:

1. Identify ClO$_2$ gas treatment conditions that can inactivate human pathogens on shredded lettuce without causing quality defects.
2. Identify a specific package design that ensures an effective gases distribution inside the package even in hard to reach areas.
3. Determine the efficacy of the packaging system in inactivating foodborne pathogens and prolonging the shelf life of shredded lettuce.

4. Evaluate a pilot scale treatment and scale up to demonstrate technical and economical feasibility.

Objective 2 involved modifying the interior of a currently used flexible package for shredded lettuce. The approach is favorable since neither the packaging line nor the basic package will be changed significantly. The design development will aim to facilitate and maximize distribution of the gas, even in hard to reach areas by building a gas reservoir within the package, ensuring that ClO$_2$ is available for an extended period. The designs will be optimized for maximum exposure between ClO$_2$ gas and the product, as the effectiveness of any antimicrobial gas treatment also depends on its surface contact with the target product (Du et al., 2002).

As part of objective 2, this preliminary study was also conducted to gather the necessary information to develop parameters for future packaging development work which will be performed by the research team, in the later parts of collaborative research project. Information regarding: 1) mass transfer of ClO$_2$ as reported in Chapter 4, specifically, the amount of ClO$_2$ gas that permeated through and was absorbed by the packaging material, 2) the exposure time of the materials to ClO$_2$ gas that will not cause any significant alteration in the films’ properties (Chapter 5), and 3) ClO$_2$-absorption behavior of lettuce leaf, will also be included as the parameters in the same future work on packaging design.
7.2. Materials and methods

Two consecutive packaging studies were conducted using 3 different designs of flexible bags; and plain flexible bags as controls. These bags were used as package for shredded lettuce that was inoculated with *E. coli* O157:H7 and stored in refrigerator at 4 ± 0.5°C. Microbial enumeration and visual inspection were performed at specific time intervals.

7.2.1. Preparation of inoculums

*Escherichia coli* O157:H7 SEA13B88 (human feces, apple cider-associated disease outbreak), maintained at -80°C in trypticase soy broth (TSB; Becton Dickinson, Sparks, MD) and 10% (v/v) glycerol, was grown for 18-24 hours in TSB at 35 ± 0.5°C, transferred to a trypticase soy agar (TSA; Becton Dickinson, Sparks, MD) slant, and this working stock culture was stored at 4°C for no more than 21 days. Inoculum was prepared by transferring a loopful (1 μl) of the working stock to 10 ml TSB, which was incubated in a shaking incubator for 6 - 8 hours at 35 ± 0.5°C. Following incubation, 2.4 ml of the culture was transferred to 24 L of TSB, and then incubated at 35 ± 0.5°C in a shaking incubator for 18-24 hours. The culture was then centrifuged (6740 × g) at 4°C for 15 min. After decanting the supernatant, the resulting pellet was resuspended in sterile deionized water and centrifuged (6740 × g) for 15 min at 4°C. The supernatant was decanted and the pellet was resuspended in 3 L of sterile deionized water. The concentration of the inoculum was determined by serially diluting the inoculum in 0.1% peptone water (PW; Becton Dickinson, Sparks, MD) and plating on TSA. The
resuspended culture was then stored at 4°C as a culture concentrate, for overnight. The culture was diluted to 24 L before use.

7.2.2. Inoculation of shredded lettuce

Commercially available Romaine lettuce (*Lactuca sativa* L. var. *longifolia*) was purchased at a local supermarket in Wyndmoor, PA, US, and stored at 4 ± 2°C for a maximum of 24 hours before use in experiments. Damaged outer leaves were removed from each head of lettuce, the lettuce was cut into pieces approximately 4 - 6 cm² and immediately submerged into the *E. coli* O157:H7 inoculum suspension for 5 min. Excess liquid culture on the lettuce was removed using a salad spinner (OXO Good Grips Salad Spinner, OXO International, Ltd., New York, NY;) for 1 min (Figure 7-1) and then the lettuce was placed into an open container and allowed to dry for 2 hours at 22 ± 2°C in a biosafety cabinet. The lettuce was then placed into plastic bags and stored for 18-24 hours at 4 ± 2°C to allow bacteria attachment.
Figure 7-1. Inoculation of shredded lettuce; shredded lettuce were submerged in *E. coli* O157:H7 inoculums for 5 min and then spin-dried
7.2.3. Packaging design

7.2.3.1. Study 1

For the first study, two different bag designs (Figure 7-2) were used. These bags were modified from commercially available bag for leafy green products, PD-961EZ (Cryovac Inc., Duncan, SC) (see Appendix 6 for bag characterization) by placing gas reservoirs (GRs) in different locations. A thermal impulse heat sealer (Vertrod Crop., San Rafael, CA) was used to create the GRs by sealing the already existing bag. The seal, with small openings throughout, divided the bag into a large chamber for lettuce sample; and lengthwise ClO₂ GRs, where ClO₂ precursor was placed and humidity-activated to released ClO₂ gas. The openings allowed gas to enter the bag chamber.

Bags with different numbers of GRs and GRs with different numbers of openings were created, i.e. Design 1 with one-GR and 6 openings; and Design 2 with two-GRs, along each side of the bag, and 3 openings on each GR.

7.2.3.2. Study 2

In the second study, the GR was a low-density poly(ethylene) (LDPE) tube, with a permeability coefficient (P) of 66.0 ± 1.09 × 10^{-17} kgClO₂•m•m^{-2}•s^{-1}•Pa^{-1} (Netramai et al., 2009), that included ClO₂ precursors. The tube was packed together with shredded lettuce and placed in the middle of the bag as shown in Figure 7-2.
Figure 7-2. Bag designs for study 1 and 2
Design 2 – Two-GR design

Design 3 – Middle-GR design

Figure 7-2. (cont’d)
7.2.4. Chlorine dioxide treatment

For the first study, 8 g of ClO₂ precursor (Special Mix with linear release, ICA TriNova LLC, Newnan, GA) was put in paper tubes which were inserted into the interior structure of flexible package. Precursor released two levels of ClO₂; high level at 8 mgClO₂/kg lettuce per day and low level at 4 mgClO₂/kg lettuce per day (more details, see Appendix 7 and Appendix 8). The gas release profiles, determined using titration procedure outlined by ICA Trinova (ICA TriNova LLC, 2006), are shown in Figure 7-3.

In the second study, ClO₂, generated by precursor, was released by permeation through the LDPE reservoir (Appendix 8). The same two levels of ClO₂ gas were also used in this experiment, i.e. 4 and 8 mgClO₂/day (Figure 7-3).

7.2.5. Preparation of packaged shredded lettuce inoculated with *E. coli* O157:H7

Approximately 283 g of *E. coli* O157:H7-inoculated lettuce (the same weight as commercial products) were put in the bag along with paper tube that contained the mixed ClO₂ precursors (Figure 7-4). The bags were sealed, photographed of Day 0, and stored, standing upright, at 4 ± 0.5°C. Following 1, 4 and 7 days of storage, samples were collected and were photographed, visually inspected for changes in appearance and color, and evaluated for residual microbial populations.
Figure 7-3. Gas release profiles; 8 mgClO₂/day type and 4 mgClO₂/day type
Figure 7-4. Inoculated shredded lettuce in one-GR design and middle-GR design bags
7.2.6. Microbial enumeration

Results from the preliminary experiments (Appendix 9) suggested that there was no significant difference in *E. coli* O157:H7 cell reductions on lettuce samples taken from the top and the bottom portions of both one-GR and two-GR bag types. Thus, the comparison only focused on samples taken from different locations that were parallel to the gas reservoirs.

Twenty-five grams of shredded lettuce were taken from various points in each of the bag portion (Figure 7-5) (i.e. total of 75 g was taken from each bag in the first study and total of 50 g per bag in the second study) and homogenized with 75 mL Dey-Engley (DE) neutralizing buffer (Becton Dickinson, Sparks, MD) for 1 min, using stomacher blender. Undiluted DE buffer from the homogenate was serially diluted in sterile neutralizing buffer to desired dilutions, spread plated onto 2 Tryptic Soy Agar (TSA, Difco, Becton Dickinson, Franklin Lakes, NJ) plates, incubated at 37 ± 0.5°C for 2 hours (for injured cells to recover), overlaid with Sorbitol MacConkey Agar (SMAC; Remel, Lenexa, KS) with cefixime, and potassium tellurite (CT; Invitrogen, Dynal AS, Oslo, Norway), and then incubated at 37 ± 0.5°C for 22 hours and counted as *E. coli* O157:H7 (Keskinen *et al.*, 2009). Due to certain restriction, all plates obtained from 1 day of storage were incubated at 23°C for 72 hours.
Figure 7-5. Sample portions; For one-GR design, portion 1 = area next to the reservoir, portion 2 = middle area of the bag, and portion 3 = area most distant to the reservoir; For two-GR design, portion 1 and 3 = area next to the reservoirs and portion 2 = middle area of the bag; For middle-GR design, portion 1 = area next to the reservoirs and portion 2 = area further away from the reservoir from both sides of the bag.
For control samples, inoculated shredded lettuce was packed in plain bags (details in section 7.2.5) without ClO₂ gas. Log₁₀ CFU/g sample of *E. coli* O157:H7 recovered from control samples represent populations of particular microorganism in lettuce with no ClO₂ treatment. The values were reported in Table 7-2 and Table 7-3 and will be used later on for discussion purposes.

### 7.2.7. Statistical analysis

The experimental design followed randomized complete block design (RCBD), as outlined in Table 7-1, in which the experiments were repeated twice (2 blocks). Each experiment involved three replicates. The results obtained were statistically analyzed by analysis of variance (ANOVA) using the Statistical Analysis System (SAS) software (SAS Institute Inc., Cary, NC) at the confidence level of 95% (\( \alpha = 0.05 \)) with Tukey’s adjustment for comparison of means.

| Table 7-1. Randomized complete block design information for study 1 |
|-----------------------------|-----------------------------|
| **Class**                  | **Level**                  | **Value**            |
| Block                      | 2                          | -                     |
| Replicate                  | 3                          | -                     |
| Design                     | 2                          | One-GR and Two-GR     |
| Concentration              | 2                          | 4 and 8 mgClO₂/kg lettuce per day |
| Storage time               | 3                          | 1, 4, and 7 days      |
7.3. Results and discussion

To study how packaging design can affect efficacy of ClO$_2$ gas, and lead to the optimization of a packaging system that, eventually, could improve safety and extend shelf-life of the products, two consecutive studies were conducted.

Two levels of ClO$_2$ gas were used in both experiments; 4.0 and 8.0 mgClO$_2$/day. The high level of ClO$_2$ gas used in the study was predetermined by exposing shredded Romaine lettuce to different levels of ClO$_2$ gas in a closed chamber for 1 h. The level that was selected gave significant reduction in _E. coli_ O157:H7 populations and did not cause a noticeable change in appearance (Appendix 7 and Appendix 8). The low level of 4 mgClO$_2$/day was also selected to quantify the effect of lower ClO$_2$ dose.

7.3.1. Study 1

The first experiment involved plain bags as control sample, and 2 types of modified flexible bags with different internal designs, as well as a plain bag as a control (Figure 7-2). Chlorine dioxide reservoir dimensions were determined by considering bag volume, ClO$_2$ dose needed, ClO$_2$ chemical characteristics, and lettuce weight per bag. The openings, placed on GR, were aimed to distribute equal amounts of ClO$_2$ gas to the sample throughout the whole chamber. The total number of openings for both designs was the same, but the locations of the openings were varied. In Design 1 (one-GR), the openings were placed along one side of the bag; Design 2 (two-GR) had the same numbers of opening, but the openings were placed in different locations, as shown in Figure 7-2. As a result of the two designs, ClO$_2$ gas entered the main chamber from
different directions, resulting in different gas distributions. The bags were packed with shredded lettuce inoculated with \textit{E. coli} O157:H7, along with ClO$_2$ as an antimicrobial gas and stored at 4$^\circ$C.

After 1, 4, and 7 days of storage, the samples were subjected to microbiological analysis. Values of log$_{10}$ CFU/g of \textit{E. coli} O157:H7 for both packaging studies were reported in Table 7-2 and Table 7-3. The average log$_{10}$ CFU/g reductions, shown in Figure 7-6, represent the differences of \textit{E. coli} O157:H7 populations in lettuce packaged with ClO$_2$ gas, for each bag design, at each ClO$_2$ level; and without ClO$_2$ gas. Due to variation of \textit{E. coli} O157:H7 populations observed in control bag at different storage times, the discussions will focus on effects of different bag designs and different ClO$_2$ levels (obtained from the same storage time).

For one-GR design, increasing the amount of ClO$_2$ gas generated per day (from 4 to 8 mgClO$_2$/day) significantly increased the average log$_{10}$ CFU/g reductions in the packaged sample after 1 and 4 days of storage, however, increasing the dose of ClO$_2$ gas per day by 100% did not double the reduction in cell population of the pathogen.
Table 7-2. Log₁₀ CFU/g of *E. coli* O157:H7 of sample portions stored for 1, 4, and 7 days in one-GR<sup>1</sup> and two-GR design bags with 4 and 8 mgClO₂/day

<table>
<thead>
<tr>
<th>Design / ClO₂ level</th>
<th>Storage time (day)</th>
<th><em>E. coli</em> O157:H7 (log₁₀ CFU/g)</th>
<th>Portion 1</th>
<th>Portion 2</th>
<th>Portion 3</th>
<th>Whole bag</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td></td>
<td>8.15 ± 0.08&lt;sup&gt;a,2,3&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td></td>
<td>8.71 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One-GR / 8 mg</td>
<td>1</td>
<td>6.99 ± 0.04</td>
<td>7.92 ± 0.05</td>
<td>7.95 ± 0.07</td>
<td>7.62 ± 0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6.93 ± 0.06</td>
<td>7.93 ± 0.08</td>
<td>7.94 ± 0.03</td>
<td>7.60 ± 0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>7.25 ± 0.12</td>
<td>8.30 ± 0.03</td>
<td>8.31 ± 0.04</td>
<td>7.95 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>One-GR / 4 mg</td>
<td>1</td>
<td>7.24 ± 0.07</td>
<td>7.95 ± 0.03</td>
<td>8.01 ± 0.04</td>
<td>7.73 ± 0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>7.27 ± 0.03</td>
<td>8.08 ± 0.06</td>
<td>8.11 ± 0.04</td>
<td>7.82 ± 0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>7.50 ± 0.10</td>
<td>8.25 ± 0.02</td>
<td>8.28 ± 0.03</td>
<td>8.01 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Two-GR / 8 mg</td>
<td>1</td>
<td>7.30 ± 0.06</td>
<td>8.04 ± 0.05</td>
<td>7.32 ± 0.06</td>
<td>7.55 ± 0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>7.30 ± 0.08</td>
<td>8.01 ± 0.04</td>
<td>7.32 ± 0.03</td>
<td>7.54 ± 0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>7.46 ± 0.05</td>
<td>8.28 ± 0.03</td>
<td>7.48 ± 0.05</td>
<td>7.74 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Two-GR / 4 mg</td>
<td>1</td>
<td>7.39 ± 0.03</td>
<td>8.02 ± 0.04</td>
<td>7.41 ± 0.03</td>
<td>7.61 ± 0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>7.31 ± 0.07</td>
<td>8.06 ± 0.10</td>
<td>7.24 ± 0.02</td>
<td>7.54 ± 0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>7.52 ± 0.05</td>
<td>8.31 ± 0.05</td>
<td>7.51 ± 0.03</td>
<td>7.78 ± 0.02</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> GR = Gas reservoir
<sup>2</sup> Mean ± standard deviation
<sup>3</sup> Means sharing the same superscript letter are not significantly different (p > 0.05; block = 2, n = 3)
Table 7-3. Log$_{10}$ CFU/g of *E. coli* O157:H7 of sample portions stored for 1, 4, and 7 days in middle-GR$^1$ design bags with 4 and 8 mgClO$_2$/day

<table>
<thead>
<tr>
<th>ClO$_2$ level</th>
<th>Storage time (day)</th>
<th><em>E. coli</em> O157:H7 (log$_{10}$ CFU/g)</th>
<th>Portion 1</th>
<th>Portion 2</th>
<th>Whole bag</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mg</td>
<td>1</td>
<td>-</td>
<td>8.16 ± 0.04$^{a,2,3}$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>-</td>
<td>6.37 ± 0.06</td>
<td>7.48 ± 0.19</td>
<td>6.93 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>-</td>
<td>6.58 ± 0.22</td>
<td>8.36 ± 0.05</td>
<td>7.47 ± 0.11</td>
</tr>
<tr>
<td>8 mg</td>
<td>1</td>
<td>6.37 ± 0.06</td>
<td>7.48 ± 0.19</td>
<td>6.93 ± 0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6.55 ± 0.03</td>
<td>7.83 ± 0.01</td>
<td>7.19 ± 0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>6.58 ± 0.22</td>
<td>8.36 ± 0.05</td>
<td>7.47 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>4 mg</td>
<td>1</td>
<td>7.54 ± 0.10</td>
<td>7.72 ± 0.02</td>
<td>7.63 ± 0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6.92 ± 0.07</td>
<td>7.81 ± 0.13</td>
<td>7.36 ± 0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>7.07 ± 0.29</td>
<td>8.45 ± 0.10</td>
<td>7.76 ± 0.17</td>
<td></td>
</tr>
</tbody>
</table>

$^1$ GR = Gas reservoir

$^2$ Mean ± standard deviation

$^3$ Means sharing the same superscript letter are not significantly different ($p > 0.05$; block = 2, n = 3)
Figure 7-6. Average log_{10} CFU/g reduction of *E. coli* O157:H7 of samples stored for 1, 4, and 7 days in one-GR design bag with 8 mg ClO₂/day (a), one-GR design bag with 4 mg ClO₂/day (b), two-GR design bag with 8 mg ClO₂/day (c), and two-GR design bag with 4 mg ClO₂/day

1 First different script letters indicates statistically differences between means at $\alpha$ of 0.05 of different bag designs with the same storage time; Second different script letters indicates statistically differences between means at $\alpha$ of 0.05 of samples with different storage times within the same design
For bags with two-GR, increasing ClO$_2$ gas from 4 to 8 mgClO$_2$/day statistically increased reduction in cell population of *E. coli* O157:H7 only on the first day of storage, but, after day 4 and 7, the log$_{10}$ CFU/g reductions of target microorganism were statistically the same, regardless of the level of ClO$_2$ gas used.

The locations of GRs (and the openings) affected antimicrobial efficacy of ClO$_2$. For example, at 8 mgClO$_2$/day, releasing antimicrobial gas from both sides of the bag (two-GR) reduced *E. coli* O157:H7 populations in shredded lettuce more than when the gas was released from only one side of the bag. This could be due to the shorter travel distance for ClO$_2$ gas in two-GR bag, as compared to the one-GR bag that the gas has to travel to reach the target surface at the other end.

The differences in log$_{10}$ reductions from one-GR and two-GR bags were more pronounced at 4 mgClO$_2$/day. From Figure 7-6, packing the samples in two-GR bags instead of one-GR bags, for 1 day, reduced *E. coli* O157:H7 by 0.07 log$_{10}$ CFU/g and by 0.13 log$_{10}$ CFU/g with 8 and 4 mgClO$_2$/day, respectively. This finding suggests an interaction between bag design and ClO$_2$ level, as supported by statistical outcomes in Appendix 11.

Comparison between one-GR bag with 8 mgClO$_2$/day (Figure 7-6(a)) and two-GR bag with 4 mgClO$_2$/day (Figure 7-6(d)) showed that both had comparable reductions in cell population, after 1 and 4 days of storage, even though the amount of ClO$_2$ gas in
the two-GR design was only half of what was released in the one-GR design (4 and 8 mgClO₂/day, respectively). Furthermore, after 7 days of storage, the log₁₀ CFU/g reduction of two-GR design bag with lower amount of ClO₂ gas was higher than that of one-GR design with 8 mgClO₂/day.

Since the design had significant impact on improving antimicrobial efficacy of ClO₂ gas, by reducing the traveling distance of the gas to its target surface, it should be possible to reduce the dose of ClO₂, and still deliver the equivalent reduction in cell population of *E. coli* O157:H7. Utilization of lower ClO₂ dose could also result in less appearance and texture changes (Ellis *et al.*, 2006; Gómez-López *et al.*, 2009).

Apart from browning normally occurred at cut and bruised areas (Cantwell *et al.*, 1998; Gómez-López *et al.*, 2009; Lopez-Galvez *et al.*, 1996; Peiser *et al.*, 1998; Rico *et al.*, 2007), some visual changes were also observed on the samples, i.e. softer, watery and/or bleached tissues, during exposure to ClO₂ gas (Du *et al.*, 2007; Han *et al.*, 2000) (Figure 7-7 and Figure 7-8). These mostly occurred on lettuce pieces that were adjacent to the GRs. Samples from the bag with one-GR including the dose of 8 mgClO₂/day showed more changes in appearance as compared to shredded lettuce in the bag with two-GR, but with 4 mgClO₂/day dose. In day 4 and 7, the changes in appearance became more pronounced.
Day 0: Browning was visible at the cut of some lettuce pieces; bruises also present (darker green color), especially on the leaf area
Day 1: Browning was more visible
Day 4: Browning occurred more extensive and darker in color, especially at the cut area of midribs
Day 7: Browning found on most of the lettuce pieces; the texture became watery and soft in many pieces, especially the ones contained midrib parts

**Figure 7-7(a).** *E coli* O157:H7 inoculated shredded lettuce packed in plain bags with 0.0 mgClO₂/kg (Control for study 1), stored at 4°C for 0, 1, 4, and 7 days
Day 0: Browning was visible at the cut of some lettuce pieces; bruises also present (darker green color), especially on the leaf area
Day 1: Browning was visible
Day 4: Browning was darker in color, especially at the cut area of midribs; bleaching was noticeable on many lettuce pieces at the area next to GRs; some pieces also became watery and soft
Day 7: Browning was more extensive; the texture became watery and soft in many pieces, especially the ones located next to GRs; more bleaching also observed

Figure 7-7(b). *E coli* O157:H7 inoculated shredded lettuce packed in one-GR bags with 8.0 mgClO₂/kg, stored at 4°C for 0, 1, 4, and 7 days
Day 0: Browning was visible at the cut of some lettuce pieces; bruises also present (darker green color), especially on the leaf area.
Day 1: Browning was visible on more lettuce pieces.
Day 4: Browning was visible; some shredded pieces at the area next to GRs became watery and soft.
Day 7: Browning was more extensive; the texture became watery and soft in many pieces, especially the ones located next to GRs; bleaching was visible in some lettuce pieces.

Figure 7-7(c). *E. coli* O157:H7 inoculated shredded lettuce packed in one-GR bags with 4.0 mgClO₂/kg, stored at 4°C for 0, 1, 4, and 7 days.
Day 0: Browning was visible at the cut of some lettuce pieces; bruises also present (darker green color), especially on the leaf area.
Day 1: Browning was darker in color.
Day 4: Browning was visible; some shredded pieces at the area next to GRs became watery and soft.
Day 7: Browning was more extensive; the texture became watery and soft in many pieces, especially the ones located next to GRs; bleaching was visible in many lettuce pieces.

**Figure 7-7(d).** *E. coli* O157:H7 inoculated shredded lettuce packed in two-GR bags with 8.0 mgClO₂/kg, stored at 4°C for 0, 1, 4, and 7 days.
Day 0: Browning was visible at the cut of some lettuce pieces; bruises also present (darker green color), especially on the leaf area
Day 1: Browning was darker in color
Day 4: Browning was more extensive and darker in color
Day 7: Browning was more extensive; the texture became watery and soft in some pieces, especially the ones located next to GRs; bleaching was visible in some lettuce pieces

Figure 7-7(e). *E. coli* O157:H7 inoculated shredded lettuce packed in one-GR bags with 4.0 mgClO₂/kg, stored at 4°C for 0, 1, 4, and 7 days
Figure 7-8. Changes observed in samples packaged with ClO₂ gas, at 8 mgClO₂/day level; bleached surfaces and watery and softer tissues.
| Day 1 | 8 mg | 1.16 ±0.04\textsuperscript{a,a,1} | 0.24 ±0.04\textsuperscript{a,b} | 0.20 ±0.07\textsuperscript{a,b} |
| Day 4 | 1.48 ±0.05\textsuperscript{a,a} | 0.48 ±0.08\textsuperscript{a,b} | 0.46 ±0.02\textsuperscript{a,b} |
| Day 7 | 1.46 ±0.12\textsuperscript{a,a} | 0.41 ±0.03\textsuperscript{a,b} | 0.40 ±0.04\textsuperscript{a,b} |
| 4 mg | 0.92 ±0.07\textsuperscript{b,a} | 0.20 ±0.02\textsuperscript{a,b} | 0.14 ±0.04\textsuperscript{a,b} |
| 4 mg | 1.14 ±0.02\textsuperscript{b,a} | 0.33 ±0.06\textsuperscript{b,b} | 0.30 ±0.04\textsuperscript{b,b} |
| 4 mg | 1.22 ±0.10\textsuperscript{b,a} | 0.46 ±0.02\textsuperscript{a,b} | 0.43 ±0.03\textsuperscript{a,b} |

**Figure 7-9.** Log\textsubscript{10} CFU/g reduction of *E. coli* O157:H7 of sample portions stored for 1, 4, and 7 days in one-GR design bag with 8 mgClO\textsubscript{2}/day (a), one-GR design bag with 4 mgClO\textsubscript{2}/day (b), two-GR design bag with 8 mgClO\textsubscript{2}/day (c), and two-GR design bag with 4 mgClO\textsubscript{2}/day

\textsuperscript{1} First different script letters indicates statistically differences between means at \( \alpha \) of 0.05 of different sample bags with the same portion; Second different script letters indicates statistically differences between means at \( \alpha \) of 0.05 of samples with different portions within the same bag.
<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th></th>
<th>Day 4</th>
<th></th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 mg</td>
<td></td>
<td>4 mg</td>
<td></td>
<td>4 mg</td>
</tr>
<tr>
<td></td>
<td>0.85 ±0.05&lt;sup&gt;b,a&lt;/sup&gt;</td>
<td>0.11 ±0.04&lt;sup&gt;b,b&lt;/sup&gt;</td>
<td>0.84 ±0.05&lt;sup&gt;b,a&lt;/sup&gt;</td>
<td>0.76 ±0.03&lt;sup&gt;c,a&lt;/sup&gt;</td>
<td>0.13 ±0.03&lt;sup&gt;b,b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 mg</td>
<td></td>
<td>2 mg</td>
<td></td>
<td>2 mg</td>
</tr>
<tr>
<td></td>
<td>1.11 ±0.08&lt;sup&gt;b,a&lt;/sup&gt;</td>
<td>0.39 ±0.04&lt;sup&gt;b,b&lt;/sup&gt;</td>
<td>1.09 ±0.03&lt;sup&gt;c,a&lt;/sup&gt;</td>
<td>1.10 ±0.06&lt;sup&gt;b,a&lt;/sup&gt;</td>
<td>0.34 ±0.09&lt;sup&gt;b,b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 mg</td>
<td></td>
<td>4 mg</td>
<td></td>
<td>4 mg</td>
</tr>
<tr>
<td></td>
<td>1.25 ±0.05&lt;sup&gt;b,a&lt;/sup&gt;</td>
<td>0.43 ±0.03&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.23 ±0.05&lt;sup&gt;b,a&lt;/sup&gt;</td>
<td>1.19 ±0.05&lt;sup&gt;b,a&lt;/sup&gt;</td>
<td>0.40 ±0.05&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Figure 7-9. (cont’d)*
The effects of proximity were further explored to evaluate how antimicrobial gas impacts the product throughout the bag, by considering log_{10} CFU/g reductions of *E. coli* O157:H7 in different areas of the bag, as shown in Figure 7-9.

After day 1, for one-GR and two-GR designs, regardless of the level of ClO₂ gas used, reductions in cell population of *E. coli* O157:H7 in the samples, adjacent to the GRs, were significantly higher than those samples further away from the reservoirs. In samples from one-GR bags, reductions in *E. coli* O157:H7 were statistically equal between samples taken from the middle area and the area most distant from the reservoir. Similar trends were observed in day 4 and 7 of storage. As expected, these results confirmed that ClO₂ gas was most effective in the area close to the GRs, thus, proximity of antimicrobial gas releasing location to the target surface is crucial to maximize the antimicrobial effect (Ellis *et al.*, 2006).

The softer and watery shredded leaves could partially block the gas flow to other areas of the bag, and further reduced accessibility to the target surfaces, especially for those areas further away from the GRs, resulting in low kills in the area distant from the releasing locations (Du *et al.*, 2002; Ellis *et al.*, 2006).

### 7.3.2. Study 2

To minimize the distance between ClO₂ gas releasing location and the shredded lettuce, the second experiment was conducted by modifying the interior of the bag and placing the reservoir in the middle of a package chamber (Figure 7-2, Figure 7-5, and Figure 7-10). Chlorine dioxide precursors were put in a LDPE tube, which has very low
barrier to ClO₂ gas. Gas delivery was regulated by permeability of the selected film, so that the gas would be readily released and available for gas-phase decontamination within the package chamber (Appendix 8). For this study, ClO₂ was released through the entire surface of LDPE tube, while, in the previous study, ClO₂ gas was mainly delivered through the 6 openings located vertically along the edges of the chamber.

The differences between microbial populations in control bag and those in middle-GR bag (Table 7-3) were reported as log₁₀ CFU/g reductions of *E. coli* O157:H7 as shown in Figure 7-11. The calculated average log₁₀ CFU/g reductions for the whole bag were shown in Figure 7-12. The results followed the same trend as those found in the first experiment; increasing ClO₂ gas in the headspace significantly increased the reduction in cell population of *E. coli* O157:H7 (Han *et al.*, 2000; Lee *et al.*, 2004).

Also, appearance changes, including bleached and watery tissues, more noticeable in lettuce from the 8 mgClO₂/day bag, with those changes most evident in lettuce pieces next to the GRs (Ellis *et al.*, 2006; Gómez-López *et al.*, 2009).

For lettuce bags containing a dose of 4 mgClO₂/day dose, no improved microbial inactivation was observed between the two-GR (Figure 7-6(d)) and middle-GR bags (Figure 7-12(b)). Thus, using either two-GR design bag or a middle-GR design bag, with 4 mgClO₂/day, yield similar microbial inactivation, even though the releasing areas of both designs were not equal. This could be attributed to the reactive nature of ClO₂ gas.
More extensive understanding of ClO₂ gas inside the package is needed to further understand this finding.

On the other hand, comparison of log₁₀ CFU/g reductions between samples taken from two-GR design bags (Figure 7-6(c)) and middle-GR design bags (Figure 7-12(a)), with 8 mgClO₂/day, showed that moving the reservoir from the edges to the center of the chamber and increasing the gas releasing surface area significantly improved the *E. coli* O157:H7 killing efficiency of ClO₂ gas, on day 1, 4, and 7, by 0.61, 0.37, and 0.27 log₁₀ CFU/g, respectively, but, at this ClO₂ dose, the appearance of the lettuce was compromised (Figure not shown).

Given that two-GR design bags, with 4 and 8 mgClO₂/day, both showed comparable average log₁₀ CFU/g reductions in the first experiment, it was likely that the soft and watery lettuce pieces, adjacent to the reservoir opening, partly blocked the flow of ClO₂ gas, preventing the gas distribution, especially, those in 8 mgClO₂/day bags. Increasing the release area from 6 small openings to the entire surface of a LDPE tube could be the main factor in improving the gas distribution in the middle-GR design (with 8 mgClO₂/day), in a more uniform fashion, resulting in the significantly higher average log₁₀ CFU/g reduction of *E. coli* O157:H7.
Day 0: Browning was visible at the cut of some lettuce pieces; bruises also present (darker green color), especially on the leaf area
Day 1: Browning was more visible
Day 4: Browning occurred more extensive and darker in color, especially at the cut area of midribs
Day 7: Browning found on most of the lettuce pieces; the texture became watery and soft in many pieces, especially the ones contained midrib parts

**Figure 7-10(a).** *E. coli* O157:H7 inoculated shredded lettuce packed in plain bags with 0.0 mgClO$_2$/kg (Control for study 2), stored at 4°C for 0, 1, 4, and 7 days
Day 0: Browning was visible at the cut of some lettuce pieces; bruises also present (darker green color), especially on the leaf area
Day 1: Browning was visible
Day 4: Browning was darker in color, especially at the cut area of midribs; bleaching was noticeable on many lettuce pieces around GRs; lettuce pieces near GRs also became watery and soft
Day 7: Browning and bleaching was more extensive; the texture became watery and soft in many pieces, especially the ones located next to GRs

**Figure 7-10(b).** *E. coli O157:H7* inoculated shredded lettuce packed in middle-GR bags with 8.0 mgClO₂/kg, stored at 4⁰C for 0, 1, 4, and 7 days
Day 0: Browning was visible at the cut of some lettuce pieces; bruises also present (darker green color), especially on the leaf area
Day 1: Browning was darker in color
Day 4: Browning was more extensive; bleaching was noticeable on some lettuce pieces around GRs; some lettuce pieces near GRs also became watery and soft
Day 7: Browning was more extensive; the texture became watery and soft in some pieces, especially the ones located next to GRs; bleaching was visible in many lettuce pieces

**Figure 7-10(c).** *E coli* O157:H7 inoculated shredded lettuce packed in middle-GR bags with 4.0 mgClO$_2$/kg, stored at 4°C for 0, 1, 4, and 7 days
<table>
<thead>
<tr>
<th>Day 1</th>
<th>1.76 ±0.06&lt;sup&gt;a,a,1&lt;/sup&gt;</th>
<th>0.66 ±0.19&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>0.43 ±0.02&lt;sup&gt;b,b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 4</td>
<td>1.87 ±0.04&lt;sup&gt;a,a&lt;/sup&gt;</td>
<td>0.59 ±0.01&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.51 ±0.07&lt;sup&gt;b,a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Day 7</td>
<td>2.13 ±0.22&lt;sup&gt;a,a&lt;/sup&gt;</td>
<td>0.34 ±0.05&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.63 ±0.29&lt;sup&gt;b,a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Figure 7-11. Log<sub>10</sub> CFU/g reduction of *E. coli* O157:H7 of sample portions stored for 1, 4, and 7 days in middle-GR design bag with 8 mgClO<sub>2</sub>/day (a) and middle-GR design bag with 4 mgClO<sub>2</sub>/day (b)

<sup>1</sup> First different script letters indicates statistically differences between means at α of 0.05 of different sample bags with the same portion; Second different script letters indicates statistically differences between means at α of 0.05 of samples with different portions within the same bag.
**Figure 7-12.** Average log_{10} CFU/g reduction of *E. coli* O157:H7 of samples stored for 1, 4, and 7 days in middle-GR design bag with 8 mg ClO₂/day (a) and middle-GR design bag with 4 mg ClO₂/day (b).

First different script letters indicates statistically differences between means at α of 0.05 of different bag designs with the same storage time; Second different script letters indicates statistically differences between means at α of 0.05 of samples with different storage times within the same design.
Even though, at the testing conditions, utilization of ClO$_2$ as an antimicrobial gas in the package gave only up to 1.24 ± 0.09 log$_{10}$ CFU/g reduction of *E. coli* O157:H7 (for middle-GR design, with 8 mgClO$_2$/day; Figure 7-12(a)), it is beneficial to note that the average values represent the average kills for a particular design and ClO$_2$ level, but the closer look specifically at each bag portion indicated that, at the area next to gas releasing locations, as high as 2 log$_{10}$ CFU/g reduction could be achieved.

7.4. Conclusion

Three types of flexible bags with different modified interior structures were used to package *Escherichia coli* O157:H7 inoculated shredded lettuce, along with 2 levels of ClO$_2$ gas, i.e. 4 and 8 mgClO$_2$/day which were released from gas reservoirs located in different areas of the bag interior, i.e. from one side of the bag, from both sides of the bag, and from center of the bag in one-GR, two-GR, and middle-GR, respectively. The samples were kept at 4°C and stored for 1, 4, and 7 days. Log$_{10}$ CFU/g reduction of *E. coli* O157:H7, from different sections of the bag, were determined. The results showed that increasing the level of ClO$_2$ gas per day increased the reduction in cell population of *E. coli* O157:H7 with the highest log$_{10}$ CFU/g reduction found in samples taken from the area next to the gas reservoir. The calculated average log$_{10}$ CFU/g reductions for the whole bag showed that ClO$_2$ gas gave up to 1.24 ± 0.09 log$_{10}$ CFU/g reduction of *E. coli* O157:H7, depending on the modified interior design, and two-GR and middle-GR bag
designs gave higher $\log_{10}$ CFU/g reduction than those from one-GR design, for both ClO$_2$ gas levels. The bag design that minimized the distance between the gas reservoir and the sample showed higher $\log_{10}$ CFU/g reduction. Changes in texture and appearance of the sample were visually observed, especially in the samples taken from the area exposed to higher level of ClO$_2$ gas and considered to be one of the factors that affects the gas distribution inside the package.

This study also indicated that; design optimization can result in applying smaller dose of ClO$_2$ gas (4.0 mgClO$_2$/day) to achieve the same level of $\log_{10}$ CFU/g reduction of *E. coli* O157:H7 with one-GR at a higher dose. Delivery of ClO$_2$ gas within the packaging system could also be adjusted and regulated to suit specific conditions of particular product requirements by selecting polymer films with different barrier properties; or by changing locations or numbers of openings of GRs.


CHAPTER 8: OVERALL CONCLUSIONS
AND FUTURE WORKS

8.1. Overall conclusions

This work presents a new approach for the implementation of chlorine dioxide (ClO₂) as an antimicrobial gas in food packaging applications, particularly, for fresh and fresh cut produce. Such antimicrobial packaging systems could also be regarded as part of a hurdle technology where the packaging system will provide the last stage of sanitation to assure a safer food product.

To be able to consider ClO₂ gas as an antimicrobial agent for vapor-phase decontamination in food packaging systems, the knowledge of its compatibilities with packaging materials, especially with polymeric films which are a major component of typical food packaging materials, is essential. The presence of ClO₂ gas in the packaging system should be a compliment to the active-protective function of the package and does not compromise the system’s performance, e.g. barrier properties as well as mechanical properties.

The understanding on how packaging could be modified to impact antimicrobial effects of ClO₂ gas is also critical in order to maximize efficiency of the gas. Therefore, packaging design could turn into an important tool to improve performance of
disinfecting gases. Although ClO₂ is being considered in this specific work as the antimicrobial gas, this approach could be implemented into other gases or vapors.

This work was aimed to study the potential use of ClO₂ gas in food packaging applications. Specific objectives, along with the corresponding hypotheses, were stated earlier in Chapter 1. A summary of the main findings are outlined here:

**8.1.1. Mass transfer of ClO₂ gas**

According to the P values for selected films which ranged between $\leq 7.0 \times 10^{-19}$ – $9.68 \times 10^{-16}$ kgClO₂·m·m²·s·Pa, BOPP, PET, PLA, nylon, and multilayer EVA/EVOH/EVA were categorized as high barrier to ClO₂, while PEs, PVC, and PS were regarded as having low barrier properties to ClO₂ gas. The activation energy of permeation (E_p) of PET and PLA films were also determined (Netramai et al., 2009).

**8.1.2. Impact of ClO₂ gas on chemical, physical, mechanical, and barrier properties of packaging materials**

After 14 days of treatment, ClO₂ exposure caused decreases in tensile and barrier properties in some films, for example, PEs and PET, while improved barrier properties to O₂ were observed in treated nylon film. Changes in permselectivity ($P_{CO2}/P_{O2}$) were found in many polymer types, with HDPE showing the most change at 46.8%.
8.1.3. Antimicrobial activity of ClO₂ gas on shredded lettuce

Antimicrobial effects of ClO₂ gas on microorganisms residing on lettuce have been reported many times, during the course of this entire study and found to increase as the applied concentration and/or exposure time increased. For example, ClO₂ used in gas-phase decontamination inside the package of shredded lettuce inoculated with *E. coli* O157:H7, ClO₂ at a level of 8 mgClO₂/kg lettuce gave higher log₁₀ CFU reduction of *E. coli* O157:H7 as compared to 4 mgClO₂/kg lettuce. But, higher ClO₂ level used also increase changes in appearance, i.e. watery and soften tissue, of shredded lettuce.

Regardless of the general trend, i.e. increasing the ClO₂ level increased average log₁₀ CFU reduction, the degree of disinfection depended largely on different packaging designs, which will be concluded in the next section.

Residual ClO₂ and ClO₂⁻ absorbed by lettuce was quantified to study the lettuce/ClO₂ absorption pattern and determine if the amount of ClO₂ absorbed by lettuce is significant enough to take into account when selecting ClO₂ dose for vapor-phase decontamination. According to the findings, increasing concentration and/or time of exposure increased absorption of ClO₂ by lettuce. The more severe treatment conditions also increased appearance changes.
8.1.4. Effects of packaging design on antimicrobial effectiveness

To study the influences of packaging design on antimicrobial effects of ClO₂ packaging system for shredded lettuce, 3 types of flexible bag with different modified interior structures were used. Each design released ClO₂ gas from different locations. The studies indicated that the bag design allowing the shortest travel distance of ClO₂ gas to the sample helped improved reduction of \( E. \text{coli O157:H7} \) population.

Through optimization, lower levels of ClO₂ could be used to achieve the comparable level of \( \log_{10} \) CFU reduction and still maintain the produce’s appearance and texture.

8.2. Applications and future works

8.2.1. Mass transfer of ClO₂ gas

Information on compatibilities of ClO₂ gas and polymeric materials, especially regarding mass transfer characteristics and the possible effects of ClO₂ exposure on different types of polymeric materials from this study is the beginning step of effectively including antimicrobial gas in the packaging system; or designing the packaging system, by selecting suitable material, for the system that included ClO₂ gas.

Polymeric materials show wide range of barriers to ClO₂ gas (Netramai et al., 2009). With this basic knowledge, one can narrow down the material choice to best fit required applications, for example, if the packaging system needs to maintain ClO₂ gas
within the package for long-term exposure, then materials with high barrier to ClO₂ gas are recommended. However, if the package needs to deliver certain amount of ClO₂ for particular time frame, the information on permeability coefficient of many polymer films could serve as initial guideline for material selection, as in the case of LDPE selection for ClO₂ gas reservoir in packaging study (Chapter 7).

Mass transfer study of ClO₂ gas should be explored further by varying the testing conditions, especially expanding the temperature range to cover typical storage temperature for fresh produce, i.e. 4°C, since the permeation behavior of the polymer is temperature dependent (Selke et al., 2004; Van Krevelen, 1997). The study on Eₚ of PET and PLA also clearly indicated this fact. The calculation on ClO₂ level for packaging study 2 (Appendix 8) also demonstrated the disadvantage of the lack of said information.

A system with a continuous supply of ClO₂ gas at a constant concentration is also desirable. The developed system described in Chapter 3 was stable for the testing durations used, i.e. ≤ 24 hours for permeation study at 23°C, but, if the longer testing time is necessary, for example, at the lower temperature where permeation process happen in slower rate, ClO₂ solution might not be a suitable gas source (even though, at lower temperature, ClO₂ gas should also degrade in a slower rate).
8.2.2. Impact of ClO₂ gas on properties and performance of packaging materials

Regarding chemical degradations of polymeric films caused by exposure to ClO₂ gas, since statistical differences exist between untreated and ClO₂-treated film after 14 days of treatment and the results were obtained at 10 mg/L of ClO₂ gas which is at the upper end of its range in food applications (refer to Table 2-2, Chapter 2), this study was conducted under an extreme scenario used in food applications. Therefore, if any further study is needed, it should be done at specific exposure condition that represents the use of ClO₂ gas in particular produce application.

On the other hand, for other applications, such as in medical products’ packaging systems which might require higher level of ClO₂ for the applications, the influences of ClO₂ gas on the package performance could be of greater concern and additional tests should be performed, before finalizing material selection.

8.2.3. Improving antimicrobial capacity of ClO₂ through packaging design

8.2.3.1. Important factors in packaging design

In the packaging study, the effects of packaging design on antimicrobial activities of ClO₂ gas were quantified by determining log₁₀ CFU reduction of *E. coli* O157:H7 in treated shredded lettuce located in different areas of the bag. Even though, at the testing conditions, utilization of ClO₂ as an antimicrobial gas in the package gave only up to
1.24 ± 0.09 log<sub>10</sub> CFU/g reduction of *E. coli* O157:H7 (for middle-GR design, with 8 mgClO<sub>2</sub>/day), it is beneficial to note that the average values represent the average kills for particular design and ClO<sub>2</sub> level, but the closer look specifically on each bag portion indicated that, at the area next to gas releasing locations, as high as 2 log<sub>10</sub> CFU/g reduction could be achieved. Thus, interior characteristics of the package, i.e. location and size of the releasing area for ClO<sub>2</sub> gas, influenced its antimicrobial effects on *E. coli* O157:H7 inoculated on shredded lettuce; minimizing the travel distance between ClO<sub>2</sub> gas and the target surface, as well as maximizing the releasing surface of antimicrobial gas, proved to increase the log<sub>10</sub> CFU/g reduction of particular pathogen. These findings could be utilized in modifying the interior of packages, for food products, that will be used in conjunction with any antimicrobial gas included in the packaging system, without changing the packaging exterior.

Many other important factors are also known to influence sanitizing effects of antimicrobial gas. Some studies showed that it was more difficult to inactivate microorganisms on injured or cut surfaces, as compared to those on smooth surfaces (Han *et al.*, 2000; Mahovic *et al.*, 2009). Therefore, shape and size of the products, as well as the surface characters, including damages and bruises, are also an important parameter to be considered (Du *et al.*, 2002; 2003; Gómez-López *et al.*, 2009; Han *et al.*, 2000; Mahovic *et al.*, 2009; Yuk *et al.*, 2006). Du *et al.* reported the different in log<sub>10</sub> CFU/site reduction of *E. coli* O157:H7 and *Listeria monocytogenes*, obtained from different areas of the fruit, after ClO<sub>2</sub> treatment (Du *et al.*, 2002; 2003). Therefore, additional studies
should be extended to the applications of ClO$_2$ gas as a headspace gas for other types of packaged produce, especially one that has different shape, size, and surface characters, such as cherry tomatoes, baby carrots, etc (Du et al., 2002; Han et al., 2000; Mahovic et al., 2009; Yuk et al., 2006), for better understanding on the factors affecting the antimicrobial gases’ efficiency in vapor phase decontamination for food packaging application.

Microbial enumeration should also be included in any future study on absorption pattern of ClO$_2$, as it could help explaining ClO$_2$-consumption behavior of produce at different treatment conditions, besides, it is important to determine if consumption of ClO$_2$ gas could also be influenced by microbial load on the food products, as mentioned earlier. Future experiments should also included testing temperature at 4°C, for both absorption test and experiment on degradation of ClO$_2$ and ClO$_2^-$ on exposed produce, since the absorption behavior might depend on 1) solubility of ClO$_2$ in solution and 2) the influences of temperature of its disinfecting capacity (USEPA, 1999).

To expand the scope of ClO$_2$-absorption study further, other commodities should be included in future works. Different types of produce provide entirely different shapes, textures, and surface characteristics, as well as different skin pigments and normal flora resided on its surface, thus, different ClO$_2$-absorption behavior, which could partially be indicated by different sanitizing results when the same treatment were applied to various food products, could be expected (Du et al., 2003; Han et al., 2000; Han et al., 2004; Rodgers et al., 2004; Sy et al., 2005).
8.2.3.2. Computational modeling of antimicrobial packaging system

Conventional way of studying the antimicrobial effect of ClO\textsubscript{2} gas in a particular packaging system would be as what has been done in this study (chapter 7), by conducting the actual experiment. Gathering enough information through repeated experiments could be time consuming and might not be applicable for certain testing conditions. In many cases, computational modeling based on principle of finite element analysis (FEA) could be a very useful tool in packaging development to help predict and, to a certain extent, to quantify the outcome of the study. Computational fluid dynamic (CFD) is the science of solving fluid flow, heat and mass transfer, chemical reactions and related phenomena. FEA is a numerical technique applied to solve complex partial differential or integral equations encountered in many continuous engineering domain, e.g. elasticity, aeronautical problem, etc. by discretize the domain into a set of ‘finite’ sub-domains, or ‘element’ (or volume or cell). Considering each finite element would simplify or even eliminate the need to solve complicated domain, giving a reasonable estimation depending on the required precision and accuracy. The overview of CFD is shown in Figure 8-1 CFD process starts with defining the model’s goals, then identifies the model domain, and designs and creates the grids (e.g. shape and size of the package and releasing location of ClO\textsubscript{2} gas). To set up an appropriate numerical model to solve for specific system, for example product/package system, first, one needs to select appropriate physical model, e.g. turbulence for gas mixing and monitoring surface contact within the package. Material properties (e.g. chemical and physical properties of ClO\textsubscript{2}, mass transfer behavior of the package, and absorption behavior of ClO\textsubscript{2} on lettuce)
and boundary conditions, are necessary in model building. After computing the solution, the results will be verified. And one might consider revise the model, if the outcome did not reflect real circumstance. Actual experiment might also be conducted to confirm the prediction of the model, but the number of real tests performed could be significantly smaller.

Gathering parameters necessary to build an appropriate CFD model could be difficult and time-consuming, especially when dealing with new type of system where many data has never been generated before, as in the case of food product/packaging/antimicrobial gas system. The information obtained from this work, i.e. compatibilities of ClO₂ gas with polymeric materials, the effects of packaging design, especially on gas releasing locations on antimicrobial activities of ClO₂, as well as, the ClO₂-absorption behavior of lettuce are among a few parameters that could be beneficial in CFD modeling for packaging study. Many additional data type are needed to develop the CFD model for food packaging study in general, for example, the effects of shape and texture of food products.
Figure 8-1. CFD modeling overview. Adapted from (Fluent Inc., 2005).
To be able to effectively monitor and quantify the behavior of ClO₂ gas inside the package, in real-time, small, precise, and accurate ClO₂-sensor is needed. Unfortunately, most sensors for ClO₂ are suitable for atmosphere measurement which requires large volume of gas sample or suitable for determining ClO₂ in solution from (USEPA, 1999). Some chemical sensors are developed for detecting trace amount of ClO₂ gas, but the size of the equipment is still too large to incorporate into the food packages, as in the case of detector used in mass transfer study (Chapter 3 and 4).

Using CFD in predicting packaging performance under various circumstances has become a useful tool for many studies in the past few years (Abdul Ghani, 2003). However, there are many challenges in applying it in antimicrobial packaging study. One of a major drawback, for example, is to correlate the outcome of the model, in terms of gas circulation, with the sanitizing efficiency of selected antimicrobial gas incorporated into the packaging system. If such approach could successfully be developed, it could be applied to any potential antimicrobial gas/packaging system.

Since the use of ClO₂ gas in antimicrobial packaging application could also be introduced as one of preservation methods in hurdle strategy, before the products are being delivered to the consumers (Allende et al., 2006; Gómez-López et al., 2009; Huang et al., 2006; Rico et al., 2007). By applying several technologies to assure safety and improve shelf-life of food products, milder treatment for each technique could be used to achieve significant results. Future studies should explore the effects of the combination of antimicrobial packaging with other mild, yet effective, technologies, such as sequential
cleaning and ultrasound (Allende et al., 2006; Huang et al., 2006; Pao et al., 2009; Singh et al., 2002), as well as the use of other packaging techniques, like modified atmosphere packaging (MAP) (Jin and Lee, 2007). Many processes can be applied throughout the food production lines to give synergistically disinfecting effect to improve safety of food products, while maintain their near-fresh quality.
BIBLIOGRAPHY


This study focuses on studying the topographic characters of *E. coli* O157:H7 biofilm on Romaine lettuce leaf surfaces, before and after being exposed to ClO₂ gas by using Scanning Electron Microscope (SEM) (Netramai et al., 2010).

**Research plan:**

- **Lettuce sample preparation:** Shredded Romaine lettuce pieces were inoculated with inoculum of *E. coli* O157:H7 SEA and kept at 4°C, for overnight, to allow bacteria attachment. The 48-hour inoculated samples had *E. coli* O157:H7 population of 8.33 ± 0.05 log₁₀ CFU/g sample.

- **Chlorine dioxide treatment of lettuce sample:** Inoculated lettuce samples were treated with 0.1 and 0.2 mg/L of ClO₂ gas (continuous exposure) for 0 - 60 min in an air-tight chamber as shown in Figure A-1. After predetermined time intervals, the samples were taken out; 25 g of treated sample was sampling for *E. coli* O157:H7 enumeration on Tryptic Soy Agar (TSA) overlaid with Sorbital MacConkey Agar with Cefixime, and Tellurite (CT-SMAC) (Han et al., 2002).

- **SEM procedure:** Both untreated and ClO₂-treated- inoculated lettuce pieces were cut into specific size (circle with diameter of 1 cm), underwent fixation and dehydration processes using the Microscopic Imaging facility at ERRC, USDA. Samples were fixed by immersion using 2.5% Glutaraldehyde for 1 hour.
followed by a 0.1M Imidazole buffer rinse (2X). Tissue was dehydrated through an Ethyl Alcohol series of 50, 80, 90 and 100% (3X), for 1 hour each. The samples were then critical point dried with Liquid CO$_2$ (Denton Vacuum, CP-1) process treatment, and sputter coated (Edwards Scan Coat Six) with gold (20 mA for 30 sec, repeated 2 times). SEM microscopic images were obtained using Quanta 200 FEG Environmental Scanning Electron Microscope using a voltage of 10.0 kV at 3.0 nm spot size.

![Figure A-1. Chlorine dioxide gas treatment of shredded lettuce in an air-tight chamber](image)

**Results:**

The untreated surfaces of inoculated lettuce leaf (48 hrs after inoculation) were covered with continuous layer of *E. coli* O157:H7 biofilm as shown in Figure 2-3. Bacteria cells were visible in some areas and distributed unevenly, accumulated more in the wrinkle surfaces.
After the inoculated surfaces were exposed to 0.2 mg/L of ClO₂ gas for up to 60 min, microbial enumeration showed *E. coli* O157:H7 reduction of \( \leq 1.57 \pm 0.05 \) log\(_{10}\) CFU/g sample, as listed in Table A-1.

The ClO₂-treated surfaces showed the absence of biofilm and the biofilm being destroyed (Figure 2-4, Chapter 2), in many area. By visual observation of the surfaces, using SEM, the number of *E. coli* O157:H7 cells visibly on the surfaces also noticeably decreased.

It should be noted that the observed results from SEM images showed possibility of biofilm being partially removed, after ClO₂ treatment, but the microbial population was only reduced by less than 2 log\(_{10}\) CFU/g sample. Furthermore, another research conducted by Ölmез and Temur on sanitizing effects of ozone (which is considered to be stronger oxidizing agent than ClO₂) and chlorine solutions, on biofilm of *E. coli* O157:H7 on lettuce leaf, indicated that there was no removal of biofilm even though the population of target microorganism was reduced up to 1.5 log\(_{10}\) CFU/g sample (Ölmез and Temur, 2010).

Further works are recommended, to confirm effects of ClO₂ gas on biofilm of *E. coli* O157:H7 on lettuce leaf.
Table A-1. Log$_{10}$ CFU reduction of *E. coli* O157:H7 and total aerobic plate count (TAPC) of ClO$_2$ treated lettuce sample

<table>
<thead>
<tr>
<th>Treatment/Time</th>
<th>ClO$_2$ Concentration (mg/L)</th>
<th>E. coli O157:H7 (log$_{10}$ CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>30 min</td>
<td>$0.69 \pm 0.04^{a,1,2}$</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
<td>$1.05 \pm 0.06^{b}$</td>
</tr>
<tr>
<td>0.2</td>
<td>30 min</td>
<td>$1.14 \pm 0.02^{a}$</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
<td>$1.57 \pm 0.05^{b}$</td>
</tr>
</tbody>
</table>

1 Mean ± standard deviation
2 Different superscript letters indicates statistically differences between means, in the same column, at $\alpha$ of 0.05
APPENDIX 2: Thiosulfate titration for determination of ClO₂ in solution

The analytical procedure for determining ClO₂ level in solution was described below. This method was modified from titration method outlined by ICA Trinova (ICA TriNova LLC, 2006; Post and Moore, 1959).

Apparatus:

- 50 ml Burette and Stand
- 50 ml Erlenmeyer flask
- pH meter

or

- Automatic titrator equipped with pH meter

Reagents:

- 10% (wt/wt) Potassium Iodide (KI) solution
- 0.1 N, 0.01 N, and/or 0.001 N Sodium Thiosulfate (Na₂S₂O₃) (Certified solution)
- 2 N Sulfuric acid (H₂SO₄)
- Starch indicator
Procedure:

The procedure outlined below is for manual titration.

1. Sampling known amount of ClO₂ solution (Vₛ mL) and put into Erlenmeyer flask.
   Add KI solution if the volume is too low.
2. (Add starch indicator, if required.)
3. Titrate the solution to a colorless end-point with Na₂S₂O₃. Record result as Vₙ.
4. Adjust pH by adding H₂SO₄ to a pH of ≤ 2.
5. Allow the solution to stand in the dark for 10 min.
6. Titrate the solution to a colorless end-point with Na₂S₂O₃. Record result as Vₐ.
7. Discard the solution.

Calculation:

To obtain results in mg ClO₂/L:

\[
\text{ClO}_2 \ (mg/L) = \frac{V_a \times N \times 67.5 \times 10^3}{4 \times V_s}
\]

where \( N = \) Normality of Na₂S₂O₃.
APPENDIX 3: Results for consistency test on isostatic method

In continuous flow permeation, the value of the permeant flow at time t, $F_t$, is described below:

$$\frac{F_t}{F_\infty} = \left(\frac{4}{\sqrt{\pi}}\right)\left(\sqrt{\frac{\ell^2}{4Dt}}\right) \sum_{n=1,3,5}^\infty \exp\left(-n^2 \frac{\ell^2}{4Dt}\right)$$  \hspace{1cm} (1)

where $\frac{F_t}{F_\infty}$ is the permeant flow ratio in the 0 partial pressure side and t is the permeation time. For short time, equation (1) explains permeation process from time $t = 0$ up to 95\% of steady state and can be simplified as:

$$\phi = \left(\frac{4}{\sqrt{\pi}}\right)X^{1/2} \exp(-x)$$  \hspace{1cm} (2)

Where $\phi$ is $\frac{F_t}{F_\infty}$ and $X$ is $\frac{\ell^2}{4Dt}$.

To be able to assume that polymeric material characteristics, permeant properties, temperature, and concentration gradient remain unchanged or constant, during permeation process, the consistency test must be performed. The test consists of 1) determining $R^2$ of a straight line plot between $\frac{1}{x_t}$ versus time to reach at $\phi_t$, with intercept pass the origin (0,0); and 2) determining $K_1$ and $K_2$ where
\[ K_1 = \frac{t_{1/4}}{t_{3/4}} \quad \text{and} \quad K_2 = \frac{t_{1/4}}{t_{1/2}}. \]

Table A-2 reported values of \( K_1, K_2, \) and \( R^2 \) obtained from consistency test for permeation experiments conducted and reported in Chapter 3.

All values indicated that there is no unacceptable variation of system parameters, such as permeant concentration and temperature fluctuation, and isostatic permeability experimental conditions can be assumed (Gavara and Hernandez, 1993).

**Table A-2.** \( K_1, K_2, \) and \( R^2 \) for consistency test

<table>
<thead>
<tr>
<th>Polymer</th>
<th>( 0.42 &lt; K_1 &lt; 0.46 )</th>
<th>( 0.65 &lt; K_2 &lt; 0.69 )</th>
<th>( R^2 ) (set to pass the origin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDPE</td>
<td>0.45 ± 0.01</td>
<td>0.68 ± 0.02</td>
<td>0.9837</td>
</tr>
<tr>
<td>LDPE</td>
<td>0.44 ± 0.02</td>
<td>0.66 ± 0.01</td>
<td>0.9703</td>
</tr>
<tr>
<td>LLDPE</td>
<td>0.43 ± 0.01</td>
<td>0.67 ± 0.02</td>
<td>0.9793</td>
</tr>
<tr>
<td>BOPP</td>
<td>0.46 ± 0.00</td>
<td>0.67 ± 0.01</td>
<td>0.9512</td>
</tr>
<tr>
<td>PS</td>
<td>0.43 ± 0.01</td>
<td>0.67 ± 0.02</td>
<td>0.9534</td>
</tr>
<tr>
<td>PVC</td>
<td>0.43 ± 0.01</td>
<td>0.66 ± 0.01</td>
<td>0.9841</td>
</tr>
<tr>
<td>PET</td>
<td>0.45 ± 0.01</td>
<td>0.67 ± 0.01</td>
<td>0.9703</td>
</tr>
<tr>
<td>PLA</td>
<td>0.44 ± 0.02</td>
<td>0.67 ± 0.01</td>
<td>0.9782</td>
</tr>
<tr>
<td>Nylon</td>
<td>0.44 ± 0.02</td>
<td>0.68 ± 0.01</td>
<td>0.9703</td>
</tr>
<tr>
<td>EVA/EVOH/EVA</td>
<td>nd (^{1})</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{1}\) Not determined
APPENDIX 4: Amperometric titration for determination of ClO₂ in solution

The analytical procedure for determining ClO₂ and ClO₂⁻ level in solution was described below. This method was modified from standard amperometric titration method for the examination of water and wastewater (4500-ClO₂ C) (Greenberg et al., 1992; Haller and Listek, 1948).

Apparatus:

- 50 ml Erlenmeyer flask or specific sample cup for titrator
- pH meter
- Titrator equipped with potentiometer

Reagents:

- 5% (wt/wt) Potassium Iodide (KI) solution
- 0.00564 N and/or 0.000564 N Phenylarsine oxide, PAO ((C₆H₅)AsO)
  (Certified solution)
- Phosphate buffer, pH 7.0
- 6 N Sulfuric acid (H₂SO₄)
- 6 N Sodium hydroxide (NaOH)
Procedure:

1. Divide sample solution into 3 portions, 200 mL each.
2. Portion 1: Adjust pH by adding NaOH to a pH of ≥ 12. After 10 min, adjust pH to 7 by adding H₂SO₄. Add 1 mL of KI solution. Titrate the solution to an end-point with PAO. Record result as A.
3. Portion 2: Adjust pH by phosphate buffer to a pH of 7. Add 1 mL of KI solution. Titrate the solution to an end-point with PAO. Record result as B.
4. Portion 3: Add 1 mL of KI solution. Adjust pH by adding H₂SO₄ to a pH of ≤ 2. After 10 min, adjust pH to 7 by adding NaOH. Titrate the solution to an end-point with PAO. Record result as C.
5. Discard the solutions.

Calculation:

To calculate ClO₂ in mg ClO₂/L:

\[ ClO_2 \ (mg / L) = 1.9(B - A) \]

To calculate ClO₂⁻ in mg Cl₂/L:

\[ ClO_2^- \ (mgCl_2 / L) = 4A - 5B + C \]
APPENDIX 5: ClO₂ degradation profile and recovery rate of the experimental setup for absorption study

The degradation profile of ClO₂ in the solution and the recovery rate of titration procedure for residual ClO₂ and ClO₂⁻ were discussed below.

Degradation profile of ClO₂ in the solutions:

The degradation rate of ClO₂ in the solutions (which give out ClO₂ gas level at 3.0 and 6.0 mg/L) was calculated from the different of ClO₂ in solution at particular time and the initial amount at the beginning of the experiment (0 min), as followed:

\[
\text{Degradation Rate (\%) = } \left( \frac{mg\text{ClO}_2, T_0 - mg\text{ClO}_2, T_t}{mg\text{ClO}_2, T_0} \right) \times 100
\]

where \( mg\text{ClO}_2, T_0 \) and \( mg\text{ClO}_2, T_t \) = mg ClO₂ in 1 L of solution at the exposure time of 0 and t min, respectively.

The degradation profiles are reported in Table A-3. As expected, the amount of ClO₂ in the solution gradually decreased with time, with slower rate for solution at higher level of ClO₂.
Table A-3. Degradation profile (%) of ClO₂ in solution according to time (min)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Degradation profile (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.0 mg/L ¹</td>
</tr>
<tr>
<td>15</td>
<td>3.96 ± 0.56</td>
</tr>
<tr>
<td>30</td>
<td>5.70 ± 0.30</td>
</tr>
<tr>
<td>45</td>
<td>5.94 ± 0.68</td>
</tr>
<tr>
<td>60</td>
<td>8.110.96</td>
</tr>
<tr>
<td>90</td>
<td>14.18 ± 1.19</td>
</tr>
</tbody>
</table>

¹ For the solutions to release 3.0 and 6.0 mg/L of ClO₂ gas into the chamber headspace, at 23°C, the actual amount of ClO₂ in 1 L of solution are approximately 87 and 175 mg.

Recovery rate of titration procedure for residual ClO₂ and ClO₂⁻:

Recovery rates of titration procedure are calculated according to equation described below, and are listed in Table A-4.

\[
\text{Recovery Rate (\%)} = \left( \frac{\text{mgClO}_2,R + \text{mgClO}_2,\text{Chlorite}}{\text{mgClO}_2,T0 - \text{mgClO}_2,T_t} \right) \times 100
\]

where \( \text{mgClO}_2,R \) = mg ClO₂ recovered from the procedure; and \( \text{mgClO}_2,\text{Chlorite} \) = mg ClO₂ as converted from mg ClO₂⁻ recovered.
Table A-4. Recovery rate (%) of titration procedure for residual ClO₂ and ClO₂⁻

<table>
<thead>
<tr>
<th>Recovery rate (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0 mg/L (^1)</td>
<td>6.0 mg/L (^1)</td>
</tr>
<tr>
<td>9.79 ± 1.22</td>
<td>42.99 ± 21.00</td>
</tr>
</tbody>
</table>

\(^1\) For the solutions to release 3.0 and 6.0 mg/L of ClO₂ gas into the chamber headspace, at 23°C, the actual amount of ClO₂ in 1 L of solution are approximately 87 and 175 mg.

Many literatures reported that, generally, ClO₂⁻ made up to 70% of end-products from reactions of ClO₂ with organic matters found in waste water (USEPA, 1999). Thus, the recovery rates at both ClO₂ levels may not represent the actual efficiency of the titration method. Also, noted that the amount of ClO₂ disappeared from the solution (through auto-degradation or other means, Table A-3) could also contributed to the reduction of ClO₂ in the solution over time. Table A-5 shows amount of ClO₂ disappeared during the experiment (Decrease, mg), which was calculated from the difference between ClO₂ at the beginning of the experiment (ClO₂@0, mg) and remaining ClO₂ at the end of the experiment (ClO₂@t, mg). Amount of ClO₂ lost through auto-degradation is listed in column ‘Auto-Degradation, mg’, while amount of ClO₂ recovered in the sample could be seen by combining residual ClO₂ and ClO₂⁻ (column ‘ClO₂-, mg’ and ‘ClO₂, mg’).
<table>
<thead>
<tr>
<th>Level, mg/L</th>
<th>Time, min</th>
<th>Wt sample, g</th>
<th>ClO2(_0), mg</th>
<th>ClO2(t), mg</th>
<th>Decrease, mg</th>
<th>Auto-Degradation, mg</th>
<th>ClO2(-), mg</th>
<th>ClO2, mg</th>
<th>Recovery rate, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0</td>
<td>30</td>
<td>48.1711</td>
<td>164.9194</td>
<td>162.9450</td>
<td>1.9744</td>
<td>1.9102</td>
<td>0.7464</td>
<td>1.43E-02</td>
<td>38.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>46.9669</td>
<td>179.8453</td>
<td>177.7275</td>
<td>2.1178</td>
<td>2.0830</td>
<td>0.6483</td>
<td>4.28E-02</td>
<td>32.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>49.7478</td>
<td>167.2313</td>
<td>165.2400</td>
<td>1.9912</td>
<td>1.9369</td>
<td>0.7196</td>
<td>1.43E-02</td>
<td>36.85</td>
</tr>
<tr>
<td>3.0</td>
<td>30</td>
<td>44.5234</td>
<td>83.7169</td>
<td>78.9244</td>
<td>4.7925</td>
<td>4.7696</td>
<td>0.4625</td>
<td>7.13E-03</td>
<td>9.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>43.4751</td>
<td>86.2228</td>
<td>81.2869</td>
<td>4.9359</td>
<td>4.9124</td>
<td>0.5139</td>
<td>4.28E-02</td>
<td>9.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>44.9116</td>
<td>83.6494</td>
<td>83.6494</td>
<td>4.7925</td>
<td>4.7657</td>
<td>0.3879</td>
<td>5.63E-02</td>
<td>9.27</td>
</tr>
</tbody>
</table>
APPENDIX 6: Characterization of PD-961EZ bag used in packaging study

PD-961EZ bags (26.67 × 43.18 cm²) were supplied from Cryovac (Cryovac Inc., Duncan, SC) in March, 2009. The bags were characterized to obtain chemical profile (as indicated by FT-IR spectrum), as well as important thermal and barrier properties (Table A-6), using procedures as previously outlined in Chapter 4 and 5. The determined characteristics were reported in Figure A-2 and thermal properties showed that the film had 2 T_m which coincided with those of LDPE and LLDPE, indicating that the film could be LDPE and LLDPE blend.

Figure A-2. FT-IR spectrum of PD-961EZ bag
FT-IR spectrum of the bag indicated that the film has chemical profile similar to those of PE materials, with the exception of the peak at around 1750 cm\(^{-1}\) region which coincided with that of carbonyl group in EVA material (Figure 5-6, Chapter 5). The film could be PE material coated with thin layer of EVA. Thermal properties showed that the film had 2 \(T_m\) which coincided with those of LDPE and LLDPE (Table 5-2, Chapter 5), indicating that the film could be LDPE and LLDPE blend.

**Table A-6. Important properties of PD-961EZ bag**

<table>
<thead>
<tr>
<th>Properties</th>
<th>Values (unit)(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(T_m)</td>
<td>~107 and 118(^{\circ})C</td>
</tr>
<tr>
<td>(P_{H2O})</td>
<td>(1.21 \pm 0.10 \times 10^{-19} \text{ kg} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \cdot \text{Pa}^{-1})</td>
</tr>
<tr>
<td>(P_{CO2})</td>
<td>(12.1 \times 10^{-17} \text{ kg} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \cdot \text{Pa}^{-1})</td>
</tr>
<tr>
<td>(P_{O2})</td>
<td>(1.03 \pm 0.12 \times 10^{-17} \text{ kg} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \cdot \text{Pa}^{-1})</td>
</tr>
<tr>
<td>(P_{ClO2})</td>
<td>(42.8 \pm 1.07 \times 10^{-17} \text{ kg} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \cdot \text{Pa}^{-1})</td>
</tr>
</tbody>
</table>

\(^1\) Mean ± standard deviation
APPENDIX 7: Identification of ClO₂ levels for packaging study

This study observed effects of different ClO₂ treatments on population of microorganism on shredded lettuce.

Research plan:

- **Chlorine dioxide treatment of lettuce sample:** Air-tight chamber (265 L) was filled with 0.1 mg/L ClO₂ gas before starting the batch treatment, and then 1.7 kg of un-inoculated shredded lettuce was laid on the bottom of the chamber. Samples were taken out at 15 and 30 min of exposure for microbial enumeration on Tryptic Soy Agar (TSA) (ClO₂ level was monitor during the experiment and the concentration read 0.0 mg/L at approximately 30 min of exposure).

Results:

Log₁₀ CFU reduction of total aerobic plate count (TAPC) of ClO₂ treated lettuce samples were shown in Table A-7. Since ClO₂ level was down to 0.0 mg/L at the end of 30 min, it is assumed that the total of 0.1 mg/L × 265 L = 26.5 mg of ClO₂ was used up by 1.7 kg of shredded lettuce; and, assumed that ClO₂ level decreased linearly, at 15 min of exposure, 26.5/2 = 13.25 mg of ClO₂ was consumed by the sample.
Based on visual observation, ClO$_2$ level at 8 (~7.79) mg/kg per day (or ~2.264 mg/283 g lettuce per bag per day) was selected as a high level ClO$_2$ treatment. The level of 4 mg/kg per day (or ~1.132 mg/283 g lettuce per bag per day) was then chosen as a logical choice to study the effect of concentration on antimicrobial effects of ClO$_2$, in the packaging study.

**Table A-7.** Log$_{10}$ CFU reduction of total plate count (TAPC) of ClO$_2$ treated lettuce sample

<table>
<thead>
<tr>
<th>Treatment time</th>
<th>TAPC (log$_{10}$ CFU/g)</th>
<th>Visual observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td>0.87 ± 0.08$^{a,1,2}$</td>
<td>-</td>
</tr>
<tr>
<td>30 min</td>
<td>1.37 ± 0.10$^b$</td>
<td>Many lettuce pieces bleached; mostly at the top layer of lettuce bed.</td>
</tr>
</tbody>
</table>

$^1$ Mean ± Standard Deviation

$^2$ Different superscript letters indicates statistically differences between means, in the same column, at $\alpha$ of 0.05
This section shows calculation of amount of ClO\textsubscript{2} precursors to be used in packaging study.

**Materials and chemicals:**

- Fast release ClO\textsubscript{2} precursor (0.5 mgClO\textsubscript{2}/g precursor per day)
- Slow release ClO\textsubscript{2} precursors (0.05 mgClO\textsubscript{2}/g precursor per day)
- LDPE tube (0.875 × 10.5 in\textsuperscript{2}) with P, at 23\textdegree C, = 66.0 ± 1.09 × 10\textsuperscript{-17} kgClO\textsubscript{2}•m\textsuperscript{-2}•s\textsuperscript{-1}•Pa\textsuperscript{-1} (Netramai et al., 2009)

**Calculations for packaging study on one-GR and two-GR designs:**

To fix the weight of precursors used in the study to 8 g, the calculations of amount of each precursor to be used in the study are as shown in Table A-8. The actual ClO\textsubscript{2} gas release profiles were shown in Figure 7-3, Chapter 7. In Table A-8, amount of fast and slow release compounds are equal to x and y g, respectively. To avoid variation of using different weights of precursors to generate 2 levels of ClO\textsubscript{2}, the weight was fixed at 8 g, i.e. x + y = 8 g.
Table A-8. Calculation of amount of precursors to be used in packaging study 1

<table>
<thead>
<tr>
<th></th>
<th>Fast release compound:</th>
<th>0.5 mg/g/d chemical will be used for:</th>
<th>x g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slow release compound:</td>
<td>0.05 mg/g/d chemical will be used for:</td>
<td>y g</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>GR:</strong></td>
<td>Total precursor wt =</td>
<td>8 g</td>
<td>x + y</td>
</tr>
<tr>
<td>Total released ClO2 wt =</td>
<td>z mg</td>
<td>0.5x + 0.05y</td>
<td></td>
</tr>
</tbody>
</table>

\[
z = 0.45x + 0.4
\]
\[
x = \frac{(z - 0.4)}{0.45}
\]

**wt-based:**

for 1 day, \( z = \frac{2.264}{283} \text{ mg ClO2 / 283 g lettuce} \)

Use:
\[
x = 4.14 \text{ g}
\]
\[
y = 3.86 \text{ g}
\]

for 1 day, \( z = \frac{1.132}{283} \text{ mg ClO2 / 283 g lettuce} \)

Use:
\[
x = 1.63 \text{ g}
\]
\[
y = 6.37 \text{ g}
\]
Calculations for packaging study on middle-GR design:

Amount of ClO₂ gas permeated through LDPE tube was calculated based on the following equation (Rodriguez et al., 2003; Selke et al., 2004):

\[
P = \frac{q \cdot \ell}{A \cdot t \cdot \Delta p} \tag{1}
\]

or

\[
q = \frac{P \cdot A \cdot t \cdot \Delta p}{\ell} \tag{2}
\]

LDPE film was selected to be used as a GR tube in study 2 due to its low barrier to ClO₂ gas (Netramai et al., 2009). Giving that 1) \(\Delta P\) is not constant, for this particular case, it varies according to the amount of ClO₂ gas constantly released (which should make up to 2.26 mgClO₂/day, for high level ClO₂ treatment) and those permeated through LDPE tube, at 4°C; and 2) actual value of \(P\) at 4°C (which should be lower than that at 23°C) was not available, amount of ClO₂ permeated per sec was estimated using equation (2), assuming 1) there was no permeation in the first second of the process; and 2) at any given second, partial pressure of ClO₂ in the tube is constant.

\[
q_t = \frac{P \times A \times \left( t - \sum_{i=1}^{t-1} q_i \right)}{t \times \text{tube volume}} \times 3.64 \times 10^6 \tag{2}
\]
where $q_t =$ amount of ClO$_2$ permeated through LDPE in 1 sec, at time $t$ (mg); $P = 66.0 \times 10^{-17}$ kgClO$_2$ m$^{-2}$ s$^{-1}$ Pa$^{-1}$ at 23$^\circ$C; $A = 1.18 \times 10^{-2}$ m$^2$; $x$ is mg ClO$_2$ assumed to be linearly released from precursor per sec, i.e. $2.62 \times 10^{-5}$ mg for high level release; $t =$ time into permeation process (sec); tube volume = $4.19 \times 10^{-2}$ L; $l = 2.79 \times 10^{-5}$ m; and $3.64 \times 10^6$ is the conversion factor from mgClO$_2$/L to Pa.

As partial pressure of ClO$_2$ gas inside LDPE tube increase, amount of permeated ClO$_2$ will continuously increase, due to partial pressure built up. When $q_t = x$, the permeated amount of ClO$_2$ becomes constant. Time to reach $q_t = x$ depends on $P$ of LDPE which is temperature dependent (Rodriguez et al., 2003; Selke et al., 2004). Theoretically, $P$ of the same polymer should be smaller as temperature decreases, resulting in longer time taking to reach $q_t = x$. Figure A-3 demonstrates $q_t$ at different $P$.

In this study, for LDPE GR to be able to deliver constant amount of ClO$_2$ per day in this particular packaging system, sealed LDPE tubes containing ClO$_2$ precursor should be left in chemical fume hood for predetermined time to achieve linear release at $q_t = x$, and then packed along with shredded lettuce. Since $P$ of LDPE at 4$^\circ$C is not known and due to time constraint, the sealed tubes were left in chemical fume hood for approximately 5 hours. The actual ClO$_2$ gas release profiles were shown in Figure 7-3, Chapter 7.
Figure A-3. Calculated amount of permeated ClO$_2$ (mg) from LDPE tube with different permeability coefficients.
APPENDIX 9: Preliminary study on sample portions for packaging study

Results of preliminary study to observe if the effects of gas distribution to the top and bottom portions of the bag are significantly different, was conducted. The procedure followed what has been outlined in Section 7.2.5 and 7.2.6 (except lettuce samples were taken from 6 different areas instead of 3 areas). The precursor used was of different type, as shown below.

Figure A-4. Gas release profile

The results were provided in Figure A-5. Since there was no different between top and bottom portions (except in two-GR at Day 4 of storage), in the same lengthwise portion, the sampling portions for packaging study were finalized to 3 lengthwise portions as applied in Section 7.2.6.
<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 4 Top</th>
<th>Day 7</th>
<th>Day 1</th>
<th>Day 4 Bottom</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.02</td>
<td>0.72</td>
<td>0.70</td>
<td>1.08</td>
<td>0.69</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>±0.03</td>
<td>±0.11</td>
<td>±0.04</td>
<td>±0.06</td>
<td>±0.08</td>
<td>±0.06</td>
</tr>
<tr>
<td></td>
<td>±0.03</td>
<td>±0.07</td>
<td>±0.04</td>
<td>±0.07</td>
<td>±0.03</td>
<td>±0.07</td>
</tr>
<tr>
<td></td>
<td>±0.02</td>
<td>±0.25</td>
<td>±0.21</td>
<td>±0.27</td>
<td>±0.26</td>
<td>±0.23</td>
</tr>
<tr>
<td></td>
<td>±0.02</td>
<td>±0.03</td>
<td>±0.20</td>
<td>±0.08</td>
<td>±0.03</td>
<td>±0.07</td>
</tr>
<tr>
<td></td>
<td>±0.09</td>
<td>±0.20</td>
<td>±0.22</td>
<td>±0.15</td>
<td>±0.21</td>
<td>±0.23</td>
</tr>
<tr>
<td></td>
<td>±0.09</td>
<td>±0.03</td>
<td>±0.21</td>
<td>±0.05</td>
<td>±0.05</td>
<td>±0.05</td>
</tr>
</tbody>
</table>

**Figure A-5(a).** Log_{10} CFU/g reduction of *E. coli* O157:H7 of sample portions stored for 1, 4, and 7 days in one-GR design bag with 8 mgClO₂/day

1 Different script letters indicates statistically differences between means at α of 0.05 of different portion (top vs bottom areas) located horizontally in the same bag.
<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 4</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 mg</td>
<td>4 mg</td>
<td>4 mg</td>
</tr>
<tr>
<td>Top</td>
<td>0.81 ±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.15 ±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.79 ±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.64 ±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.16 ±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.65 ±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.59 ±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.14 ±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.58 ±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bottom</td>
<td>0.75 ±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.12 ±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.80 ±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.59 ±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.16 ±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.62 ±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.62 ±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.12 ±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.65 ±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Figure A-5(b).** Log<sub>10</sub> CFU/g reduction of *E. coli* O157:H7 of sample portions stored for 1, 4, and 7 days in two-GR design bag with 8 mgClO<sub>2</sub>/day

1 Different script letters indicates statistically differences between means at α of 0.05 of different portion (top vs bottom areas) located horizontally in the same bag.
APPENDIX 10: Results on moisture content of shredded lettuce for packaging study

The analytical procedure for determining moisture content (%) in *E coli* O157:H7 inoculated shredded lettuce was described below. The results were reported in Table A-9 and Table A-10.

**Apparatus:**
- Aluminum dish
- Analytical balance
- Vacuum chamber or Hot-air oven
- Dessicator

**Procedure:**

1. Weight around 5 g of chopped shredded lettuce (*W_F*) into aluminum dish with known weight (*W_A*).

2. Let dry in vacuum chamber (or hot-air oven).

3. Put sample in dessicator, until the temperature cool down to room temperature.

4. Weight dried sample and repeat step 2-4 until sample weight does not change more than ± 0.0005 g; record as *W_D*.

5. Discard the sample.
Calculation:

To obtain moisture content, MC (%):

\[ MC \ (\%) = \frac{(W_F - W_D - W_{AI}) \times 100}{W_F} \]
Table A-9. Moisture content (%) of shredded lettuce samples, for packaging study on one-GR\textsuperscript{1} and two-GR designs, after storage for 0, 1, 4, and 7 days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Moisture content (%)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0</td>
<td>Day 1</td>
<td>Day 4</td>
</tr>
<tr>
<td>Control</td>
<td>95.16 ± 0.08\textsuperscript{a}</td>
<td>96.09 ± 0.13\textsuperscript{b}</td>
<td>95.77 ± 0.15\textsuperscript{b}</td>
<td></td>
</tr>
<tr>
<td>One-GR, 8.0 mgClO\textsubscript{2}/kg</td>
<td>95.67 ± 0.17\textsuperscript{b}</td>
<td>95.92 ± 0.20\textsuperscript{b}</td>
<td>95.79 ± 0.08\textsuperscript{b}</td>
<td></td>
</tr>
<tr>
<td>One-GR, 4.0 mgClO\textsubscript{2}/kg</td>
<td>95.12 ± 0.06\textsuperscript{a,2}</td>
<td>95.77 ± 0.08\textsuperscript{b}</td>
<td>95.93 ± 0.14\textsuperscript{b}</td>
<td>95.90 ± 0.26\textsuperscript{b}</td>
</tr>
<tr>
<td>Two-GR, 8.0 mgClO\textsubscript{2}/kg</td>
<td>95.68 ± 0.19\textsuperscript{b}</td>
<td>95.89 ± 0.25\textsuperscript{b,c}</td>
<td>96.00 ± 0.26\textsuperscript{c}</td>
<td></td>
</tr>
<tr>
<td>Two-GR, 4.0 mgClO\textsubscript{2}/kg</td>
<td>95.60 ± 0.34\textsuperscript{b}</td>
<td>95.41 ± 0.51\textsuperscript{b}</td>
<td>95.71 ± 0.60\textsuperscript{b}</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{1} GR = Gas reservoir

\textsuperscript{2} Different superscript letters indicates statistically differences between means, between different storage days, at $\alpha$ of 0.05
Table A-10. Moisture content (%) of shredded lettuce samples, for packaging study on middle-GR\(^1\), after storage for 0, 1, 4, and 7 days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Moisture content (%)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 1</td>
<td>Day 4</td>
<td>Day 7</td>
</tr>
<tr>
<td>Control</td>
<td>94.99 ± 0.11(^{a})</td>
<td>95.01 ± 0.06(^{a})</td>
<td>95.35 ± 0.23(^{a})</td>
<td>95.36 ± 0.91(^{a})</td>
</tr>
<tr>
<td>middle-GR, 8.0 mgClO(_2)/kg</td>
<td>94.99 ± 0.11(^{a,2})</td>
<td>94.98 ± 0.21(^{a})</td>
<td>95.13 ± 0.32(^{a})</td>
<td>95.35 ± 0.19(^{a})</td>
</tr>
<tr>
<td>middle-GR, 4.0 mgClO(_2)/kg</td>
<td>95.08 ± 0.28(^{a})</td>
<td>95.07 ± 0.61(^{a})</td>
<td></td>
<td>95.19 ± 0.13(^{b})</td>
</tr>
</tbody>
</table>

\(^1\) GR = Gas reservoir

\(^2\) Different superscript letters indicates statistically differences between means, between different storage days, at \(\alpha\) of 0.05
APPENDIX 11: Statistical analysis result for packaging study

Table A-11. Statistical analysis, for Study 1, on interaction between different bag design, ClO₂ gas level, and storage time (at α of 0.05), as indicated by Log₁₀ CFU/g reduction of *E. coli* O157:H7

The SAS System
The GLM Procedure

<table>
<thead>
<tr>
<th>Class Level Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class</td>
</tr>
<tr>
<td>design</td>
</tr>
<tr>
<td>conc</td>
</tr>
<tr>
<td>block</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>7</td>
<td>1.88267292</td>
<td>0.26895327</td>
<td>61.60</td>
<td>&lt;.0001</td>
</tr>
<tr>
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R-Square | Coeff Var | Root MSE | logred Mean |
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