

©2015  
CAROL SAADE  
ALL RIGHTS RESERVED

SYSTEM FEASIBILITY: DESIGNING A CHLORINE DIOXIDE  
SELF-RELEASING PACKAGE LABEL TO IMPROVE FOOD SAFETY

By

CAROL SAADE

A dissertation submitted to the  
Graduate School-New Brunswick  
Rutgers, The State University of New Jersey

In partial fulfillment of the requirements

For the degree of

Doctor of Philosophy

Graduate Program in Food Science

Written under the direction of

Kit L. Yam

And approved by

---

---

---

---

New Brunswick, New Jersey

October, 2015

## **ABSTRACT OF THE DISSERTATION**

System Feasibility: Designing a Chlorine Dioxide  
Self-Releasing Package Label to Improve Food Safety

By CAROL SAADE

Dissertation Director:

Professor Kit L. Yam

Fresh produce consumption is associated with foodborne illnesses. Gaseous chlorine dioxide ( $\text{ClO}_2$ ) has the ability to penetrate and inactivate human pathogens attached to inaccessible sites on produce surfaces. The aim of this work was to evaluate the feasibility of an innovative and practical  $\text{ClO}_2$  self-releasing package label made of synthetic or biobased polymers. These labels, embedded with  $\text{ClO}_2$  precursors, have the ability to generate and release  $\text{ClO}_2$  in a controlled manner within the package in response to a controlled activation mechanism to improve the safety of packaged fresh produce.

The package labels were manufactured using extrusion or solution casting. Synthetic labels were extruded with different thickness. Biobased labels were casted onto glass frame and dried. Gaseous  $\text{ClO}_2$  release was triggered by a controlled activation mechanism. Concentration of  $\text{ClO}_2$  released from labels was quantified over time using UV spectrophotometer at 360 nm. The antimicrobial effectiveness of both the synthetic and biobased labels was evaluated against the growth of *Salmonella* Montevideo G4639 on trypticase Soy Agar (TSA) plates and on inoculated mung bean seeds.

The manufacturing of synthetic and biobased labels was feasibly viable. Both labels generated and released ClO<sub>2</sub> over time following controlled activation. Biobased labels achieved better control over ClO<sub>2</sub> release as compared to the synthetic labels. As to their antimicrobial effectiveness, synthetic labels achieved up to 2.3 log reductions in *Salmonella* populations on TSA plates, whereas biobased labels achieved up to 8 log reductions on TSA plates and up to 2 log reductions on inoculated mung bean seeds.

The findings of this research support the technical feasibility of the package label in terms of label manufacturing, ClO<sub>2</sub> generation and release after controlled activation at concentrations effective to inactivate *Salmonella*, either partially or totally on TSA plates and the surface of mung bean seeds. Both synthetic and biobased labels showed practical feasibility in terms of release of ClO<sub>2</sub> in the range of effective concentration used in the disinfection of fresh produce.

## **ACKNOWLEDGMENTS**

I would like to express my special appreciation and thanks to my advisor Dr. Kit L. Yam for his constant guidance and support. The learning and training acquired under his mentorship have shaped the scientist and critical thinker I am today. Dr. Yam has always encouraged me to think inside and outside the box and offered me with both the opportunity and confidence to display my greatest potentials and stand out on the personal and professional level. I am deeply grateful for enlightening me throughout my Ph.D. journey.

I would like to thank Dr. Bassam Annous from USDA for his guidance, support and patience all throughout my research. Dr. Annous has constantly challenged me to think differently making me a better scientist.

Also, I am grateful for Dr. LinShu Liu's willingness to help out in every possible way to make my Ph.D. journey a lot smoother. Not to mention Dr. Nazir Mir's support in this project. Thank you all for your brilliant comments and suggestions that helped me advance greatly on this research.

I would like to thank Mr. Tom Yip for his technical expertise and for allowing us to use his facility, Specialty Extrusion Co., to extrude synthetic package labels required for the first part of this research.

I would like to thank my dear friend Saifanassour Arabi for making my Ph.D. project fun, interactive and creative. Thanks for the stimulating discussions and engaging brainstorming sessions that enabled me to face challenges with an open mind.

This dream wouldn't have become a reality without the endless support and encouragement of my parents, family and friends. Thank you mom and dad for believing

in my potentials and supplying me with strength, love and hope to make it through this long journey triumphant and proud. Thank you Marie, Julie, Boutros, Ralf, Rudy, Mayou and Myrna for providing me with positive energy and patience needed to lighten my load and reach my destination.

I am forever thankful for Dr. Ricardus Haber who helped me choose Food Science as my career. I still vividly recall the conversation we had in Ehden about my graduate studies which had since shaped my future.

## DEDICATION

*I dedicate this thesis to Rachad Itani who lit every step of my way in this challenging journey, and time after time has given me new courage to face life cheerfully. His beautiful mind, sharp vision, pure kindness and immense generosity never cease to fuel my imagination and creativity in Science and Life.*

## TABLE OF CONTENTS

ABSTRACT OF THE DISSERTATION .....	ii
DEDICATION .....	vi
TABLE OF CONTENTS.....	vii
LIST OF TABLES .....	xi
LIST OF FIGURES .....	xii
1. INTRODUCTION .....	1
2. LITERATURE REVIEW .....	5
2.1. Fresh produce.....	5
2.1.1. Fresh produce consumption and safety .....	5
2.1.2. Fresh produce sanitation .....	6
2.1.3. Defining the Practical Application of ClO <sub>2</sub> Releasing Package Label .....	11
2.2. Chlorine dioxide.....	12
2.2.1. Chlorine Dioxide Generation.....	15
2.2.2. Reaction with Organic Compounds .....	18
2.2.3. Chlorine Dioxide Use in the Food Industry .....	18
2.2.4. Disinfection Efficacy and Pathogen Inactivation by Chlorine Dioxide.....	19
2.2.5. Chlorine Dioxide Patents .....	21
2.2.6. Chlorine Dioxide - regulations and safety concerns .....	24
2.3. Chlorine Dioxide Precursors.....	25
2.3.1. Citric Acid (C <sub>6</sub> H <sub>8</sub> O <sub>7</sub> ).....	25
2.3.2. Sodium Chlorite (NaClO <sub>2</sub> ).....	26
3. DESIGN OF ClO <sub>2</sub> SELF-RELEASING PACKAGE LABEL .....	27
3.1. System Description and Uniqueness.....	27
3.2. System Components.....	28



3.2.1.	Polymer Matrix .....	28
3.2.2.	ClO <sub>2</sub> Precursors .....	29
3.2.3.	Processing Method .....	30
3.2.4.	Controlled Activation Mechanism .....	30
3.3.	Defining “Controlled” Aspect of Package Label .....	34
3.4.	Usefulness of this System .....	35
3.5.	Package Label Intended Use .....	36
4.	PROBLEM FORMULATION .....	37
4.1.	Rationale and Problem Statement .....	37
4.1.1.	Rationale .....	37
4.1.2.	Problem Statement .....	38
5.	APPROACH -I-: EXTRUSION .....	41
5.1.	Concept Development .....	41
5.1.1.	Synthetic Label Manufacture .....	41
5.1.2.	Controlled Activation Mechanism .....	41
5.2.	Extrusion .....	42
5.3.	Synthetic Polymers .....	44
5.3.1.	Ethylene Vinyl Acetate (EVA) .....	44
5.3.2.	Poly-ε-caprolactone (PCL) - Aliphatic Polyester .....	47
5.3.3.	Aromatic Polyether-based Thermoplastic Polyurethane (TPU) .....	49
6.	APPROACH -II-: COATING .....	54
6.1.	Motivation .....	54
6.2.	Concept Development .....	54
6.2.1.	Biobased Label Manufacture .....	55
6.2.2.	Controlled Activation Mechanism .....	56

6.3. Coating.....	56
6.4. Biobased Polymers.....	57
6.4.1. Pectin.....	57
6.4.2. Gelatin.....	62
7. EXPERIMENTAL DESIGN .....	68
Approach -I-: Extrusion .....	68
7.1. Materials and Methods.....	68
7.1.1. Materials .....	68
7.2. Methods.....	70
7.2.1. Compounding of Polymer Resins .....	70
7.2.2. Synthetic Package Label Manufacture.....	71
7.2.3. Label Characterization Analyses .....	74
7.2.4. Quantitative Determination of ClO <sub>2</sub> from EVA Package Labels .....	76
7.2.5. Antimicrobial Effectiveness of EVA Package Labels .....	79
Approach -II-: Coating.....	81
8. Materials and Methods.....	81
8.1. Materials .....	81
8.2. Methods.....	81
8.2.1. Biobased Package Label Manufacture.....	81
8.2.2. Multilayer Label Assembly.....	86
8.2.3. Controlled Activation Mechanism of Labels .....	87
8.2.4. Quantitative Determination of ClO <sub>2</sub> from Biobased Labels .....	90
8.2.5. Antimicrobial Effectiveness of Biobased Labels on Media Plates (TSA)...	91
8.2.6. Antimicrobial Effectiveness of Biobased Labels on Inoculated Mung Bean Seeds	92
9. RESULTS AND DISCUSSION .....	95

Approach -I-: Extrusion .....	95
9.1. Synthetic Package Label Characterization Results.....	95
9.1.1. Scanning Electron Microscopy (SEM) .....	95
9.1.2. Tensile Properties.....	98
9.1.3. Differential Scanning Calorimetry (DSC) .....	101
9.2. Quantitative Determination of ClO <sub>2</sub> from EVA Package Labels.....	105
9.3. Antimicrobial Effectiveness of EVA Package Labels .....	113
Approach -II-: Coating .....	117
9.4. Quantitative Determination of ClO <sub>2</sub> from Biobased Labels .....	118
9.4.1. Multilayer Labels Without Barrier Layers (ML w/o BL) .....	119
9.4.2. Multilayer Labels With Barrier Layers (ML w/ BL) .....	124
9.5. Antimicrobial Effectiveness of Biobased Labels.....	128
9.5.1. Antimicrobial Effectiveness of Labels on <i>Salmonella</i> Growth on TSA Plates 128	
9.5.2. Antimicrobial Effectiveness of Labels on <i>Salmonella</i> Growth on Inoculated Mung Bean Seeds .....	129
10. CONCLUSIONS.....	136
11. FUTURE WORK.....	139
11.1. Synthetic Labels.....	139
11.2. Biobased Labels .....	141
12. REFERENCES .....	144

## LIST OF TABLES

Table 1. Physical properties of chlorine dioxide (Masschelein, 1979).....	14
Table 2. General physical properties of PCL, EVA (28% VA) and TPU. ....	69
Table 3. Processing conditions of PCL, EVA (28 % VA) and TPU compounding with citric acid using twin-screw extruder. ....	70
Table 4. Extrusion conditions: temperature and screw speed, of PCL, EVA (28 % VA) and TPU package labels incorporated with citric acid.....	73
Table 5. Synthetic package labels: citric acid loading and thickness. ....	74
Table 6. EVA (28% VA) package labels. ....	77
Table 7. Antimicrobial effectiveness of EVA package labels on the growth of <i>Salmonella</i> Montevideo on TSA plates after 2, 4, and 6 hours. ....	114
Table 8. Effect of ClO <sub>2</sub> concentration released from ML w/o and w/barrier layers on <i>Salmonella</i> Montevideo growth (log CFU/mL) on TSA plates.....	129
Table 9. Effect of ClO <sub>2</sub> concentration released from ML w/o and w/barrier layers on <i>Salmonella</i> Montevideo Population (log CFU/g) on Mung Bean Seeds. ....	131

## LIST OF FIGURES

Figure 1. Chlorine dioxide chemical structure (Masschelein, 1979). .....	12
Figure 2. Citric acid chemical structure. ....	26
Figure 3. Conceptual framework for developing Controlled Release Packaging (Zhu, 2008) . ....	34
Figure 4. Synthetic label concept development: extrusion, controlled activation and application. ....	43
Figure 5. Ethylene-vinyl acetate (EVA) copolymer. Building blocks of EVA molecule: ethylene and vinyl acetate and chemical structure with backbone of covalently linked atoms with pendant hydrogens and acetoxy side chains. ....	44
Figure 6. Schematic representation of EVA copolymer structure. EVA copolymer structure is made with short-chain branches and butyl groups, long-chain branches occurring at random intervals along the backbone with possible branching and acetate branches (*). ....	45
Figure 7. Synthesis of polycaprolactone by ring-opening polymerization of $\epsilon$ -caprolactone structural unit upon exposure to catalyst and heat. ....	48
Figure 8. Polycaprolactone chemical structure. ....	48
Figure 9. TPU structural formula. ....	50
Figure 10. Segmented polyurethane structure: alternation of soft and hard segments with hard block chain packing (Mackey, 2002). ....	53
Figure 11. Schematic representation of pectin backbone showing alternation between smooth regions of linear galacturonan homopolymer and hairy regions of rhamnogalacturonan and side chains (Axelos & Thibault, 1991). ....	58
Figure 12. Gelatin molecule at pH of 8.4. Gelatin molecule shows repeating pattern of (glycine-proline-hydroxyproline) triplets. At pH 8.4, gelatin displays a polyampholytic character due to presence of charged amino acids residues on its backbone. ....	63
Figure 13. Zones of a single-screw extruder. ....	72
Figure 14. Manufacture of gelatin functional layer incorporated with sodium chlorite. A solution of native proteins (sol) is heated to denature proteins (progel). This step is irreversible. The second step takes place upon cooling of gelatin progel to below 35-40°C leading to formation of a 3-D gel network. The aggregation of three helices through inter-chain hydrogen bonding forms junction zones conferring long-range rigidity to these regions. ....	84
Figure 15. Inter-layer binding of pectin and gelatin layers. Application of the positively charged gelatin glue solution on the surface of slightly negatively charged pectin followed by pressing onto the negatively charged gelatin layer to promote binding and achieve contact between the layers. ....	85

Figure 16. Activation of multilayer label. Application of a continuous film of gelatin glue solution onto the pectin layer followed by label assembly and pressure to promote binding between pectin and gelatin functional layers and trigger ClO <sub>2</sub> release.....	87
Figure 17. Diagram of multilayer label without gelatin barrier layers showing pectin (left-hand side) and gelatin (right-hand side) functional layer molecular structures as well as junction zones maintained by hydrogen bonding. ....	88
Figure 18. Diagram of multilayer label with gelatin barrier layers showing the pectin functional layer (left-hand side), gelatin barrier layer (center) and gelatin functional layer (right-hand side) molecular structure as well as junction zones maintained by hydrogen bonding. ....	89
Figure 19. Antimicrobial effectiveness of biobased labels on Mung bean seeds – Experimental setup.....	94
Figure 20. SEM Photomicrograph of labels 1- PCL 8.6% citric acid 13.5 mil and 2- PCL 8.6% citric acid 25 mil thickness at 100X magnification. ....	96
Figure 21. SEM Photomicrograph of label 7- TPU 15 % citric acid 32.5 mil thickness at 50X magnification. ....	96
Figure 22. SEM photomicrographs of EVA (28 % VA) labels at 100 X magnification. Label 3- 7.5% citric acid 13.3 mil; label 4- 7.5% citric acid 26.6 mil; label 5- 15% citric acid 11.9 mil and label 6- 15% citric acid 19.2 mil. ....	97
Figure 23. Tensile strength and elongation-to-break of the synthetic package labels. ....	99
Figure 24. Young's modulus and toughness of the synthetic package labels.....	99
Figure 25. DSC thermograms of PCL labels. ....	102
Figure 26. DSC thermogram of TPU label. ....	102
Figure 27. DSC thermograms of EVA labels. ....	104
Figure 28. Effect of extrusion processing conditions and storage on the structural integrity of PCL and TPU incorporated with citric acid.....	107
Figure 29. Release of ClO <sub>2</sub> (mg/L air) from EVA package labels over 6 hours, after controlled activation.....	108
Figure 30. SEM photomicrographs of EVA labels at 50 X magnitude reflecting sample variability within the same label with regard to citric acid particle size and distribution on the surface of these labels. A: Label 3; B: Label 4. ....	111
Figure 31. SEM photomicrographs of EVA labels at 50 X magnitude reflecting sample variability within the same label with regard to citric acid particle size and distribution on the surface of these labels. C: Label 5 and D: Label 6. ....	112
Figure 32. Polymer relaxation-controlled penetration of water into pectin or gelatin polymeric film. One surface of the film is exposed to the moisture (left-hand side). Three different zones can be distinguished inside the film: (1) swollen network (high water content, high mobility of ClO <sub>2</sub> precursors and water molecules); (2) swelling zone (polymer chain relaxation); and (3) non-swollen network (low mobility of ClO <sub>2</sub> precursors, no water).....	118

Figure 33. Schematic representation of the multilayer label without barrier layers (5 layers) at the molecular level. The moisture content, water activity ( $a_w$ ) of each layer as well as loading % on dry basis (db) of each layer were shown to indicate the direction of water diffusion as well as diffusion of $\text{ClO}_2$ precursors ( $\text{H}^+$ and $\text{ClO}_2^-$ ) after controlled activation.....	121
Figure 34. Release of $\text{ClO}_2$ (mg) from multilayer label without gelatin barrier layers (5 layers) in the absence and presence (high relative humidity condition) of water in the vial, over 25 hours after controlled activation. ....	122
Figure 35. Release of $\text{ClO}_2$ (mg) from multilayer labels without (5 layers) and with (9 layers) gelatin barrier layers over time after controlled activation at 10 and 23 °C. ....	124
Figure 36. Release of $\text{ClO}_2$ (mg) from original multilayer labels without (5 layers) and with (9 layers) and new multilayer labels without (3 layers) and with (5 layers) over 96 hours after controlled activation, at 23°C. ....	127

## 1. INTRODUCTION

Shelf-life of food is defined as the duration of time when food maintains its quality attributes (flavor, color, aroma, texture and nutritional value) and safety under defined environmental conditions. During storage and distribution, quality and safety of food products may be compromised resulting in unacceptable products for consumption.

Packaging not only serves as an inert, passive barrier to protect foods against environmental factors such as effects of temperature, relative humidity and oxygen, but has also evolved in ways to actively interact with packed food to maintain safety, quality and extend shelf-life.

Controlled release packaging (CRP) is a new generation of functional active packaging where active compounds (AC), antimicrobials or antioxidants, are released from packaging system in a controlled fashion, to counteract microbial and quality deterioration kinetics and extend overall shelf-life. In other words, this packaging system provides continuous replenishment of active compounds at an adequate concentration, when it is needed the most, over the time period corresponding to shelf-life of a specific food. Depending on food commodity and required shelf-life, CRP can be customized through manipulating packaging parameters (polymer composition, processing methods and structure) and active compound to suit different applications.

Fresh produce consumption continues to rise due to their important role in healthy diet and the availability of affordable fresh fruits and vegetables year-round from increased US imports. The 2010 dietary guidelines for Americans recommend the increase in intake of fresh fruits and vegetables per day to promote good health ("The Dietary Guidelines for Americans,," 2010).



In the US, around 46 % of total foodborne illnesses between 1998 and 2008 were attributable to consumption of fresh produce (Painter et al., 2013). The increase in the reported illnesses is due to a combination of factors, namely increase in fresh produce consumption, improved surveillance, changes in consumers' habits, and increase in global trade with large share of produce from countries with lower sanitation standards and complex distribution systems (Harris et al., 2003).

Fresh produce is susceptible to microbial contamination. The surface irregularities of produce offer protective areas for bacteria to survive and multiply, preventing access of sanitizers and microbial inactivation due to hydrophobicity of these areas. Physical injuries of produce such as bruising or punctures break the protective epidermal barrier of the produce and provide nutrients for microbial survival and proliferation. Internalization of microorganisms in produce before harvesting or during processing has also been the cause in contaminated produce. Biofilm formation subsequent to microbial attachment on the surface of produce has increased resistance to antimicrobial treatments lessening effect of decontamination (Gomez-Lopez et al., 2008; Harris et al., 2003).

The current industrial practice is the washing of fruits and vegetables with aqueous sanitizers, mostly chlorine. These disinfectants in solution are unable to penetrate pores, channels, crevices and natural openings in the skin of produce to inactivate microorganisms residing in these areas due to surface tension, lowering therefore their effectiveness in killing pathogens (Beuchat & Ryu, 1997). Given their greater penetration ability, gaseous sanitizers such as chlorine dioxide ( $\text{ClO}_2$ ) may be more effective than aqueous disinfectants at inactivating microorganisms located in hard to reach areas (Gómez-López et al., 2009; Han et al., 2000).

$\text{ClO}_2$  is a greenish yellow gas at room temperature with effective biocidal activity over a wide range of pH (3-8). It is a selective and strong oxidizing agent, which unlike chlorine does not chlorinate organic compounds to produce carcinogenic trihalomethanes (THMs). Also,  $\text{ClO}_2$  does not react with ammonia to form chloramines.  $\text{ClO}_2$  is explosive at concentrations higher than 10 % v/v at atmospheric pressure. Due to its instability, this compound is usually generated on-site upon demand to eliminate safety hazards associated with its storage and transportation.

One way to address the safety concerns associated with  $\text{ClO}_2$  storage and distribution would be to generate this compound safely and conveniently from an active packaging system. This would serve as an additional hurdle for bacteria to overcome.  $\text{ClO}_2$  has received FDA approval in 2001 to reduce or eliminate microorganisms in a wide variety of food products such as fruits and vegetables (Rulis, 2001).

Chlorine dioxide generating systems, in the form of packaging films or sachets, have been developed on a lab scale with both reactants mixed together or separated from each other by a water soluble barrier to prevent premature  $\text{ClO}_2$  production. Sachet systems require a moisture source for  $\text{ClO}_2$  to form after dissolution of water-soluble membrane. Packaging films with both reactants embedded in the solid form, result in  $\text{ClO}_2$  losses upon exposure to moisture from environment during storage and handling. Besides, reactants in this system are often non-uniformly distributed leading to lower yields of  $\text{ClO}_2$ . Not to mention the strong dependence of these packaging films to moisture, restricting their use only to food products that generate moisture such as fresh produce during respiration.

Preliminary work in our lab has proven the technical feasibility of a  $\text{ClO}_2$ .

releasing packaging film made of polylactic acid incorporated with ClO<sub>2</sub> precursors (organic acid and sodium chlorite). ClO<sub>2</sub> was released from the packaging film in response to moisture generated from respiration of grape tomatoes at concentrations effective to achieve more than 3 log reduction of *Salmonella* spp. and *Escherichia coli* O157:H7 on grape tomatoes, without compromising quality attributes (Ray et al., 2013).

There is a need to design a simple, safe, practical and convenient ClO<sub>2</sub> packaging system that 1) avoids premature reaction of ClO<sub>2</sub> precursors upon exposure to moisture during handling and storage, 2) offers better control over ClO<sub>2</sub> release by incorporating only one of reactants into the packaging system followed by a controlled activation mechanism for an optimum ClO<sub>2</sub> production, 3) minimizes safety hazards associated with ClO<sub>2</sub> transportation and storage as well as sachet disruption and subsequent chemical contamination of food, and 4) offers ease of handling, activation and application into primary package.

The main objective of this work was to evaluate the feasibility of an innovative and practical ClO<sub>2</sub> self-releasing package label made of synthetic and biobased polymers with the ability to generate and release gaseous ClO<sub>2</sub> in a controlled manner within the package system in response to controlled activation to improve microbial safety and maintain quality of fresh produce. Subsequent to activation, the package label maintains a self-generating character, allowing release of ClO<sub>2</sub> until consumption of reactants. Feasibility of packaging label can be demonstrated by the ability of such label to act as a ClO<sub>2</sub> controlled delivery system after activation whereby the amount of ClO<sub>2</sub> released with time is sufficient to inhibit microbial growth without compromising quality attributes and which can be manipulated to suit different food applications.

## **2. LITERATURE REVIEW**

### **2.1. Fresh produce**

#### **2.1.1. Fresh produce consumption and safety**

Fresh produce consumption in the United States continues to rise due to a number of factors. The major one being health benefits associated with the consumption of such commodities. Another factor is the active promotion of fruits and vegetables as part of a healthy lifestyle and in disease prevention. Also, the increased global trade is making fresh produce easily accessible all year-round at affordable prices. The 2010 Dietary Guidelines for Americans recommends the increase in intake of vegetables and fruits to promote a healthy diet and reduce the risks of chronic diseases such as cardiovascular disease and certain types of cancer ("The Dietary Guidelines for Americans,," 2010). In the US, per capita consumption of fresh fruits and vegetables increased by 32 % from 1982 to 1997 indicating the growing demand for these commodities (Harris et al., 2003).

Outbreaks of foodborne illness associated with the consumption of raw fruits and vegetables have increased due to many factors, the minimal processing required for these foods, improved surveillance, significant increase in the consumption of these foods, complex distribution system with temperature abuse and increased imports from countries with different agricultural and post-harvesting practices (Harris et al., 2003). According to a study conducted by CDC in 2013, 46 % of foodborne illnesses in the US from 1998-2008 had been attributed to consumption of fresh produce (Painter et al., 2013).

Several factors influence the survival and growth of pathogens on fresh produce:

type of produce and its surface characteristics, microorganism of concern, pre-harvest and postharvest conditions. The surface irregularities of produce offer protective areas for bacteria to survive and multiply preventing access of sanitizers due to hydrophobicity of these areas, and subsequent microbial inactivation. Physical injuries of produce such as bruising or punctures break the protective epidermal barrier of the produce and provide nutrients and moisture essential for microbial survival and proliferation. Internalization of microorganisms in produce before harvesting or during processing and washing has also been the cause of contaminated produce. Biofilm formation subsequent to microbial attachment on produce surface has increased resistance to antimicrobial treatments due to reduced diffusion of sanitizer, physiological changes and production of enzymes degrading antimicrobial compounds, lessening effect of decontamination treatment (Gomez-Lopez et al., 2008; Harris et al., 2003).

#### 2.1.2. Fresh produce sanitation

##### **Traditional sanitizing agent: chlorine**

The most common industrial practice is the washing of fruits and vegetables with aqueous sanitizers, mostly chlorine based chemicals (liquid chlorine and hypochlorite) at concentrations between 50 and 200 ppm with a contact time of 1 to 2 minutes to sanitize the surface of produce (Parish et al., 2003).

Chlorine's efficacy is strongly dependent on the presence of organic matter and metals, and exposure to temperature, light and air. Chlorine reacts with organic molecules to produce trihalomethanes (THMs) and chloramines, hazardous to human health. Also, these disinfectants in solution are unable to penetrate pores, channels, crevices and natural openings in the skin of produce to inactivate microorganisms residing in these

areas, lowering therefore their effectiveness in killing pathogens (Beuchat & Ryu, 1997).

The efficacy of chlorine treatment in terms of log reduction depends on several factors: target microorganism, type of produce, and environmental conditions of application (concentration, contact time and temperature).

Mixed results have been reported in the literature with regard to the efficacy of the chlorine treatment on fresh produce. Overall, chlorine wash of fruits and vegetables has accomplished less than 2 log CFU/g reduction of bacterial populations (Beuchat, 1992; Beuchat, 1999; Cherry, 1999). Work performed by Zhang and Farber (1996) showed a reduction in *L. monocytogenes* population by 1.2 and 1.7 log when shredded cabbage and lettuce were treated with 200 ppm chlorine for 10 min (Zhang & Farber, 1996). Shredded lettuce treated with 100 and 300 ppm of chlorine for 10 min decreased population of *Yersinia enterocolitica* by 2 to 3 log (Escudero et al., 1999). However, *Salmonella* Montevideo was able to withstand 100 ppm chlorine treatment when it was inoculated into cracks of mature green tomatoes (Wei et al., 1995).

### **Aqueous chlorine dioxide**

Unlike chlorine wash, chlorine dioxide reacts less with organic matter and have greater activity at neutral pH.  $\text{ClO}_2$  has 2.5 times the oxidizing capacity of chlorine. Besides, it does not chlorinate organic compounds and ammonia to produce carcinogenic trihalomethanes and chloramines.

A maximum of 3 ppm residual concentration  $\text{ClO}_2$  is allowed for contact with whole produce as long as it is accompanied by an additional step: rinsing with potable water, blanching, cooking or canning ([CFR] & Regulations, 2000c).

Similarly to any aqueous antimicrobial wash treatment, effectiveness of the treatment depends on target microorganism and environmental conditions of treatment.

Work reported by Pao et al., 2007 showed that washing tomatoes freshly inoculated with *Salmonella enterica* and *Erwinia carotovora* with 20 and 10 mg/L of aqueous ClO<sub>2</sub> respectively for 1 min reduced these populations by 5 log CFU/cm<sup>2</sup> (Pao et al., 2007). Washing of shredded lettuce inoculated with *E.coli* O157:H7 with 10 mg/L aqueous ClO<sub>2</sub> for 5 min reduced its population by 1.2 log CFU/g (Singh et al., 2002). The effectiveness of ClO<sub>2</sub> wash treatment (3mg/L for 10 min) on reduction of *Listeria monocytogenes* inoculated onto injured and uninjured green bell pepper surface, showed a higher reduction (3.77 CFU/5g) in the former than the latter (0.44 CFU/5g) (Han et al., 2001). Washing mung bean sprouts inoculated with *Salmonella* Typhimurium and *Listeria monocytogenes* with 100 mg/L ClO<sub>2</sub> reduced their population by 3 and 1.5 log units respectively after a contact time of 5 minutes (Jin & Lee, 2007).

### **Gaseous chlorine dioxide**

Given their greater penetration ability, gaseous sanitizers such as ClO<sub>2</sub> may be more effective than aqueous disinfectants at inactivating microorganisms located in hard to reach areas (Gómez-López et al., 2009; Han et al., 2000).

Many factors affect the biocidal activity of the vapor-phase ClO<sub>2</sub> treatment including produce type, target microorganism, characteristics of produce surface, concentration of sanitizer and contact time.

ClO<sub>2</sub> treatment has shown effectiveness against bacterial pathogens and spoilage microorganisms of fresh produce. Decontamination level of produce with gaseous ClO<sub>2</sub>

was as high as  $> 8$  logs of *E. coli* O157:H7 on green bell pepper (1.2 mg/L for 30 min) and as low as 1.53 log of *Listeria monocytogenes* on lettuce (1.4 mg/L ClO<sub>2</sub> for 29.3 min) (Han et al., 2000; Sy, McWatters, et al., 2005). Yeast and mold population on whole apples and peaches was decreased by 1.8 and 2.65 log respectively when subjected to 4.1 mg/l ClO<sub>2</sub> for 20-25 min (Sy, Murray, et al., 2005). When treated with 8.0 mg/l ClO<sub>2</sub> for 120 min, reductions in yeast and mold populations of 2.06-2.32, 4.07-4.16 and 2.56 log CFU/g were achieved in blueberries, strawberries and raspberries respectively (Sy, McWatters, et al., 2005). ClO<sub>2</sub> gas treatment of tomatoes prevented postharvest decay of the treated fruits as compared to non-treated ones after 3 days of storage at ambient temperature (Mahovic et al., 2007).

### **Seeds sprouts**

Mung bean constitutes a popular component of oriental diet owing to its high nutritional profile (proteins, carbohydrates, minerals and vitamins), low cost and numerous health benefits associated with its consumption including detoxifying, anti-inflammatory, antitumorigenic cholesterol-lowering and diuretic properties (Li et al., 2006).

Consumption of mung bean sprouts has gained popularity worldwide in the past decade; this commodity is usually consumed raw or slightly cooked in salads and sandwiches (Weiss & Hammes, 2003). Seed sprouts are agricultural commodities that do not undergo similar sanitation treatment as fresh produce.

Contamination of seeds with bacterial pathogens has been the cause of many outbreaks linked to their consumption. In the summer of 2011, one of the largest recorded outbreak of a food-borne infection took place in Germany after consumption of *Escherichia coli*



O104:H4 contaminated sprouts resulting in a large number of seriously ill patients and a substantial number of deaths (Choffnes et al., 2012).

Many of bacterial pathogens are able to survive dry storage conditions of seeds, for months. The optimal conditions of temperature, water activity and pH presiding during the sprouting process (3-10 days) allow microbial pathogens to grow 2-3 logarithmic units higher than initial population in seeds (Feng, 1997) and survive on sprouts surface during refrigerated shelf-life (Harris et al., 2003).

FDA recommends decontamination of the seeds before sprouting process to prevent internalization of bacteria into the sprout tissues making them physically inaccessible to sanitizers (Caetano-Anollés et al., 1990).

Numerous methods have been investigated for disinfection of seeds, including heat treatment (Weiss & Hammes, 2003), exposure to ionizing radiation (Thayer et al., 2003), chemical treatments such as chlorine and hypochlorite (Beuchat et al., 2001), and combination treatments of high pressure, temperature and antimicrobial products (Peñas et al., 2010) in efforts to inactivate microorganisms at the surface and interior of seeds without affecting seed viability and germination capability.

The combined treatment high pressure (250 MPa), temperature and disinfectant agent (hypochlorite at 18000 ppm or carvacrol at 1500 ppm) reduced sprouts natural microbial populations by > 5 log units without affecting germination (20 % reduction in the case of hypochlorite + high pressure and 40 % reduction in the case of carvacrol + high pressure) (Peñas et al., 2010). Work conducted by Prodduk et al. 2014 has shown increased reduction in *Salmonella* populations upon exposure of mung bean sprouts to prolonged exposure time to 0.5 mg/ L air ClO<sub>2</sub>, without mechanical mixing (3, 3 and 4 log CFU/g at

15, 30 and 60 minutes respectively). When mixing (tumbling) was performed, higher log reductions were achieved at the same  $\text{ClO}_2$  exposure concentration and time (3, 4 and 5.5 log CFU/g at 15, 30 and 60 minutes respectively) (Prodduk et al., 2014).

### 2.1.3. Defining the Practical Application of $\text{ClO}_2$ Releasing Package Label

The purpose of this work is to design a practical  $\text{ClO}_2$  package label that will release gaseous  $\text{ClO}_2$  in adequate amounts (target concentration), suitable rates and at the right time to inactivate pathogenic microorganisms, maintain product quality and extend overall shelf-life.

The target concentration of  $\text{ClO}_2$  specific to fresh produce commodities is defined as the concentration of  $\text{ClO}_2$  needed to achieve total or partial inhibition of microorganism of interest on a specific fresh produce without compromising its quality attributes. Target concentration could vary with time (different than single exposure) and is dependent on 1) the release kinetics of labels, a function of label design (polymer type, loading, surface area and thickness), 2) the volume of package, and 3) temperature (Arrhenius kinetics).

The efficacy of gaseous  $\text{ClO}_2$  decontamination treatment depends mainly on surface characteristics of the produce and localization of microorganisms, initial populations of pathogenic and natural microflora, concentration of applied  $\text{ClO}_2$ , contact time, and effect on quality attributes.

The end of shelf-life of fresh produce is usually reached when either bacterial population reaches a pre-established maximal count or a specific quality attribute reaches an unacceptable score, whichever of these events takes place first.

It is worth noting that often times decontamination treatment applied to reduce microbial counts of initial populations and prolong shelf-life of produce does not guarantee produce

safety from the potential growth of pathogenic bacteria from availability of more space and nutrients and less competition from spoilage population (Gomez-Lopez et al., 2008).

## 2.2. Chlorine dioxide

Chlorine dioxide was discovered in 1811 by Sir Humphry Davy after reacting potassium chlorate with sulfuric acid. Chlorine dioxide, previously termed “euchlorine”, is a neutral oxychlorine compound that is unstable in the gaseous form and remains a true gas when dissolved in solution (Masschelein, 1979).  $\text{ClO}_2$  cannot be compressed nor liquefied since it becomes spontaneously explosive at a partial pressure higher than 76 mmHg in air or concentrations higher than 10 % by volume at atmospheric pressure. Due to its instability, this compound is usually generated on-site upon demand (Masschelein, 1979).

### *Chemical Structure and Properties*

Chlorine dioxide exists entirely or almost entirely as an angular free radical monomer (Figure 1) with oxidizing properties even in dilute aqueous solutions (Eckenfelder et al., 1992; Gordon et al., 1972).

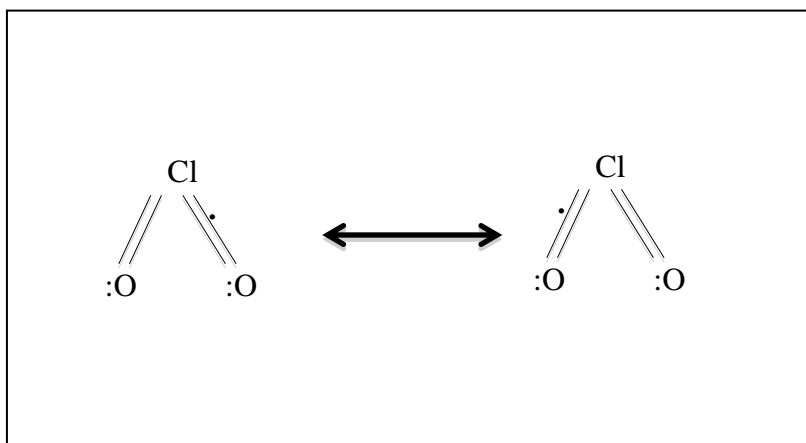
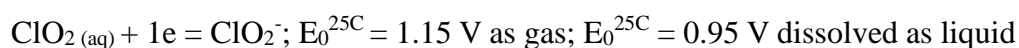


Figure 1. Chlorine dioxide chemical structure (Masschelein, 1979).

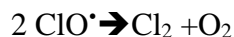
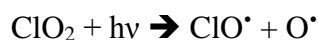
Liquid chlorine dioxide has an oily consistency, a dark amber color and is extremely unstable at temperature above  $-40^{\circ}\text{C}$ . This compound shares the same appearance and smell attributes as chlorine. Besides, it is highly soluble in chilled water, tenfold more soluble in water than chlorine and has over twice its oxidizing capacity (Masschelein, 1979). Due to its unique one-electron-transfer mechanism,  $\text{ClO}_2$  is considered a highly selective oxidant. The reduction-oxidation potential of chlorine dioxide follows those two consecutive reactions (Masschelein, 1979):



The vapor pressure in millimeters mercury is expressed as  $\text{Log } p = 7.7427 - (1375.1)/T^{\circ}\text{K}$ ; at  $p = 760 \text{ mmHg}$ .

Below is a summary of chlorine dioxide physical properties (Table 1).

*Photodegradation:* gaseous  $\text{ClO}_2$  is very light sensitive, it degrades through the homolytic fission of the chlorine-oxygen bond to form free radicals  $\text{O}^{\bullet}$  and  $\text{ClO}^{\bullet}$  according to the following equations (Masschelein, 1979):



In the presence of moisture, gaseous chlorine dioxide undergoes photolytic decomposition with production of a complex mixture of acids with no chlorine production according to the following mechanism (Nielson & Woltz, 1952):

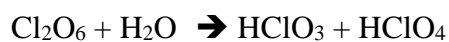
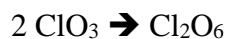
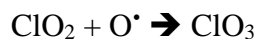


Table 1. Physical properties of chlorine dioxide (Masschelein, 1979).

Molecular weight (g/mol)	67.47
Melting Point (°C)	-59
Boiling Point (°C)	+11
Density liquid (0°C)	1.64
Density Vapor	2.4
Heat of Evaporation (kcal/mol)	6.52
Vapor Pressure (Torr at 0°C)	490 or 512
Heat of Dissolution in water (0°C) (kcal/mole)	6.6
Solubility in water	3.01 g/L at room temperature  Maximum safe concentration: 8-9 g/L at 20 °C  In chilled water, soluble up to 20 g/L
Partition coefficient (L) between water and gaseous phase: $L = C_{\text{ClO}_2}(\text{aq}) / C_{\text{ClO}_2}(\text{g})$	$70 \pm 0.7$ at 0 °C, 45 at 15 °C; $26.5 \pm 0.8$ at 35° C (Masschelein, 1979)
Aspect	Solid: red Liquid: orange Gas: orange

Aqueous chlorine dioxide is stable in solution at neutral pH, in the absence of light and at room temperature and or cooler (Kazcur & Cawfield, 1992). Chlorine dioxide decomposes into chlorite and chlorate ions through hydrolysis and disproportionation, with increased hydrolysis rate encountered at elevated temperatures and pH (>10). Hydrolysis reaction is as follows (Kazcur & Cawfield, 1992):



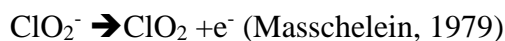
*Thermal decomposition:* gaseous chlorine dioxide thermodegradation takes place following the overall reaction:  $\text{ClO}_2 = 0.5 \text{Cl}_2 + \text{O}_2$ . Chlorine dioxide is not affected by the light used in spectrophotometric measurements (Gordon et al., 1972).

#### 2.2.1. Chlorine Dioxide Generation

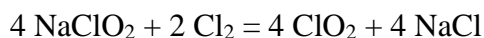
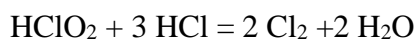
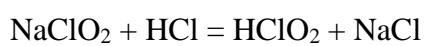
A number of commercial chlorine dioxide generators have been developed throughout the years to synthesize chlorine dioxide for diverse applications such as water treatment, chemical oxidation, disinfection, pulp bleaching and paper manufacture. Whereas conventional systems rely on chemical reaction between sodium chlorite and acid, aqueous chlorine or gaseous chlorine, other novel methods generate chlorine dioxide from emerging technologies such as electrochemical systems, solid chlorite inert matrix (flow-through gaseous chlorine) and chlorate-based technology with hydrogen peroxide and sulfuric acid as precursors.

Sodium chlorite has a strong oxidizing power. It is used as a bleaching agent and oxidative disinfectant in whitening of sugar, starches, grease and wax. Sodium chlorite exhibits a mild oxidizing capability under cold alkaline conditions. However, when medium becomes acidic, sodium chlorite solutions gain strong oxidizing ability with or

without heating, even at temperatures slightly above normal and at pH values of 3 to 5 (Masschelein, 1979). Sodium chlorite consists of a source of chlorine dioxide that needs to be oxidized in order to generate such compound, according to the following equation:



*Acid-chlorite solution:* the basis of this reaction consists of treating sodium chlorite solution with an acid that could be either a strong acid (hydrochloric acid, sulfuric acid) or weak acid (acetic acid, phosphoric acid or citric acid). HCl is usually preferred since production of chlorates is avoided (Masschelein, 1979). Upon the addition of acid to sodium chlorite solution, unstable chlorous acid forms which undergoes disproportionation to produce chlorine dioxide according to the following reactions (Masschelein, 1979):



Acidification of sodium chlorite is a simple and easy to operate system that is suitable to small and medium scale chlorine dioxide production. This process usually results in  $\text{ClO}_2$  yields of 60-80% of the theoretical yield, with starting proportions of chlorite and acid as well as mixing affecting overall reaction process. For instance, at a low pH of less than 0.5, chlorine dioxide's yield reached 100 % when equal weight proportions of sodium chlorite and HCl were used (Masschelein, 1979). The rate of chlorine dioxide production from acidified chlorite solutions takes place at measurable rates with the rate generally increasing with an increase in temperature and a decrease in pH (Masschelein, 1979). The

maximum rate of chlorine dioxide formation takes place at a pH of around 2-2.7 corresponding to pKa of chlorous acid (pKa=2.31) (Kieffer & Gordon, 1968). Simultaneously to reaction occurring above, another reaction sequence of acidic chlorite decomposition or chlorous acid self-disproportionation takes place resulting in the production of chlorine dioxide, chlorate and chloride, according to the following chemical reaction (Kieffer & Gordon, 1968):



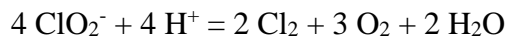
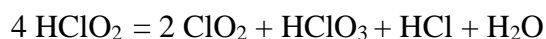
It is worth noting that as this reaction proceeds more chloride ions get generated causing the decomposition reaction to occur at a faster rate and a different stoichiometry given the catalytic nature of chloride ions (Deshwal & Lee, 2005). In this case, the chemical reaction will approximate the following reaction in the presence of chloride (Deshwal & Lee, 2005):



When a highly concentrated chlorite solution is treated with a weak acid, the following reaction predominates instead (Deshwal & Lee, 2005):



Other side reactions have also been observed (Masschelein, 1979):



Overall, chlorine dioxide generated is often impure and is present with chlorine and sometimes chlorates (Masschelein, 1979).



### 2.2.2. Reaction with Organic Compounds

Chlorine dioxide acts as a selective and versatile oxidant for many organic compounds by removing an electron without adding an atom of its own to the oxidized product.  $\text{ClO}_2$  is therefore reduced to  $\text{ClO}_2^-$  (compound of health concern) following this equation  $\text{ClO}_2(\text{aq}) + \text{e}^- = \text{ClO}_2^-$  where  $E^\circ = 0.95 \text{ V}$  (Hoigné & Bader, 1994). Unlike chlorine or hypochlorite, which react via oxidation and electrophilic substitution,  $\text{ClO}_2$  reacts only by oxidation and does not chlorinate organic compounds to form chlorinated hydrocarbons such as trihalomethanes (THMs), known to be carcinogenic (Aietta & Berg, 1986). Besides, chlorine dioxide does not react with ammonia to form chloramines. The major chlorine dioxide by-products are inorganic by-products such as chlorite, chloride and chlorate. About 0.5-0.8 mg of chlorite and 0.3 mg of chlorate are produced per mg of chlorine dioxide consumed or applied (Andrews & Ferguson, 1996).

### 2.2.3. Chlorine Dioxide Use in the Food Industry

Chlorine dioxide has long been utilized in the food industry. Its use covers a wide range of applications going all the way from sterilization and disinfection of equipment and materials, disinfection of fruits and vegetables, use in poultry chiller to control undesirable quality changes occurring during processing, such as odor and color, to increased safety through microorganisms inactivation on food products. Other uses of chlorine dioxide include sterilization of spices, removal of medicinal odor from cooked shrimp as well as shelf-life extension of tomatoes (Masschelein, 1979).

$\text{ClO}_2$  inactivates microorganisms in fish, fruits and vegetables without affecting their organoleptic and nutritive values. Disinfection can be achieved by immersion for 30

minutes in aqueous solution containing 50 to 1000 mg/l (50 to 1000 ppm) or by exposure to air containing 2000 to 3000 ppm of  $\text{ClO}_2$  (g). Pepper, for instance, may be sterilized by using gaseous  $\text{ClO}_2$  concentrations between 1000 to 20000 ppm (Masschelein, 1979). When added at a concentration of 1.3 ppm to the cooling water used for pickling cucumbers, chlorine dioxide optimally controlled bacterial populations that were reaching high loads during daily operations (Reina et al.). It is worth mentioning that after treatment with chlorine dioxide, remaining or residual products on fruits and vegetables are chloride and chlorite traces (Thomas, 1979).

#### 2.2.4. Disinfection Efficacy and Pathogen Inactivation by Chlorine Dioxide

Since most of the chemical inactivation compounds are oxidative in nature, they have the tendency to be reduced by organic and inorganic compounds present in the medium to be treated. Chemical demand is defined as the amount of disinfectant consumed by compounds present in the medium. In other words, it's the difference between initial amount of chemical applied and the quantity of chemical available after a specified contact period otherwise known by residual amount (Weavers & Wickramanayake, 2001). Residual levels of chemical in the medium after demand should be maintained in adequate concentrations to ensure desired extent of microbial inactivation. Physical state of microorganisms in media affects the inactivation process since most of microorganisms are found as clumps and attached to other suspended particles and not as discrete particles in free suspension (Weavers & Wickramanayake, 2001).

Given the selective nature of chlorine dioxide towards organic compounds and its non-reactivity with ammonia, only small quantities of this compound are needed to reach desired residual levels. Inactivation of microorganisms by chlorine dioxide is achieved

through oxidation of the amino acids (cysteine, tryptophan and tyrosine). As to viral inactivation, it can be caused in some cases by modification of viral capsid proteins while in others by reacting with viral RNA and affecting RNA synthesis (EPA, 1999).

Chlorine dioxide also affects physiological functions, such as disruption of protein synthesis (Bernarde et al., 1976a) or permeability of outer membrane through alteration of membrane proteins and lipids (Aieta & Berg, 1986; Ghandbari & al., 1983; Olivieri & al., 1985).

The disinfection efficiency of chlorine dioxide is affected by environmental factors such as pH, temperature and pathogen aggregation.  $\text{ClO}_2$  is an effective biocide over a wide range of pH (3 to 9), but its efficiency towards pathogen inactivation varied from an increasing degree of inactivation upon an increase in pH in the case of poliovirus 1 and *Naegleria gruberi* cysts to no noticeable change in its bactericidal activity in pH range 6 to 10 (EPA, 1999).

As to the effect of temperature on disinfection efficiency of  $\text{ClO}_2$ , a temperature decrease would lead to a reduction in the degree of inactivation of pathogens. For instance, LeChevallier et al. (1997) (LeChevallier et al., 1997) observed a 40 percent decrease in  $\text{ClO}_2$  effectiveness on *Cryptosporidium* when temperature was reduced from 20°C to 10°C, similarly to what has been obtained for *Giardia* and viruses. Pathogen aggregates are more resistant to  $\text{ClO}_2$  treatment and usually require a longer time to get inactivated than single state pathogens or clumps of smaller size (EPA, 1999). Gaseous chlorine dioxide has shown sporicidal activity that has been confirmed by work conducted by Jeng and Woodworth (1990a) (Jeng & G., 1990a).

#### 2.2.5. Chlorine Dioxide Patents

A good number of patents have been generated over the years from mid 1900s to present time on chlorine dioxide production from different systems, demonstrating the growing interest in this compound and its wide range of applications.

All patents have addressed in their inventions one or numerous limitations associated with chlorine dioxide production with the aim to come up with a simple, convenient and safe way to generate such compound at high yield and quality.

ClO<sub>2</sub> needs to be generated on-site given its unstable and explosive nature in addition to safety hazards associated with its storage and distribution.

Many patents have developed systems whereby chlorine dioxide was produced either readily or in a sustained and controllable manner in aqueous or gaseous form subsequent to acidification of chlorite (and/or chlorination using aqueous or gaseous chlorine), acidification (and/or reduction) of chlorates in solution or in dry form with or without water or water vapor trigger.

Chlorine dioxide generated is usually used for disinfection, sterilization or deodorization purposes and applied to disinfect drinking water, water used in washing, water in swimming pools, packaged fruits and vegetables during shipment as well as chemosterilization of porous and nonporous surfaces of medical and dental articles.

Many chlorine dioxide generator apparatuses have also been designed to produce in-situ chlorine dioxide at low cost for direct utilization given the safety concerns arising from its storage and distribution.

A number of patents have developed solid compositions where ClO<sub>2</sub> active ingredients were mixed alone or in combination with other inert compounds to produce ClO<sub>2</sub>

generators of different shapes and forms (tablet, capsule, pouch, sachet, powder, etc.) having the ability to release  $\text{ClO}_2$  in the presence or absence of liquid water or water vapor in the environment.

Solid compositions containing specific ratios of water-soluble sodium chlorite, an oxidizing chlorine releasing agent and a proton donor used to lower pH of the aqueous solution to less than 3 were also developed. In such system, chlorine dioxide was generated at nearly 100 % yield after a short time when dissolved in a suitable amount of water. Given the nature of the reaction (fast and nearly complete), side effects resulting from residual by-products were limited, allowing the use of this system as a skin, mucous membrane antiseptic (Marzouk & Gutman, 1995).

Chlorine dioxide tablets have been also prepared by mixing  $\text{ClO}_2$  active ingredients in solid form (metal chlorite, solid acid and/or oxidizing chlorine releasing agent) with inactive compounds (fillers, binders and coloring agents) to form solid compositions of different shapes. Given the sensitivity of  $\text{ClO}_2$  generation reaction to moisture, storage of this solid mix at relative humidity above 10 % is to be avoided to prevent premature chlorine dioxide production. Tablet composition was manipulated by combining metal chlorite (e.g. sodium chlorite), metal bromide (e.g. sodium bromide) with solid acid (e.g. sodium hydrogen sulfite) at different proportions to obtain fast and high chlorine dioxide release ranging from 68-657 ppm upon contact with water (Sanderson, 2010).

Other chlorine dioxide generating compositions have also been developed where gaseous  $\text{ClO}_2$  was released over an extended period of time regardless of the surrounding environmental condition such as R.H. This composition contained solid metal chlorite such as sodium chlorite, solid citric acid, a moisture slow-release agent with the ability to

absorb moisture from the environment and slowly release some of it by evaporation to allow dissolution of chlorite and acid and their subsequent reaction, a solid deliquescent agent that has the ability to take up moisture from the environment which can be furthered used by moisture slow-release agent (retained or released), a gelling agent and liquid water where liquid water can be gelled and used in dissolution and reaction of  $\text{ClO}_2$  precursors in the absence of moisture. All the different agents making up this composition allow release of gaseous chlorine dioxide in a stable manner regardless of surrounding environment's relative humidity and can be applied to spaces of different sizes. This mix can be placed in a bag composed of air-permeable non-woven fabric enabling air to penetrate inside and chlorine dioxide to be released into the external space. Applying proper ventilation will permit uniform distribution of  $\text{ClO}_2$  into the space in question (Shibata & Abe, 2009).

Pouch systems have also been developed where chlorine dioxide precursors (sodium chlorite and acid in dry or solution form, in the presence or absence of water) alone or in combination with other materials are separated from each other by a physical barrier (e.g. wall) and then allowed to react together following application of pressure (e.g. squeezing or puncturing) to generate chlorine dioxide at a controlled rate (Leifheit & Hutchings, 1992). In some cases, pre-determined amounts of chlorine dioxide precursors were placed in sealed water-soluble bags or pouches and allowed to release chlorine dioxide upon dissolution of envelope when in contact with water. Given the sensitivity of such pouches to ambient R.H., they are usually stored in airtight and re-sealable containers to allow multiple usages and avoid premature chlorine dioxide production (Tarbet, 2005).

Chlorine dioxide generating polymer packaging films have been developed whereby

chlorine dioxide was released over an extended period of time after exposure to atmospheric moisture. A coating was developed by mixing a hydrogen-bonded matrix loaded with chlorite salt, with another hydrophobic phase containing a hydrolysable acid anhydride component. This coating was applied onto the substrate as a hot melt and allowed to solidify at room temperature. Chlorine dioxide generated from this system after exposure to moisture from environment was used as a biocide or insect repelling agent (Wellinghoff, 1994).

In addition to the different chlorine dioxide systems discussed above, many patents devised a multitude of cost-effective processes to produce higher quality and yield of chlorine dioxide. For instance, electrolysis of aqueous chlorite solution performed in the presence of a water-soluble sulfate was able to produce chlorine-free chlorine dioxide (Rempel, 1955).

In summary, numerous patents were developed for the purpose of generating chlorine dioxide via a multitude of delivery systems, given the attractive features this compound displays including oxidizing, biocidal potential, versatility and wide range of application in medical, agricultural and food fields. So far, very few patents have focused on developing packaging films with the ability to generate gaseous chlorine dioxide suitable for food applications.

#### 2.2.6. Chlorine Dioxide - regulations and safety concerns

Chlorine dioxide has been approved for use by FDA under 21 CFR §173.300 as an additive, more specifically as an antimicrobial agent at residual levels not exceeding 3 parts per million (ppm) in water used for poultry processing and washing of fruits and vegetables not used as raw commodities and which require further treatment such as

rinsing with potable water, blanching, cooking or canning before consumption (FDA, 2005). The use of chlorine dioxide precursors in food packaging materials for uncooked meat such as poultry and seafood received approval from FDA in 2001 (FDA, 2001). As to safety considerations linked to the use of chlorine dioxide, Occupational Safety and Health Administration (OSHA) has set a low threshold limit value (LTV) of 0.1 ppm (0.3 milligram per cubic meter ( $\text{mg}/\text{m}^3$ )) as an 8-hour time-weighted average (TWA) exposure concentration to workers (OSHA, 1996).

### **2.3. Chlorine Dioxide Precursors**

In this research, we chose to synthesize  $\text{ClO}_2$  from chemical reaction between citric acid, an organic triprotic acid and sodium chlorite salt in the presence of moisture. The stoichiometry of this chemical reaction is as follows (Masschelein, 1979):

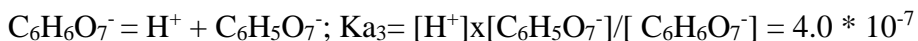
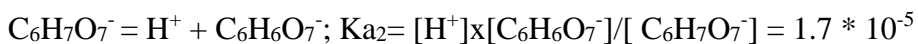
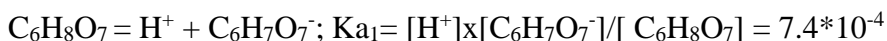


#### **2.3.1. Citric Acid ( $\text{C}_6\text{H}_8\text{O}_7$ )**

Weak acids are usually preferred over strong acids because they minimize violent reactions that are difficult to control (Masschelein, 1979). Besides, citric acid is food-grade that is permitted for use in direct contact with food. Citric acid is a polyprotic acid that contains three acid hydrogens per molecule (Figure 2). It has a molecular weight of 192.124 g / mol, a solubility in water of 73 g / 100 mL at 20°C and a melting point ( $T_m$ ) at 153°C. This acid ionizes in a stepwise manner with an ionization constant at each step. Primary ionization of citric acid is stronger than secondary and the secondary is stronger than the tertiary because it takes extra energy to overcome the second and third negative charges being so close to the first negative charge. Dissociation of citric acid is shown



below (Mortimer, 1967):



The principal source of  $\text{H}^+$  is the primary ionization; the  $\text{H}^+$  produced by the other ionizations as well as that from ionization of water, is negligible in comparison.

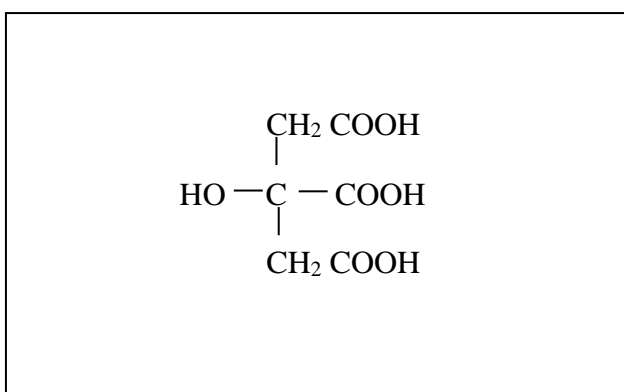


Figure 2. Citric acid chemical structure.

### 2.3.2. Sodium Chlorite ( $\text{NaClO}_2$ )

In the solid form, sodium chlorite acts as a corrosive agent with the ability to explode when subjected to heat (absolute limit  $176^\circ\text{C}$ ), in contact with organic materials or undergoing a violent shock. That is why it is preferably dealt with in solution. Sodium chlorite decomposes in an explosive manner at temperatures of  $180\text{--}200^\circ\text{C}$ :  $\text{NaClO}_2 = \text{NaCl} + \text{O}_2$ . Sodium chlorite has a molecular weight of  $90.44 \text{ g/mol}$  and a solubility of  $39 \text{ g/100 mL}$  at  $17^\circ\text{C}$ . This salt acts as a source of chlorine dioxide. In a neutral solution, sodium chlorite is not oxidative toward the attack of most organic compounds. However, when the medium becomes acidic, activation of sodium chlorite occurs leading to the formation of unstable chlorous acid and eventually chlorine dioxide (Masschelein, 1979).

### **3. DESIGN OF ClO<sub>2</sub> SELF-RELEASING PACKAGE LABEL**

#### **3.1. System Description and Uniqueness**

This packaging system can take multiple shapes (label, film, etc.), and be embedded with one of the ClO<sub>2</sub> precursors to avoid premature reaction and subsequent chlorine dioxide loss. The release of ClO<sub>2</sub> occurs upon controlled activation of the packaging system by supplying the other precursor in different forms (sprayed from solution, embedded in another layer of polymer, etc.) coupled with an auxiliary treatment step (application of pressure, heat, a combination of thereof, UV, ultrasound, etc.) to accelerate and facilitate the movement of particles within polymer matrix and enhance chemical reaction of ClO<sub>2</sub> formation.

The system in question is a ClO<sub>2</sub> self-releasing package label: a polymer matrix embedding within it ClO<sub>2</sub> chemical reaction, when triggered at the right time by controlled activation mechanism, facilitates the movement of reactants to meet one another, react, generate and release gaseous chlorine dioxide at effective concentrations to inhibit microbial growth without compromising quality attributes of food products. The success of this system depends on matching release kinetics of ClO<sub>2</sub> (how much ClO<sub>2</sub> is being released and how fast/slow it's being released over time) with microbial and quality deterioration kinetics of target food product.

The uniqueness of this system stems from the design and manufacturing aspects of the label where ClO<sub>2</sub> package label is required to meet design requirements (size, thickness, shape, ease of integration in primary package, loading of ClO<sub>2</sub> precursors, etc.) and withstand processing methods (extrusion, coating, etc.) to achieve desired

functionality (generating and releasing  $\text{ClO}_2$  in a controlled manner to match microbial and quality deterioration kinetics). Another unique feature of this label is controlled activation mechanism performed on label through administration of the other  $\text{ClO}_2$  precursor followed by the application of external means (pressure only or combination of heat and pressure) for a short period of time (5-10 s) to trigger the generation and release of  $\text{ClO}_2$  in a controlled manner in the package headspace when is needed, at the point of use, until the consumption of  $\text{ClO}_2$  precursors, reflecting the self-releasing character of this label.

### **3.2. System Components**

The components of this system are summarized in Figure 3.

#### **3.2.1. Polymer Matrix**

Polymer matrix is the internal polymer network with free space serving as a reservoir of  $\text{ClO}_2$  precursors and reaction site where chemical compounds come into contact with each other and react after controlled activation mechanism to generate chlorine dioxide. Polymer composition can be manipulated to achieve the desired release of  $\text{ClO}_2$ . Polymer type dictates polymer morphology (molecular orientation and crystallinity), glass transition temperature ( $T_g$ ) and polarity.

In semi-crystalline polymers, the crystalline domains prevent diffusion of permeant through matrix, while amorphous domains possess more free volume facilitating diffusion of permeants through the matrix.

At temperatures above  $T_g$ , transition from glassy to rubbery state increases polymer chain mobility and therefore permeant diffusion. Polarity decreases diffusion of permeant

through matrix because of tight packing of polymer chains. Swelling of hydrophilic polymers on the other hand due to use of plasticizers (water or ethyl glycol, glycerol or other) increases diffusion of permeant through matrix. Thickness of the polymer matrix can be manipulated to influence release of  $\text{ClO}_2$ , the thicker the label, the slower the diffusion and release rate of  $\text{ClO}_2$  due to the distribution of precursors within the bulk of polymer matrix.

### 3.2.2. $\text{ClO}_2$ Precursors

$\text{ClO}_2$  is produced from chemical reaction between chlorite ion ( $\text{ClO}_2^-$ ) of  $\text{NaClO}_2$  salt and  $\text{H}^+$  of citric acid.

Molecular weight and size of these compounds affect their localization and mobility within the polymer.

Incorporation of these compounds in the solid versus dissolved form as well as their loading percentage in the matrix affect their entrapment, distribution pattern and mobility within the matrix, and their availability for chemical reaction and  $\text{ClO}_2$  generation. Whereas citric acid and sodium chlorite salt in the solid form do not diffuse throughout the polymer matrix due to their occurrence in the polymer matrix as aggregates and cluster of molecules, their dissolved counterparts have the ability to diffuse in the polymer matrix due to their small molecular size and higher mobility.

Compatibility of  $\text{ClO}_2$  precursors with polymer molecules through molecular association (chemical bonding or physical interaction) affect availability of these compounds for chemical reaction, their mobility within the matrix and also alter the structure of polymer matrix.

### 3.2.3. Processing Method

The processing method used to manufacture the package label influences label's morphology as well as dispersion and localization of  $\text{ClO}_2$  precursors in the polymer matrix, controlling therefore release property of this package system.

Two processing methods have been used to manufacture the  $\text{ClO}_2$  package label: extrusion and solution casting. While the former relies on thermoplastic behavior of polymers at low moisture levels, the latter involves dispersion or solubilization of biobased polymers in a film-forming solution followed by the evaporation of the solvent (water in this case). During extrusion, long polymer chain molecules undergo some level of molecular orientation during processing and cooling resulting in a polymer product with defined crystallinity, and final polymer properties (mechanical, optical and chemical)(Chung, 2000).

Films produced by solution casting method display different structural and morphological properties, depending on the drying conditions (temperature, relative humidity and type of energy source, etc.) and film forming solution composition.

The extrusion processing method was used to manufacture synthetic labels made from three synthetic polymers: ethylene vinyl acetate (28 % vinyl acetate), polycaprolactone and aromatic polyether-based thermoplastic polyurethane, while solution casting method was used to manufacture the biobased labels from pectin and gelatin.

### 3.2.4. Controlled Activation Mechanism

Controlled activation mechanism consists of an activation step necessary to trigger the chemical reaction of  $\text{ClO}_2$  generation and release from package label. This step is usually

performed when needed, at the point of use, at the same time of fresh produce packing, to prevent  $\text{ClO}_2$  loss. The controlled aspect of this step provides the system with an ON switch, which can be activated when desired by the user, conferring a self-releasing character to the package label until the consumption of  $\text{ClO}_2$  precursors.

From a molecular level standpoint, the activation mechanism influences molecular diffusion of  $\text{ClO}_2$  precursors and moisture through the polymer matrix to enable chemical reaction and generation of  $\text{ClO}_2$  after which, this compound diffuses through polymer matrix and out into headspace.

In this research, two controlled activation mechanisms were explored: 1) combination of spraying and heat-pressing performed on synthetic labels, and 2) pressing only applied to biobased labels.

Optimizing the variables such as concentration of solution being sprayed, temperature, pressure and time applied is essential to maximize  $\text{ClO}_2$  chemical reaction and its release profile from package label.

### **Pressure**

Applying pressure to the package label will force  $\text{ClO}_2$  precursors and moisture to come in close proximity with one another in polymer matrix increasing likelihood of such molecules to react together and liberate chlorine dioxide.

In some cases, pressure ensures contact between layers of the multilayer biobased package label. Depending on the duration of pressing step, pressure applied could decrease the space between the polymer molecules by reducing free volume, restricting their mobility and affecting the diffusion of  $\text{ClO}_2$  through the polymer matrix.

## Temperature

While applied pressure situates reactants in close proximity with one another, the temperature supplied by heat step influences reaction rate of ClO<sub>2</sub> generation and diffusion of reactants through the polymer matrix and of ClO<sub>2</sub> out of the polymer matrix. Temperature dependence of reaction rates is demonstrated by Arrhenius equation:  $k = A e^{-E_a/RT}$ , where  $k$  is the rate constant of a chemical reaction,  $A$  is the pre-exponential factor,  $E_a$  the activation energy,  $R$  the universal gas constant and  $T$  is absolute temperature (K). Similarly, diffusivity of compounds in the polymer matrix, be it reactants or products, is related to temperature by Arrhenius equation  $D = D_0 e^{(-E_a/RT)}$ , where  $D$  is the diffusion coefficient (cm<sup>2</sup>/s),  $D_0$  is a constant,  $E_a$  the activation energy for the diffusion process (J/mol),  $R$  the universal gas constant (J/mol K) and  $T$  is absolute temperature (K). Increase in temperature increases kinetic energy and mobility of ClO<sub>2</sub> precursors in the polymer matrix resulting in increased frequency of effective collisions and faster rate of ClO<sub>2</sub> generation as well as an increase in the diffusion and release of ClO<sub>2</sub> from the polymer matrix. Also, depending on applied temperature and duration of heat step, softening of the polymer matrix could occur, leading to increased polymer chain mobility and diffusion of compounds through matrix and of ClO<sub>2</sub> out of matrix.

## Relative Humidity (RH)

Relative humidity is needed to dissociate molecular compounds into their respective ionic form and enable chemical reaction to take place especially when reactants used are in the solid, undissociated form. Also, RH causes swelling and relaxation of hydrophilic polymer matrix facilitating therefore diffusion of reactants and ClO<sub>2</sub> release from the

package label.

### **Parameters influencing chlorine dioxide reaction kinetics**

Reaction kinetics is how fast/how slow reactants react together to generate products.

Many parameters affect reaction rate (Mortimer, 1967):

1. Temperature, a parameter accounted for by Arrhenius equation and incorporated into rate constant  $k$  ( $k = A e^{-E_a/RT}$ )
2. Concentration of reactants, where an increase in the concentration of reactants will increase the number of effective collisions and therefore the amount of  $\text{ClO}_2$  produced, frequency of effective collisions is included in rate constant as well.
3. Nature of reactants (covalent versus ionic compounds): reaction between ionic compounds occurs very fast as it involves exchange of ions already separated in aqueous solutions during their dissolution. However, covalent compounds or compounds in the solid form need to be broken down and ionized first, which could be complete or incomplete depending on the molecule and solvent used. For instance, whereas all the sodium chlorite salt is ionized in aqueous solution, organic acid is only incompletely ionized in such a way dissolved molecules will exist in equilibrium with ions in aqueous solutions.
4. Surface area of reactants is of primary importance when reactants are used in solid form, the larger the surface area (the smaller or finer) particles are, the more surface area will be available for reaction to take place, the faster the reaction rate.



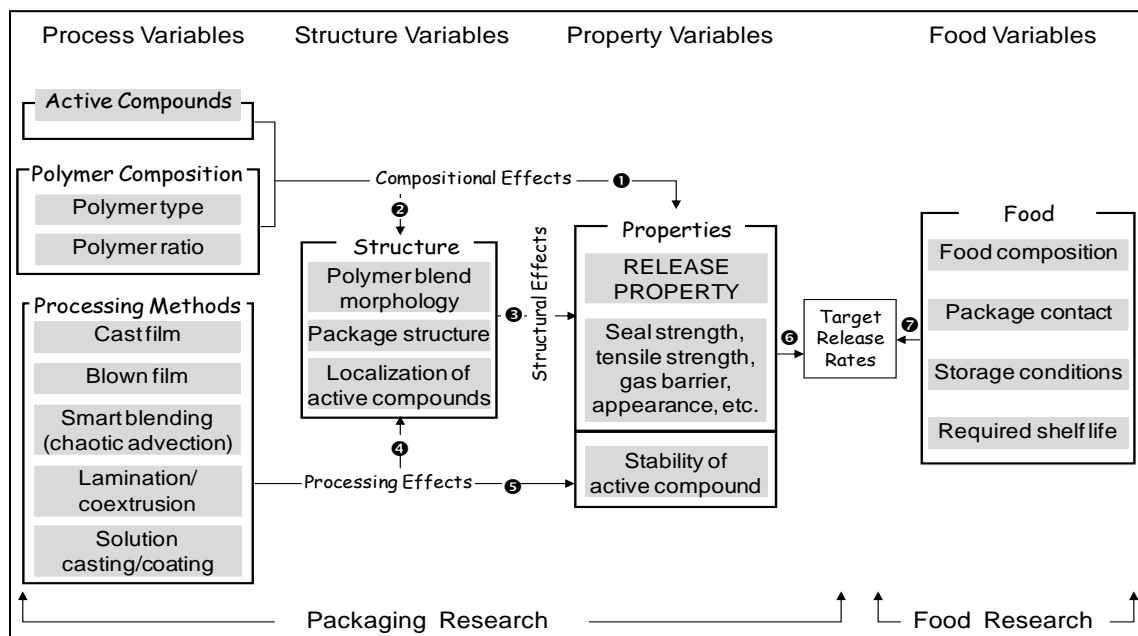


Figure 3. Conceptual framework for developing Controlled Release Packaging (Zhu, 2008) .

### 3.3. Defining “Controlled” Aspect of Package Label

Controlled Release Packaging (CRP) is an innovative type of active packaging that deliberately regulates the release of active compounds based on targeted need (food deterioration kinetics). The importance of CRP lies in the “controlled” part, which constitutes the key aspect of such package label. This provides packaging engineers with precision to tailor package systems suitable for different food applications. CRP’s main goal is to match delivery rate of active compounds from package system with deterioration kinetics of food, be it quality deterioration kinetics or microbial growth kinetics to enhance food safety and quality and extend shelf-life.

In this research, the term “controlled” refers to 1) controlled activation mechanism serving as the trigger or switch to  $\text{ClO}_2$  release, the time at which release of  $\text{ClO}_2$  from the package label is desired, and 2) controlled or time release of  $\text{ClO}_2$  from the package

label as in the amount of  $\text{ClO}_2$  released and how fast or slow this compound is released from package label and 3) desirable matching between  $\text{ClO}_2$  release kinetics and microbial growth and quality deterioration kinetics. Whereas the former type of control depends on the operator, the latter ones depend on release kinetics which in turn depends on polymer composition, processing method, polymer morphology; activation mechanism condition (RH, pressure, and temperature); package volume; fresh produce of interest and target microorganism.

### **3.4. Usefulness of this System**

To date, most of systems developed for  $\text{ClO}_2$  generation have both reactants (acid and salt) either mixed together or separated from each other by a barrier (for instance, water-soluble membrane) and require a source of moisture (food or environment) to trigger chemical reaction and release  $\text{ClO}_2$ .

Not only are these systems dependent on a moisture source, but also their handling and storage at inappropriate conditions especially relative humidity ( $\text{RH} \geq 40\%$ ) result in  $\text{ClO}_2$  losses. Use of chlorine dioxide as a sanitizer in solution is effective in removing dirt and reducing microbial load on food products.

Complementing this step with timely exposure to gaseous chlorine dioxide will constitute an additional hurdle minimizing even further microbial count, enhancing safety and extending shelf-life.

Gaseous chlorine dioxide, given its molecular size and gaseous nature, has the ability to penetrate hard to reach small pores, channels and crevices otherwise inaccessible by water-based sanitizers due to surface tension.

The current system addresses the drawbacks mentioned above by combining  $\text{ClO}_2$

precursors only at the point of use and offering a controlled activation upon demand, minimizing losses of chlorine dioxide during handling and storage and expanding its intended use to cover all food products regardless of their ability to generate moisture. This system offers therefore better control over activation step and increased flexibility and versatility to suit diverse food applications. Besides, through varying polymer composition, processing conditions as well as controlled activation conditions, release rate of chlorine dioxide can be manipulated to match microbial growth and quality deterioration kinetics associated with a specific food product. Not to mention, the safety, convenience and user-friendliness of adopting such a system at the industrial level since it doesn't require huge investments to integrate it into existing operations, making it both a practical and useful system.

### **3.5. Package Label Intended Use**

The package label is intended to be applied to the inside of primary package and gaseous chlorine dioxide allowed to release into headspace in a controlled manner to inactivate microbial growth, without compromising quality of food product and package integrity. The release will be initiated in response to controlled activation mechanism. Given the self-releasing character of this label after activation, it can be adapted to different food products (respiring and non-respiring) making it a versatile system.

## 4. PROBLEM FORMULATION

### 4.1. Rationale and Problem Statement

#### 4.1.1. Rationale

Preliminary work related to developing chlorine dioxide releasing system, in the form of a packaging film, was conducted in our lab.

The system considered was polylactic acid (PLA) film incorporated with both  $\text{ClO}_2$  reactants (food-grade acid and sodium chlorite) in solid form.

$\text{ClO}_2$  was produced in response to a trigger, moisture generated from respiration of grape tomatoes, subsequent to reaction between its precursors.

Not only did  $\text{ClO}_2$  release from packaging film into the headspace, but also its concentration was effective at achieving more than 3 log reductions in *Salmonella* spp. and *Escherichia coli* O157:H7 on grape tomatoes (Ray et al., 2013).

This system showed however several drawbacks mainly linked to chlorine dioxide loss during handling and storage.

There is a need to design a simple, safe, practical and convenient  $\text{ClO}_2$  package system that 1) avoids premature reaction of  $\text{ClO}_2$  precursors upon exposure to moisture during handling and storage, 2) offers better control over  $\text{ClO}_2$  release by incorporating only one of reactants into the packaging system followed by a controlled activation mechanism for an optimum  $\text{ClO}_2$  production, 3) provides prolonged exposure to  $\text{ClO}_2$  released from a package system as opposed to one-time exposure only by gas flushing, 4) minimizes safety hazards associated with  $\text{ClO}_2$  transportation and storage as well as sachet disruption and subsequent chemical contamination of food, and 5) offers ease of handling,

activation and application into primary package. This can be achieved through designing a self-releasing package label that can release  $\text{ClO}_2$  in a controlled manner at concentrations effective enough to improve food safety and maintain quality, which could be used in different applications.

#### 4.1.2. Problem Statement

The objective of this research is to evaluate the feasibility of an innovative and practical self-releasing package label made of synthetic and biobased polymers with the ability to generate and release gaseous chlorine dioxide in a controlled manner within the package system in response to controlled activation to improve microbial safety and maintain quality of fresh produce. The innovative aspect of the package label stems from the fact that label incorporates within it a chemical reaction, when triggered at right time by controlled activation mechanism generates and releases chlorine dioxide with time until the consumption of  $\text{ClO}_2$  precursors to inactivate microorganisms and improve fresh produce safety.

Technical feasibility of this package label consists of the design and manufacture of this label and its ability to generate and release  $\text{ClO}_2$  at concentrations effective to inactivate microorganisms' growth on fresh produce under the preset experimental conditions to improve food safety and extend shelf life. Under the applied controlled activation mechanism, would  $\text{ClO}_2$  precursors be able to diffuse through the matrix to become spatially close to one another? If so, would they react and produce  $\text{ClO}_2$  gas? Would this generated  $\text{ClO}_2$  be able to diffuse in the package label and release from the matrix? If so, would the released  $\text{ClO}_2$  concentration be adequate to inactivate microorganisms on the surface of fresh produce?

Evaluating the technical feasibility of this package label, confirming label's activity and antimicrobial effectiveness, is achieved by a combination of methods: 1) quantification of the concentration of  $\text{ClO}_2$  released from the label using UV spectrophotometry, the output of this method being different delivery profiles of  $\text{ClO}_2$  from activated package labels and 2) testing the antimicrobial effectiveness of the package label against growth of pathogenic bacteria (*Salmonella* Montevideo) on TSA plates. This method serves as a validation step of the first method. If microbial inactivation is achieved upon exposure to activated label, then  $\text{ClO}_2$  was 1) generated from reaction between citric acid and sodium chlorite, and 2) released from such label at an adequate concentration and a rate matching microbial growth kinetics to achieve the desired antimicrobial effect. The findings of these two methods used in combination would provide supporting evidence on the technical feasibility of this package label.

In order to meet the main objective of this research, a series of specific sub-objectives are formulated and need to be achieved in order to either validate or contradict label's feasibility.

- Sub-objective 1: Manufacture the synthetic package label incorporated with  $\text{ClO}_2$  precursors based on design specifications using commercial-scale extrusion.
- Sub-objective 2: Quantify the concentration of  $\text{ClO}_2$  released from the synthetic package labels over time after controlled activation mechanism (spraying and heat-pressing).
- Sub-objective 3: Test the antimicrobial effectiveness of activated  $\text{ClO}_2$  synthetic package labels against *Salmonella* Montevideo growth on media (Tryptic Soy Agar) plates.

- Sub-objective 4: Manufacture the biobased package label incorporated with  $\text{ClO}_2$  precursors based on design specifications using solvent casting technique.
- Sub-objective 5: Quantify the concentration of  $\text{ClO}_2$  released from the biobased package labels over time after controlled activation mechanism (pressing only).
- Sub-objective 6: Test the antimicrobial effectiveness of activated  $\text{ClO}_2$  biobased package labels against *Salmonella* Montevideo growth on media (Tryptic Soy Agar) plates and fresh produce.
- Sub-objective 7: Evaluate the practical feasibility of the biobased labels by comparing the range of  $\text{ClO}_2$  concentration released from these labels and inactivation levels achieved with target concentration range reported in literature.
- Sub-objective 8: Optimize the design of synthetic package label to achieve desired controlled release of  $\text{ClO}_2$  to match microbial growth kinetics and improve fresh produce safety.
- Sub-objective 9: Optimize the design of biobased package label to achieve desired controlled release of  $\text{ClO}_2$  to match microbial growth and quality deterioration kinetics of fresh produce and extend label's practical application to suit a variety of food applications.

## 5. APPROACH -I-: EXTRUSION

### 5.1. Concept Development

#### 5.1.1. Synthetic Label Manufacture

Synthetic ClO<sub>2</sub> package label is a polymer matrix extruded with citric acid in the solid form using a commercial single screw extruder at Specialty Extrusion Co (Figure 4).

The polymer resins were first compounded with citric acid using twin-screw extruder to achieve proper mixing between resin and citric acid. Three different polymers (ethylene vinyl acetate (28% vinyl acetate), polycaprolactone and thermoplastic polyurethane) were used to manufacture package label with two final citric acid concentrations (7.5 and 15 %) and two levels of thickness.

The synthetic package labels were prepared by manually cutting the extruded films into 10 cm<sup>2</sup> surface area (5 cm x 2 cm).

#### 5.1.2. Controlled Activation Mechanism

##### **Spraying**

Since only citric acid is incorporated into the polymer matrix, the other reactant is supplied in the form of an aqueous solution by spraying (Figure 4). Chlorite ions in solution diffuse along with water molecules into polymer matrix to react with solid citric acid embedded in package label. Citric acid in the solid form needs to be dissociated first and it is only when ionized chlorites collide with protons of the acid that the reduction reaction takes place and chlorine dioxide forms.



### **Heat-Pressing**

If label activation is only limited to spraying step, depending on polymer composition and structure, diffusion of chlorite ions and moisture, and subsequent reaction with protons would only be restricted to surface of the polymer matrix, the bulk of label being inaccessible (Figure 4).

This would cause burst release of chlorine dioxide at concentrations lower than expected. Applying pressure would force and speed up movement of chlorites and water molecules to reach reactive sites within label where citric acid is located. Once spatially close to citric acid molecules, heat step will increase kinetic energy of these particles and frequency of effective collisions with protons to generate  $\text{ClO}_2$ . Chlorine dioxide generated will then release from the polymer matrix depending on polymer composition and structure.

Following the activation mechanism, the package label is inserted into primary package and allowed to release  $\text{ClO}_2$  in controlled manner to inactivate microbial growth.

### **5.2. Extrusion**

Extrusion process is widely used in the plastic industry to convert thermoplastics into different packaging forms (i.e. films, bags, bottles, etc.).

Polymer extrusion starts by feeding polymer resin into a heated chamber containing the feed screw through the use of a hopper.

Extrusion involves three main steps, melting of a solid polymer from shear action of feed screw and heated barrel, shaping of the molten polymer and exit of melted resin out of the chamber at a steady rate through a die. Solidification of the molten polymer into a plastic film is achieved by cooling the melt onto chilled rolls.

In addition to heating and melting the plastic, the extruder performs two types of mixing action: dispersive mixing resulting in component size reduction and distributive mixing leading to spatial rearrangement of different components (Chung, 2000).

The resulting extruded plastic film possesses different structures and properties depending on orientation and stretching during extrusion and chain alignment and crystallization during the cooling step.

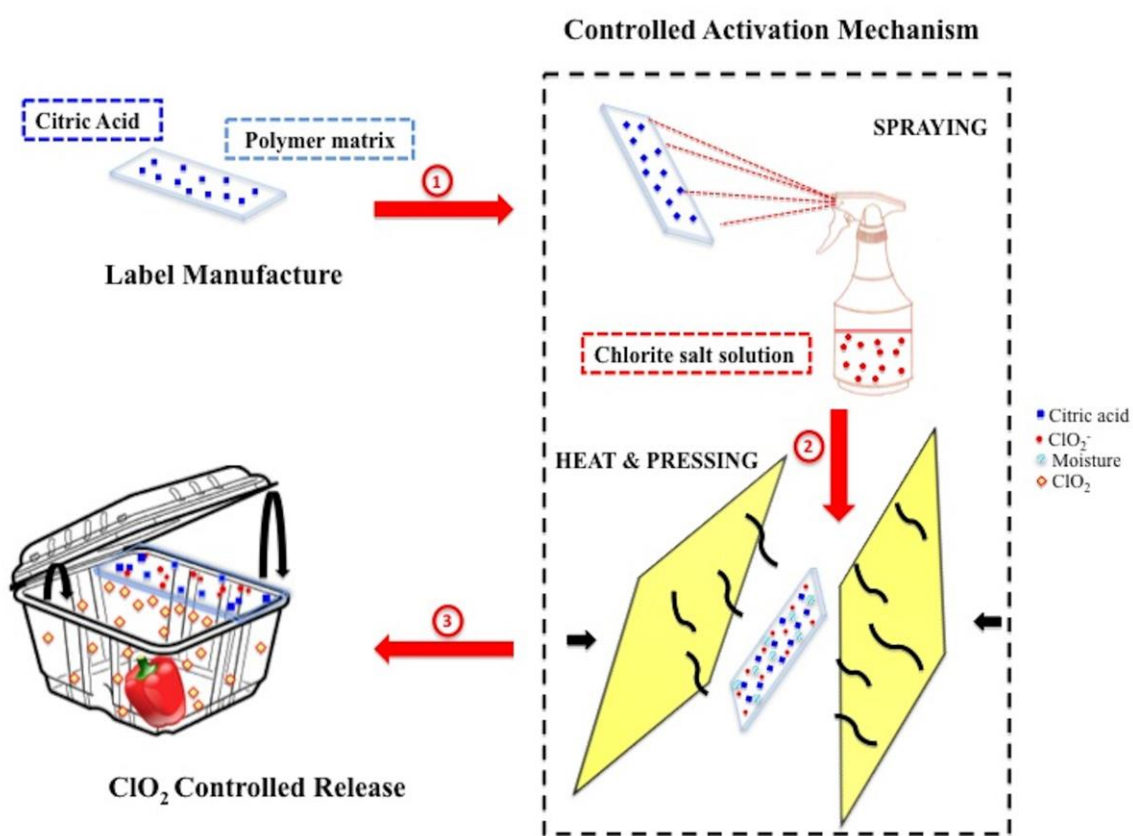


Figure 4. Synthetic label concept development: extrusion, controlled activation and application.

### 5.3. Synthetic Polymers

#### 5.3.1. Ethylene Vinyl Acetate (EVA)

Polyethylene (PE) is a crystalline polymer made of a backbone of covalently linked carbon atoms with pendant hydrogens (Figure 5). The final properties of PE can be manipulated by modifying its regular chain structure through incorporation of different functional groups ranging from alkyl groups to acids and esters (Henderson, 1993).

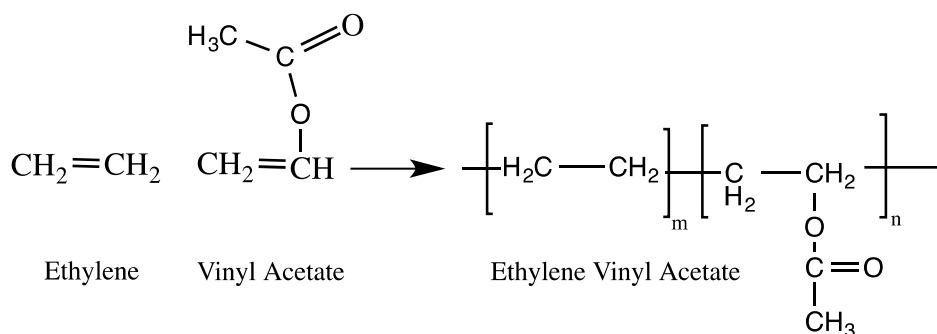


Figure 5. Ethylene-vinyl acetate (EVA) copolymer. Building blocks of EVA molecule: ethylene and vinyl acetate and chemical structure with backbone of covalently linked atoms with pendant hydrogens and acetoxy side chains.

During high-pressure polymerization process, vinyl acetate (VA) comonomer is randomly distributed along the ethylene chain causing PE's crystal structure to be disrupted providing EVA with amorphous properties, thus semi-crystalline nature of EVA (Henderson, 1993). VA is a polar group that adds functionality to EVA therefore, affects its polarity, chemical reactivity and crystallinity in a magnitude proportional to VA incorporation level (Henderson, 1993). Apart from acetoxy side chains, EVA copolymer chain is also composed of short- and long- chain side branches influencing chain packing, crystallinity and density similarly to low-density polyethylene (LDPE) (Figure 6) (Henderson, 1993). Properties of EVA copolymers are primarily determined by the

following structural attributes: 1) content of vinyl acetate, 2) molecular weight and distribution, 3) molecular branching (Henderson, 1993).

### VA Content

Ethylene vinyl acetate is a semicrystalline polymer with alternating crystalline and amorphous regions, resulting from the addition of vinyl acetate co-monomer units into PE backbone chain. As a result of VA incorporation, crystallinity and crystalline structure, melting point, solubility, density and glass transition temperature of the polymer product are affected with overall impact on flexibility and thermoplastic characteristics of EVA (Almeida et al., 2011).

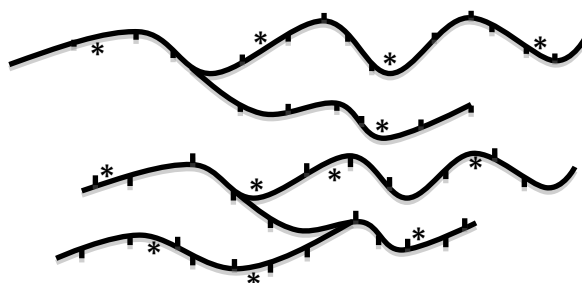


Figure 6. Schematic representation of EVA copolymer structure. EVA copolymer structure is made with short-chain branches and butyl groups, long-chain branches occurring at random intervals along the backbone with possible branching and acetate branches (\*).

Increasing VA content increases EVA elasticity and flexibility due to lower rigid amorphous content, decreases crystallinity by reducing polymer regularity and increases polarity with improved gas, moisture, fats and oils permeability as compared to LDPE. At high VA levels, EVA material becomes more clear, tacky and rubbery (Henderson, 1993).

## **Molecular Weight and Distribution**

Increasing molecular weight increases viscosity, toughness, heat seal strength, hot tack and flexibility of EVA copolymers. Number average molecular weight ( $M_n$ ) affects a number of physical properties such as melt viscosity, melt index (MI), impact strength, solubility and processability (Henderson, 1993). MI is the number of grams of polymer that will extrude from a cylinder through a die of specified dimensions in ten minutes under standard conditions of temperature and pressure. For EVA copolymers, pressure is standardized at a dead weight of 2.26 kg and a temperature of 190°C. MI is inversely related to molecular weight. When the level of vinyl acetate is increased, chain transfer increases leading to broader molecular weight distributions and changes in melt flow characteristics. Broad MWD polymers are composed of short chains and long entangled chains. Due to entangled character of long chains, these can withstand large amounts of any deformational stress and demonstrate above average elastic response (Henderson, 1993).

## **Molecular Branching**

Molecular branching caused by the use of chain transfer agents (pendant methyl groups from the use of propylene and butyl groups) disrupts crystallinity of polyethylene segments. Short chain branches with less than 6 carbon atoms exert similar effects on EVA copolymer properties as wt % VA. Long chain branches with 6 carbon atoms formed by chain transfer upon polymerization increase EVA copolymer's melt elasticity due to entanglement (Henderson, 1993).

While EVA softening point depends on both MW and degree of crystallinity of the

polymer, peak melting temperature corresponding to crystallites melting, is only affected by degree of crystallinity (Henderson, 1993). Incorporation level of VA affects EVA degree of crystallinity leading to changes in physical properties (thermal and mechanical properties). Thermal properties, such as softening points and crystalline melting points, are lowered upon an increase in VA content and a decrease in crystallinity. Mechanical properties are also affected by VA level with a decrease in stiffness or flexural modulus values observed upon increasing VA content. EVA copolymers are known to be tough and low modulus materials (Henderson, 1993).

EVA that was used in this study has a VA level of 28 %. This copolymer has a high molecular weight of 101,600, a crystallinity degree of 17.1 % and melting and glass transition temperatures of  $72.8 \pm 0.9$  °C and  $-28.6 \pm 0.5$  °C, respectively. Due to its low glass transition temperature, EVA exists in the rubbery state at room temperature conferring high flexibility to the copolymer (Almeida et al., 2011). Release of chlorine dioxide from this copolymer depends on a combination of parameters namely, copolymer degree of crystallinity (VA content), porosity of matrix caused by citric acid loading, as well as processing temperatures used during extrusion process and their effect on changing proportion between crystalline and amorphous domains in the final polymer (Almeida et al., 2011).

### 5.3.2. Poly- $\epsilon$ -caprolactone (PCL) - Aliphatic Polyester

Poly- $\epsilon$ -caprolactone (PCL) is an aliphatic synthetic polyester generally produced by ring-opening polymerization of  $\epsilon$ -caprolactone (Figure 7). PCL is a hydrophobic, semi-crystalline and biodegradable linear polyester (Figure 8).

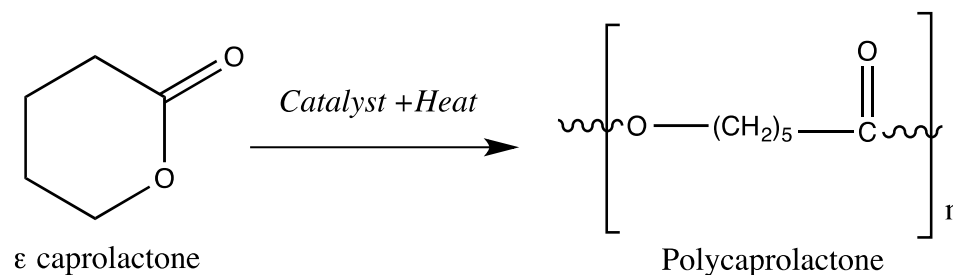


Figure 7. Synthesis of polycaprolactone by ring-opening polymerization of ε-caprolactone structural unit upon exposure to catalyst and heat.

Hydrophobicity of PCL depends on the length of the hydrocarbon chain.

Crystallinity level is inversely related to molecular weight, with a value of about 40 % for molecular weights exceeding 100,000 and 80 % as molecular weight decreases to 5000 (Pitt, 1990). Melting point also depends on degree of crystallinity, with PCL T<sub>m</sub> falling between 59-64°C (Pitt, 1990).

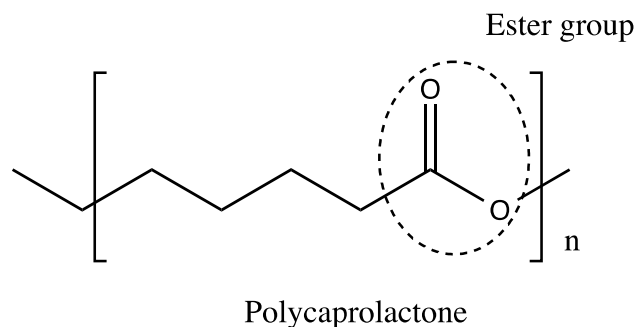


Figure 8. Polycaprolactone chemical structure.

Glass transition temperature (T<sub>g</sub>) of PCL is -60°C making this polymer rubbery and more permeable at room temperature. Heat of fusion required to melt 100% crystalline PCL is 139.5 J/g, a value used to determine PCL degree of crystallinity from differential scanning calorimetry (DSC). Physical, thermal and mechanical properties of PCL depend

on its molecular weight and degree of crystallinity. The latter affects permeability and biodegradability of PCL making the polymer inaccessible to water and other permeants by reducing solute solubility and increasing tortuosity of the diffusional pathway. Biodegradation involves random hydrolytic chain scission of PCL molecular chain through cleavage of ester bonds. This hydrolytic degradation is autocatalyzed by carbonyl end group on polymer chain (Woodruff & Hutmacher, 2010). Biodegradation rate is reduced by limiting access of water to the ester bonds through increasing crystallinity and polymer hydrophobicity (Pitt, 1990). The higher the molecular weight of polymer, the longer the molecular chain and the greater the number of ester bonds that need to be cleaved in order to cause polymer degradation, extending the hydrolysis process. Biodegradation of PCL is usually slow making this polymer suitable for long-term delivery systems (period of more than one year) (Woodruff & Hutmacher, 2010) .

#### 5.3.3. Aromatic Polyether-based Thermoplastic Polyurethane (TPU)

The building blocks of polyurethane are isocyanate, polyol and chain extender. The resulting polymer is a linear chain of high molecular weight (with thermoplastic properties (low melting point) along with required degree of toughness and performance (Lee, 1998). Diisocyanates are divided into two distinct categories: aromatic and aliphatic. Aromatic diisocyanates form the dominant category with diphenylmethane diisocyanate (MDI), toluene diisocyanate (TDI) and 1,5-naphthalene diisocyanate (NDI) being the most commonly known. Aliphatic diisocyanates are much less reactive and more expensive than aromatic ones besides; they do not yellow upon exposure to light and are therefore used whenever light stability is needed. Among aromatic diisocyanate products, MDI is produced in the largest volumes worldwide and constitutes the major



material currently used in the production of thermoplastic polyurethanes. MDI is a mixture of pure 4,4' two-ring product and other isomers. The pure 4,4'-MDI isomer is the material used in the manufacture of thermoplastic polyurethane (Lee, 1998).

Polyols are molecules possessing several hydroxyl functional groups. While the low molecular weight polyols (ethylene glycol, glycerine, butanediol, etc.) are used as chain extenders or crosslinkers, the higher molecular weight compounds (average molecular weight up to 5000) serve as main building blocks in the manufacture of polyurethane polymer and can belong to either polyether or polyester class (Oertel & Abele, 1994).

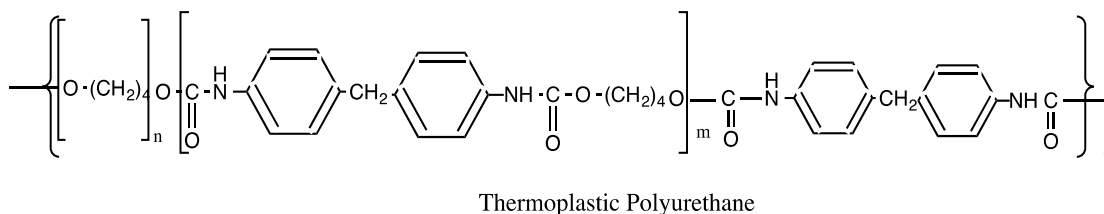


Figure 9. TPU structural formula.

### Aromatic Polyether-Based Thermoplastic Polyurethane (TPU)

Polyether-based thermoplastic polyurethanes are segmented block copolymers  $(AB)_n$  composed of alternating hard (polar and high melting point) and soft (nonpolar and low melting point) segments resulting in phase-separated structure.

Incompatibility between hard segments of higher melting point and polarity and relatively non-polar soft segments results in phase segregation with micro domains of soft and hard phases at room temperature (Figure 10) (Mackey, 2002).

Soft blocks consist of long molecular chains of polyether (propylene oxide and propylene oxide/ethylene oxide polyethers, polytetramethylene glycol polyethers, etc.) with high molecular weight that remain soft and flexible at ambient temperature. These long molecules possess a random coil conformation (tangled mass of string), which can undergo deformation and uncoiling upon application of force, thus imparting flexibility and elasticity to TPU. The soft segments are interconnected via physical intermolecular interactions (physical cross-links) enabling them to assume their original shape once deforming force is removed. Without the important feature of polymer chain interconnectivity, chains would flow upon deformation. Hard block are formed by reaction between isocyanate (mostly MDI) and chain extender (low molecular diol with the main ones being ethylene glycol, 1,4-butane diol and bis(hydroxyethyl) hydroquinone (HQEE)), these consist of the stiff oligourethane units that confer elastomeric resiliency to TPU (Mackey, 2002).

Rigid or hard sequences are connected to each other through flexible soft segments via covalent bonding; however hard blocks of different polymer chain molecules are interconnected through hydrogen bonding forming crystalline domains. Hard segments act as multifunctional crosslinks. Depending on the degree of incompatibility of hard blocks with polyol and affinity between hard segments, different morphologies can be observed within hard block domain. For instance, microcrystals form when hard segments stack and develop sufficient order and packing density. Interphase areas, neither soft nor hard, are not ideal domain structures, they form when portions of hard block are located outside or at interface of the micro domain. While flexible soft segments impart elasticity to material, crystalline microphases prevent soft segments from permanently

deforming when polymer chains are stretched.

By varying the percentage of hard segments and chain length of soft segment, elasticity and toughness of a polymer can be manipulated. Hardness of elastomer depends on degree of phase separation, higher hardness is obtained from well-ordered hard blocks rather than high level of interphase material for a given isocyanate level. Also, a higher percentage of isocyanate is needed for a high level of interphase material to attain an equivalent hardness level. Phase separation also increases upon increase in the hydrocarbon length of polyethers because of decrease in polarity of polyol compared with urethane hard segment. Due to low compatibility between polyethers and MDI, resultant polyurethane possesses high degree of phase separation with large and more complex hard segment domains. It is worth noting that content of hard block influences mechanical properties in a linear fashion, while dynamic properties are affected by structure of hard block domain in a more complex manner (Bosman, 2002; Mackey, 2002). Polyethers possess excellent hydrolysis and microbial resistance as well as good low temperature flexibility. However, very strong concentrated acids and bases cause hydrolytic degradation through cleavage of ether linkages on the polyethers. At higher temperatures, oxygen sensitivity of ether linkage becomes very noticeable leading to production of peroxides, acids, aldehydes and esters through oxidation process (Oertel & Abele, 1994).

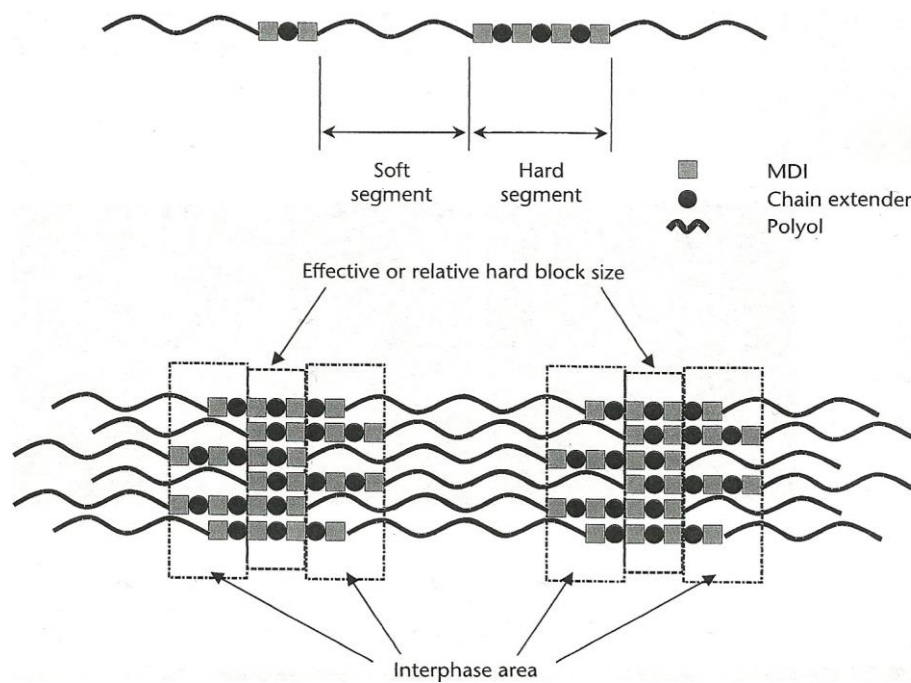


Figure 10. Segmented polyurethane structure: alternation of soft and hard segments with hard block chain packing (Mackey, 2002).

## **6. APPROACH -II-: COATING**

### **6.1. Motivation**

Chlorine dioxide releasing labels made from extruded synthetic polymers in the first part of this research did not achieve controlled release of this compound over time. The release of chlorine dioxide was merely a surface effect, with a burst release of this compound observed from labels following controlled activation mechanism. The synthetic polymers used were predominantly hydrophobic.

Substituting the predominantly hydrophobic synthetic polymers used in the first part of this research with hydrophilic alternatives using extrusion technique is not feasible. Extrusion of hydrophilic polymers requires temperatures above the melting point of citric acid, leading to detrimental structural changes of extruded polymers from enhanced reactivity of citric acid with these polymers.

The use of biopolymers, such as gelatin and pectin, in coating application offers better control over release of chlorine dioxide due to swelling property of these polymers upon exposure to moisture. Moisture induces morphological changes in polymer matrix influencing mobility of reactants (salt and acid) within label, their reaction and diffusion of  $\text{ClO}_2$  out of the label with time to achieve desired release profile suitable for a specific food application.

### **6.2. Concept Development**

The biobased label is a multilayered  $\text{ClO}_2$  self-releasing package label made of continuous layers of pectin embedded with citric acid and gelatin incorporated with sodium chlorite, using solution coating technique. The constituent layers of this label are

first assembled, then instantly activated by application of pressure at the point of use for a short period of time (5 s). Two biobased labels were manufactured, one without barrier layers and one with barrier layer in between functional layers of pectin with citric acid and gelatin with sodium chlorite.

#### 6.2.1. Biobased Label Manufacture

##### **Biobased Label 1: Multilayer label with functional layers only, without barrier layers**

The label shown in Figure 17 is composed of the top information layer displaying consumer information about the product. The bottom part of the label, the functional part, consists of five continuous layers: two gelatin layers embedded with 4 % (w/w, wet basis) sodium chlorite sandwiched between three pectin layers containing 2 % (w/w, wet basis) citric acid. These layers are glued together using 7 % gelatin solution and assembled to form the package label. The surface area of this label is 10 cm<sup>2</sup> and of 1.4 mm (50 mil) thickness.

##### **Biobased Label 2: Multilayer label with functional layers separated by barrier layers**

This label is similar to the label described above with the exception of the added barrier layer in between pectin and gelatin functional layers allowing control over release of chlorine dioxide from label (Figure 18). This multilayered label is composed of nine continuous layers of pectin (2 % citric acid, w/w, wet basis), gelatin (4 % sodium chlorite salt, w/w, wet basis) and gelatin barrier layers of 2 x 5 cm<sup>2</sup> surface area. The thickness of this label is 3 mm (102.4 mil).

### 6.2.2. Controlled Activation Mechanism

Activation of these biobased labels is performed at the point of use through the application of pressure (100 KPa) for a short time (5 s). On the macroscopic level, applied pressure promotes binding between pectin and gelatin layers to form a cohesive label. On the microscopic level, pressure reduces gap between layer surfaces promoting intermolecular bonding and forces the reactants to become spatially close to one another favoring chemical reaction and chlorine dioxide generation.

## 6.3. Coating

The coating technique is a method used for thin film production. It consists of dissolving polymers along with other components in a solvent forming a solution with minimum solid content and viscosity, followed by pouring the resulting solution onto a mold and allowing the solvent to evaporate leading to the formation of a residual film that can be stripped from the surface. Water is the solvent used in the making of food-grade films made of biopolymers such as pectin and gelatin. Drying conditions (temperature, relative humidity and drying rate) are important to ensure equal drying across film. The composition of the film forming solution as well as drying conditions influence physical and mechanical properties of the resultant film. Unlike extrusion technique, solvent-casting method does not subject the polymer solution to thermal and mechanical stress resulting in insignificant degradation reactions (Siemann, 2005).

## 6.4. Biobased Polymers

### 6.4.1. Pectin

#### **Structure**

Pectin is a structural polysaccharide widely present in land plants. It has been widely used in the food industry as a gelling agent and has gained interest in packaging field for its film forming ability and its potential in controlled drug delivery applications. The primary sources of commercial pectin are citrus peels and apple pomace. Pectin exists in a helical shape, it is a linear polysaccharide of smooth regions of galacturonan homopolymer made of repeating (1→4 linked)  $\alpha$ -D-galacturonic acid residues with varying degrees of carboxyl groups methylated, amidated or in the free carboxylic form. These smooth regions are interrupted with hairy regions of rhamnogalacturonan of (1→2) linked  $\alpha$ -L-rhamnopyranosyl residues to which side chains made of D-galactopyranose and L-arabinofuranose residues are attached (Figure 11). This branching introduces irregularities to pectin structure limiting the extent of chain association and the size of junction zones formed between regular, unbranched pectin chains upon gelation (Damodaran et al., 2008).



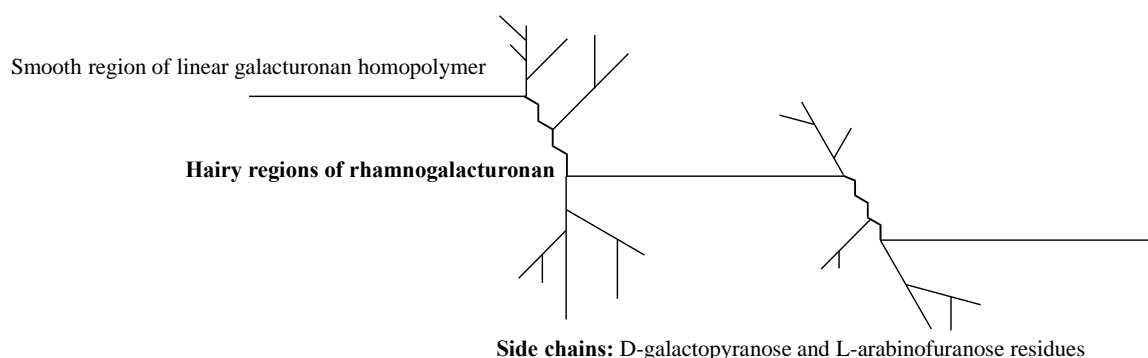


Figure 11. Schematic representation of pectin backbone showing alternation between smooth regions of linear galacturonan homopolymer and hairy regions of rhamnogalacturonan and side chains (Axelos & Thibault, 1991).

### **Degree of Esterification (DE) or Degree of Methylation (DM) and Types of Pectin**

Degree of esterification is the percentage of esterified galacturonic acid residues to total galacturonic acid groups on a pectin chain. Degree of esterification influences pectin solubility, firming and gelling properties. Based on the degree of esterification (DE), two types of pectin are commercially available: low methoxyl (LM) pectin with DE < 50 % and high methoxyl (HM) pectin with DE > 50 %, with the nonesterified carboxylic groups being present as a mixture of free acid (-COOH) and salt (e.g. -COO<sup>-</sup> Na<sup>+</sup>). LM pectin are linear polyanions (polycarboxylates) with molecular weight ranging from 50,000-150,000 Da, they form thermoreversible gels that can be melted and reformed repeatedly. Pectins are often sold as monovalent cation salts, typically in the sodium salt form, highly ionized in solution, promoting hydration of polymer chains due to ionic charges distributed along molecules, preventing therefore molecular aggregation.

## **Gelling Mechanism**

Pectin gels consist of a three dimensional network of cross-linked pectin polymer molecules forming junction zones and entrapping a large volume of continuous liquid phase within the network, with interjunction portions made of relatively mobile and extended pectin chains (pectin layer in Figure 17). Junction zones are formed from interchain associations of adjacent pectin molecules by hydrogen bonding, hydrophobic interactions (e.g. Van Der Waals attractions), ionic cross bridges, molecular entanglement or covalent bonds. The liquid phase is an aqueous solution of low-molecular-weight solutes and segments of polymer chains (Thakur et al., 1997). Many structural and physical parameters influence pectin physical properties and gelation namely DE, charge distribution along backbone, average molecular weight of the sample, ionic strength, pH, temperature and the presence of cosolute (Axelos & Thibault, 1991). Depending on DE and pH conditions, negative charge density of pectin molecules can vary. At high pH, pectin molecules carry higher negative charge density causing coulombic repulsion of pectin chains and the hydration of individual pectin molecules in solution. At this pH, pectin chains adopt an extended conformation (two-fold) structure. Lowering the pH decreases the charge density on pectin molecules promoting a more compact conformation close to three-fold structure stabilized by intramolecular hydrogen-bonding between O(2) of galacturonic acid residue and  $\text{-COOH}$  of the next residue in the direction of the reducing end (Gilsenan et al., 2000). In concentrated pectin solutions, branching of pectin chains enhances entanglement and limits the size of intermolecular junction regions. Pectins of high molecular weight form stronger gels than those of lower molecular weight.

In this research, LM pectin (32 % DM) was used to produce packaging film layer incorporated with citric acid. pH of pectin slurry after citric acid addition was  $2.6 < pK_a$  pectin (3.5). At this pH, pectin chains exist in the three-fold arrangement (compact structure). Gelling mechanism of LM pectin at low pH consists of the development of a network structure resulting from association of antiparallel three-fold pectin helices into larger aggregates forming intermolecular junction zones made not only from intermolecular dimers but also larger assemblies such as trimers or tetramers entrapping within it aqueous solution of solute molecules (Gilsenan et al., 2000). At very low pH, the majority of the carboxylic groups on galacturonan homopolymer chain is in the protonated, uncharged form (-COOH) favoring the formation of junction zones resulting from hydrogen bonding between -COOH of one pectin chain and the O(3) of hydroxyl group on adjacent (antiparallel) chain and between -COOH of neighboring antiparallel chains, where at least one of the carboxyl groups is protonated and act as hydrogen bond donor. Hydrophobic interactions may also occur between the methyl ester groups of two polymer chains Figure 17 (Gilsenan et al., 2000).

### **Swelling**

Pectin hydrogel is a physical hydrogel made of clusters of distinct domains of molecular entanglements, free chain ends, and molecular 'hairpin' and 'kinks' or 'loops' held together by weak hydrophobic associations, ionic interactions or hydrogen bonding (Farris et al., 2011). These gels are reversible given their sensitivity to water and can disintegrate completely in water besides; they are thermo-reversible and melt to a polymer solution when subjected to heat.

It is worth noting that LM pectin slurry is solution coated and allowed to dry under ambient conditions of temperature, pressure and relative humidity. The dry pectin layers act as dry hydrogels and possess the ability to undergo volume change through hydration and swelling depending on chemical composition of pectin and moisture content of surrounding environment (Farris et al., 2011). Hydration begins at the most polar and hydrophilic functional groups causing swelling of matrix. As the hydration of the polar groups continues, the hydrophobic groups are also exposed and interact with water. Pectin network keeps drawing water molecules through osmotic driving force of the network chains towards infinite dilution, which will be entrapped in the space of the network and center of pores or voids forming hydration layers around pectin chains (Farris et al., 2011).

### **Pectin Stability**

Acids and/or enzymes catalyze the hydrolysis of glycosidic linkages joining the galacturonic acid units leading to breakdown of pectin molecules with consequences on structure of pectin films or hydrogels (Damodaran et al., 2008). The extent of depolymerization depends on pH, temperature, time at this pH and temperature and pectin structure. Moreover, acidic conditions hydrolyze esters of methylated carboxylic acid residues converting them back to free carboxylic acid form, lowering therefore DE. Usually, adding more pectin in the formulation or using a higher viscosity grade could compensate for breakdown and overcome defects associated with depolymerization (Damodaran et al., 2008).

#### 6.4.2. Gelatin

Gelatin is derived from hydrolysis of insoluble collagen protein from different sources such as pigskin, cattle skin (hide) and cattle bone (Harris, 1990). The resulting gelatin is a mixture of random coiled chains having a range of molecular weight. Gelatin molecular structure exhibits repeating pattern of (Glycine-Proline-Hydroxyproline) triplets with glycine occurring every third residue and pyrrolidine imino acids (proline and hydroxyproline) accounting for 10 % of residues (Figure 12). Gelatin possesses a polyampholyte character due to the presence of charged amino acid residues on its backbone enabling it to complex both negatively and positively-charged polyelectrolytes at pH conditions below or above its isoelectric point (pI) (Figure 12) (Farris et al., 2011). Two types of gelatin are available depending on the collagen pretreatment process: acid-treated type A gelatins and alkaline-treated type B gelatins. The basic treatment hydrolyzes the amide groups of asparagine and glutamine into carboxyl groups leading to the formation of gelatin with a higher density of carboxyl group and negative charge and lowering its pI. Pigskin gelatin is obtained from acid pretreatment process while that from bovine hide is prepared from alkaline pretreatment process using lime as the alkaline agent (Harris, 1990).

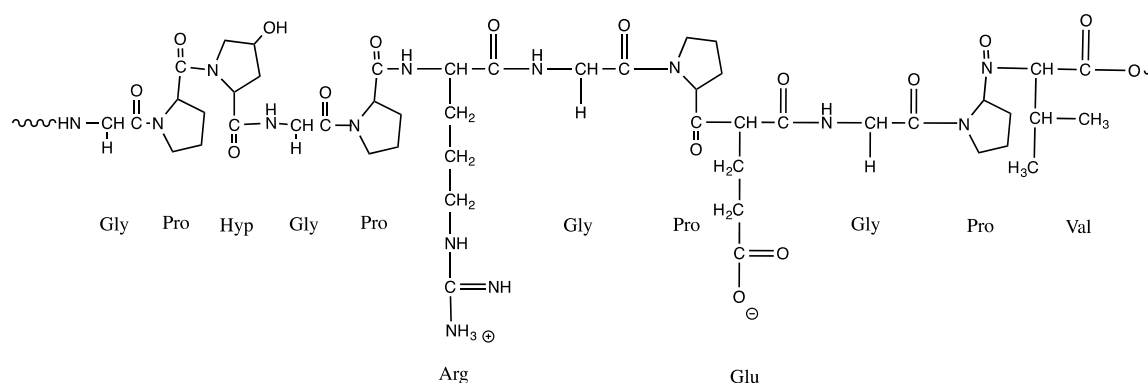


Figure 12. Gelatin molecule at pH of 8.4. Gelatin molecule shows repeating pattern of (glycine-proline-hydroxyproline) triplets. At pH 8.4, gelatin displays a polyampholytic character due to presence of charged amino acids residues on its backbone.

## Physical Characteristics

### pH and pI

Commercial gelatins have a pH ranging between 5.0-5.8 with the exception of pigskin gelatins with pH 4.0-4.5. The isoelectric point (pI) of gelatin varies with the type of pretreatment process. While acid pigskin gelatins have isoelectric points ranging from 7.5-9.0, limed-processed gelatins have pI between 4.8-5.0. Type B gelatins carry a slight negative charge at pH greater than 5.5 (Harris, 1990).

### Gel strength and viscosity

Gelatin is characterized by its bloom value, a function of gel strength, and viscosity, a function of solution properties. Gel strength is dependent on the sum of  $\alpha$ - and  $\beta$ - and higher molecular weight chain components of gelatin, whereas viscosity is dependent on average molecular weight and gelatin fractions of higher molecular weight. Gelatin gel strength can be quoted 'bloom strength' only when 'bloom' value is defined as the following: the weight required to push a cylindrical plunge, of 13 mm diameter, to

depress the surface of gelatin gel (6.67% w/w concentration and matured at 10°C for 16-18 h) by 4 mm. The high-molecular-weight fractions of gelatin are responsible of initial conformations necessary for onset of gelation influencing therefore setting time. Melting point is related to the proportion of low-molecular-weight peptides that are not involved in formation of gel network. Gelatin viscosity in solution is proportional to its concentration, pH, ionic strength and specific viscosity of gelatin (high molecular weight fractions) and temperature (Harris, 1990).

### Gelling Mechanism

Formation of gelatin gel occurs in two steps. First, the moderately concentrated gelatin solution is heated converting proteins from “sol” (solution of native proteins) state to “progel” (solution of denatured proteins) state by denaturation. This step is irreversible. The second step takes place upon cooling of gelatin progel to below 35-40°C leading to formation of a gel network (Figure 14). The decrease in thermal kinetic energy promotes the formation of stable noncovalent bonds with hydrogen bonding contributing more than hydrophobic interactions and electrostatic interactions. The rate of association of soluble complexes via hydrogen bonding during cooling is slow leading to the formation of an ordered translucent gel network that does not scatter light (Damodaran et al., 2008). Charged groups distributed on protein are important for maintaining protein-water interactions and water-holding capacity of gels. Gelation involves the aggregation and alignment of gelatin random coils into junction zones stabilized by hydrogen bonding. Segments of gelatin molecules rich in (glycine-proline-hydroxyproline) triplets possess a helical structure and act as nucleation sites for the formation of junction zones (Ledward, 2000). ‘Useful’ junctions, triple helical crosslinks, are formed by the aggregation of three

of such helices with inter chain hydrogen bonding conferring long-range rigidity to these regions (Figure 14). Slow cooling promotes growth of existing junction zones as well as formation of new ones from regions with lower content of pyrrolidine residues leading to gels with increased rigidity and strength at low temperatures. Gelatin gel is thermoreversible and dissolves back into solution upon melting at 35-40°C because it is primarily stabilized by temperature sensitive intermolecular hydrogen bonds (Ledward, 2000). Gelatin hydrogels are physical hydrogels composed of a three dimensional network of amorphous regions of randomly-coiled gelatin chains interrupted with junction regions of spatially ordered molecules known as microcrystallites stabilized by hydrogen bonding between NH of glycine and C=O of proline residues (Figure 14). This gel network entraps water that possesses chemical potential similar to that in dilute aqueous solutions but lacks fluidity and cannot be easily expressed out. The majority of the water held in gelatin gels is hydrogen bonded to C=O and N-H groups of peptide bonds, bound to charged groups in the form of hydration shells and /or exists in hydrogen-bonded ice-like water-water networks. Water can also act as hydrogen bond cross-linker between C=O and N-H groups of peptide segments within the restricted environment of the microstructure of the gel network. Gelatin gel strength is related to multiple parameters such as concentration, distribution of pyrrolidine residues and the overall size and shape of the molecules. The relationship between gel strength ( $G$ ) and gelatin concentration ( $C$ ) follows a power law where  $G \propto (C-C_0)^n$ ;  $C_0$  is the least concentration endpoint (0.6 % for gelatin) and  $n$  varies from 1 to 2. The optimum pH for gel formation is about 7-8 for most proteins (Damodaran et al., 2008). In concentrated gelatin systems, gel strength in the pH range of 4-10 is virtually independent of pH



(Harris, 1990).

### **Effect of Additives on Protein Structure**

The addition of small molecular weight solutes influence protein structural stability in aqueous solutions through stabilization or destabilization mechanism related to the preferential interaction of these additives with aqueous phase and the protein surface, in other words their effect on bulk water structure.

For instance, the addition of simple sugars, glycerol and other non-electrolytes stabilize the native structure of proteins by binding very weakly to the protein surface, enhancing both hydrogen-bonded structure of bulk water and preferential hydration of the protein surface. These low-molecular-weight compounds also increase the gel strength (Damodaran et al., 2008; Harris, 1990).

The addition of electrolytes such as salts influence protein structure in two different ways. It is worth noting that structure of proteins is affected more by anions than cations. At low concentrations ( $\leq 0.2$  M ionic strength), ions interact with proteins by nonspecific electrostatic interactions resulting in neutralization of protein charge and stabilization of protein structure. At high concentrations ( $> 1$  M), salts exert specific effects and affect structural stability of protein differently.  $\text{NaClO}_4$  for instance, acts as a strong denaturant that weakens protein structure and changes protein hydration properties.  $\text{NaClO}_4$  binds strongly to the protein surface causing protein dehydration due to high salt concentration in the region surrounding protein excluding water access to this region. This salt also breaks down the structure of bulk water, rendering it a better solvent for apolar molecules. These effects of  $\text{NaClO}_4$  cause the protein to unfold by destabilizing the hydrophobic interactions in proteins and exposure of nonpolar surface to favorably interact with the

salt (Damodaran et al., 2008).

When the protein is exposed to a mixture of stabilizing and destabilizing additives, the net effect on structural protein stability follows additivity rule. In the presence of glycerol for instance, the concentration of destabilizing salt required to denature protein increases with increase of glycerol concentration. Changes in water structure caused by the destabilizing salt are opposed by the addition of glycerol (Damodaran et al., 2008).

### **Gelatin Adhesive Properties**

Gelatin has been used as animal glue for a long time due to its adhesive properties. All animal glues are known for their film forming and bonding properties. Gelatin glue possesses an amphoteric and polyelectrolyte character due to the presence of acidic and basic functional groups as well as reactive and ionizable amines and carboxyl terminal groups. Depending on pH of the solution, gelatin protein can carry either an overall positive or negative charge and can act as a cation or anion, respectively. These characteristics influence interactions among protein and other molecules and between protein molecules and water (Engineers, 2007).

Gelatin glue forms a gel upon cooling of a viscous and tacky deposit of a hot aqueous solution enabling the formation of immediate strong initial bond. During gelation, both intra- and intermolecular associations are formed from random primary and secondary bonds. Intermolecular network between molecular chains is stabilized through cross-links such as hydrogen bond, ionic and covalent bond. Natural or forced air drying ensures the formation of final permanent bond of high strength and resiliency, between polar materials (Engineers, 2007).

## 7. EXPERIMENTAL DESIGN

### Approach -I-: Extrusion

#### 7.1. Materials and Methods

##### 7.1.1. Materials

Three different polymers were used in the manufacture of synthetic ClO<sub>2</sub> self-releasing package label. Ethylene vinyl acetate (28 % VA) commercialized under the trade name Elvax® 260 was purchased from Dupont Company, Wilmington, DE, USA. Thermoplastic poly-(ε)-caprolactone sold as CAPA™ FB100 was acquired from Solvay Caprolactones, Warrington, Cheshire, WA4 6HB, UK. Aromatic polyether-based thermoplastic polyurethane under the commercial name Estane® MVT 75AT3 was obtained from The Lubrizol Corporation, Cleveland, OH, USA. The list of polymers along with their physical properties are shown in Table 2. Chemical precursors used in the generation of chlorine dioxide were citric acid, ACS reagent > 99.5 % acquired from Sigma Aldrich, MO, USA and sodium chlorite 80% unstabilized obtained from Acros Organics, New Jersey, USA.

Table 2. General physical properties of PCL, EVA (28% VA) and TPU.

Polymer type / General properties	CAPA™ FB100	Elvax® 260	Estane® MVT 75AT3
Description and Composition	High molecular weight partly crosslinked polyester	Ethylene-vinyl acetate copolymer resin containing 28 % by weight vinyl acetate and thermal stabilizer (BHT antioxidant)	Aromatic Polyether – based thermoplastic polyurethane
Mean Molecular weight	100,000	101,600 (Almeida et al., 2011)	-
Melting point (°C)	58 (supplier datasheet)	75 (supplier datasheet)	136 (supplier datasheet)
Glass transition temperature (°C)	-60	-28.6 (Almeida et al., 2011)	-68
Tensile strength (MPa)	11.74 (129.9 mil thickness)	15 (39.37 mil thickness)	34.5 (30 mil thickness)
Elongation (%)	-	750 (39.37 mil thickness)	700 (30 mil thickness)
Crystallinity (%)	40-50 (Jenkins & Harrison, 2006)	17.1 (Almeida et al., 2011)	-
Enthalpy of fusion (J/g)	139.5 (Pitt et al., 1981)	277.1 (Almeida et al., 2011)	101.6 (Bosman, 2002)

## 7.2. Methods

### 7.2.1. Compounding of Polymer Resins

Polymer resins of each polymer type used in the preparation of package labels were first dried at a temperature of 55°C for 3 hours. Polymers and citric acid were then blended using a Brabender, USA non-intermeshing counter-rotating twin-screw batch-type mini-extruder equipped with stainless steel screws. Processing conditions of each of the polymers such as temperature at the different zones along twin-screw extruder as well as screw speed are shown in Table 3 below. After extrusion, blends were pelletized using a pelletizer after being subjected to a prior cooling step in the case of PCL and TPU polymers only. The final citric acid loading percentage achieved for each of the three polymers was 20 %.

Table 3. Processing conditions of PCL, EVA (28 % VA) and TPU compounding with citric acid using twin-screw extruder.

Processing conditions	CAPA™ FB100	Elvax® 260	Estane® MVT 75AT3
Zone 1	70 °C	75 °C	90 °C
Zone 2	80 °C	110 °C	110 °C
Zone 3	82 °C	120 °C	120 °C
Die	85 °C	120 °C	130 °C
Screw speed (rpm)	48	90	62

### 7.2.2. Synthetic Package Label Manufacture

Package label was manufactured by extrusion technique using commercial-scale single screw extruder from Specialty Extrusion, Co. The extruder used to produce label is a single-screw extruder of 20:1 L/D ratio, 2" screw diameter that can be operated at speed range from 0-60 rpm with maximum output of 80-85 lbs per hour. Barrel is heated by electrical resistance and cooled by air. Screw speed was set at 10-11 rpm all throughout the extrusion process for the three different polymers used in the production of the package label (Figure 13).

#### **PCL Label Manufacture**

PCL virgin resins as well concentrated pellets (20% citric acid) were dried prior to handling at 79.4°C (175°F) for 3 hours. 5.5 lbs of the concentrated resin (20% citric acid) were mixed with 7.3 lbs of virgin PCL resin in a bucket prior to adding the mix into feed hopper. The resulting mix of 12.8 lbs (8.6 % citric acid) was extruded at conditions shown in Table 4. The hot melt exiting the die was cooled by passing it through chill rolls cooled by tap water (12°C). Two package films of two different thicknesses (13.5 and 25 mils) with similar final citric acid concentration (8.6 %) were rolled onto cardboard tube and stored in a plastic bag for subsequent testing.

#### **EVA Label Manufacture**

Extruder was set at conditions suitable for EVA processing and purged with virgin EVA 28% VA prior to adding the mixture to be extruded. EVA, compounded (20 % citric acid) and virgin, were thoroughly mixed in a bucket prior to adding them into feed hopper to

ensure proper mixing. 13.5 lbs of concentrated EVA resin (20% citric acid) were mixed with 4 lbs of virgin EVA. The resulting mix (17.5 lbs) had a final concentration of ~15 % citric acid. EVA processing conditions used in extrusion are shown in Table 4. The hot melt exiting the die was cooled as it was passed through chill rolls (12°C) and two EVA films of ~15 feet each were produced with similar citric acid concentration (15% citric acid) but two different thicknesses (11.9 and 19.2 mils). 3 lbs of the remaining mix (15% citric acid) were diluted further with virgin EVA resins, fed into hopper and extruded at similar conditions to obtain two films of similar final citric acid concentration (7.5 %) and different thickness (13.3 and 26.6 mil). The films (~15 feet) were rolled onto a cardboard tube and stored in a plastic bag for further analysis.

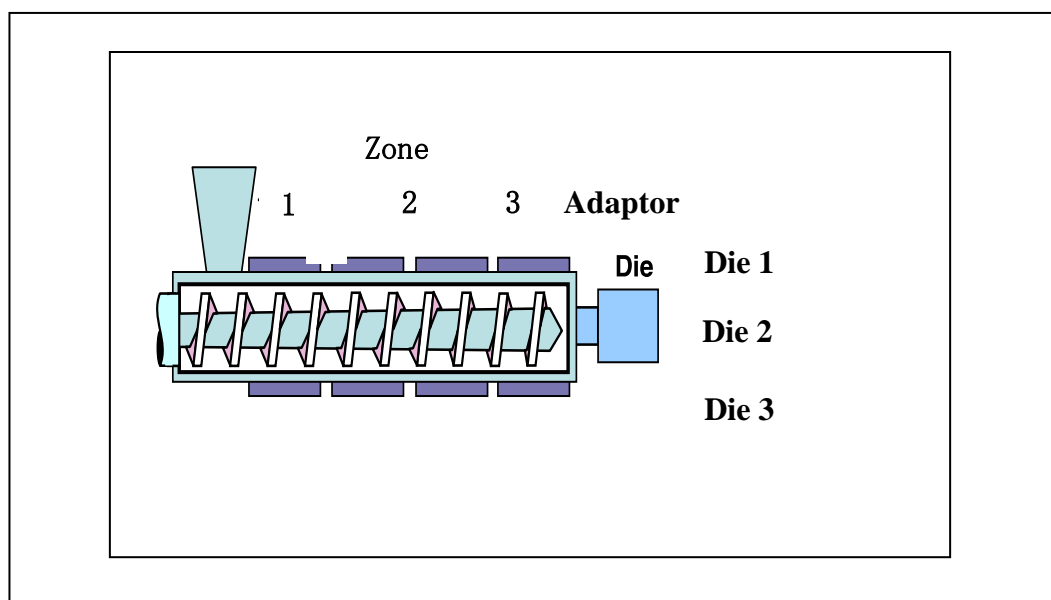


Figure 13. Zones of a single-screw extruder.

### TPU Label Manufacture

Extruder conditions were set to match TPU processing conditions as indicated by

manufacturer followed by purging with virgin TPU prior to film extrusion. TPU virgin resins as well as compounded resins (20% citric acid) were dried for about an hour at 79.4 °C (175°F) prior to processing. 6 lbs of compounded TPU (20% citric acid) were mixed with 2 lbs virgin TPU to obtain a final concentration of 15% citric acid. Given the high concentration of citric acid used in the polymer film and the high operating temperature ( $T > 153^{\circ}\text{C}$ ,  $T_m$  of citric acid) used in extrusion, the polymer melt degraded upon exiting the die and did not form a coherent film (Table 4). Despite this issue, strips of the melt (15 % citric acid and 32.5 mil thickness) were cooled by passing them through chill rolls and stored for further analysis.

Table 4. Extrusion conditions: temperature and screw speed, of PCL, EVA (28 % VA) and TPU package labels incorporated with citric acid.

Processing conditions	CAPA™ FB100	Elvax® 260	Estane® MVT 75AT3
Zone 1	82 °C	93 °C	176 °C
Zone 2	93 °C	107 °C	182 °C
Zone 3	91 °C	105 °C	182 °C
Adapter	92 °C	104 °C	177 °C
Die Zone 1 (side of die)	99 °C	112 °C	148 °C
Die Zone 2 (middle of die)	101 °C	114 °C	147 °C
Die Zone 3 (side of die)	118 °C	131 °C	175 °C
Screw speed (rpm)	10-11	10-11	10-11



The composition and thickness of different labels that were manufactured are shown in (Table 5).

Table 5. Synthetic package labels: citric acid loading and thickness.

Synthetic Package Labels							
Polymers	PCL		EVA (28% VA)				TPU
Citric Acid Loading (wt %)	8.6		7.5		15		15
Thickness (mil)	13.5	25	13.3	26.6	11.9	19.2	32.5
Label number	1	2	3	4	5	6	7

### 7.2.3. Label Characterization Analyses

#### **Scanning Electron Microscopy (SEM)**

Package labels were subjected to SEM to determine their surface structure (topography) and more specifically surface distribution of citric acid crystals. A Quanta 200 FEG field emission gun scanning electron microscope (FEI Co., Inc., Hillsboro, OR), operated at a high vacuum with secondary electron imaging mode was used to conduct this analysis. Prior to testing, package labels were coated with a thin layer of gold in order to increase electrical conductivity. SEM images of both rough and smooth sides of packaging label were generated using five different magnifications (50, 100, 250, 500 and 1000 X), and further analyzed to study surface texture and distribution of citric acid crystals.

### **Tensile Properties**

Mechanical properties of different package labels were determined using an Insight 5 materials tester, model 1122 (Instron, Norwood, MA), and TestWorks 4 data acquisition software (MTS Systems Corp., Eden Prairie, MN) following standard testing method used for tensile properties of thick plastic sheeting ASTM D882. Tensile strength, elongation to break, Young's modulus and toughness (fracture energy) of labels were quantified in order to study the effect of citric acid incorporation on the final mechanical properties of labels by comparing values obtained, to those of films solely composed of the polymer under study without any addition of active compounds. Prior to analysis, samples were cut into rectangular strips of 1 x 10 cm (W x L) along the extrusion direction, conditioned and tested at 22°C and 65 ± 5% RH. Samples were analyzed in the machine direction at increasing crosshead speed to 250 mm/min with a grip distance of 5 cm. Each test was conducted on six replicates / film sample and an average value was obtained.

### **Differential Scanning Calorimetry (DSC)**

A heat flux TA Instruments (New Castle, DE, USA) Q-2000 DSC with an autosampler on single punched out disks from the films under nitrogen purge was used for the analysis. About 10 mg of the SA-RPESO sample was accurately weighed in an aluminum pan and sealed with pin-perforated lids. The DSC oven was ramped at 10 °C/min, from ambient to 110 °C/min, to eliminate thermal history, and possible moisture. A refrigerated cooling system was used to equilibrate the sample at -60 °C, from 110 °C, at a rate of 5 °C/min. The sample was held isothermal at -60 °C for 2 minutes, then data was recorded while the

oven temperature was raised from -60 °C to 150 °C at a rate of 5 °C/min. The DSC method applied an inert atmosphere by purging the oven with nitrogen at 50 mL/min. Thermal Advantage and Universal Analysis software provided by TA instruments were used for data analysis.

DSC thermograms are endothermic down mode. Crystallization is an exothermic transition. Crystallization temperature and enthalpy correspond to the highest point of crystallization peak and the area under crystallization curve, respectively on DSC thermograms. Melting is an endothermic transition, with both melting temperature and enthalpy of fusion corresponding to lowest point of the melting dip and area under melting dip, respectively on thermograms. All the samples were analyzed in triplicate.

#### 7.2.4. Quantitative Determination of ClO<sub>2</sub> from EVA Package Labels

##### **Materials**

Extruded ethylene vinyl acetate package labels embedded with two citric acid loadings (7.5 and 15 %) and two levels of thickness were used in both experiments (Table 6). Chemical precursors for the generation of ClO<sub>2</sub> were citric acid, ACS reagent > 99.5 % from Sigma-Aldrich, MO, USA and sodium chlorite 80% unstabilized from Acros Organics, New Jersey, USA. Glycerol, ACS reagent ≥ 99.5 % was purchased from Sigma-Aldrich, MO, USA. The activation of labels consisted of two operational steps: spraying the surface of these labels with sodium chlorite and glycerol solution, followed by heat-pressing for a short period of time. Spraying step was conducted using Widetrack Wide Area Ultrasonic Spraying System, Sono-Tek Corporation, Milton, NY, USA. Heat pressing of labels was achieved using heat-press (Sencorp Systems, Inc., Hyannis, MA, USA). The activated package labels were placed in 40 mL EPA, amber glass vials

assembled with caps with TFE/silicone septa purchased from Fisher Scientific, Pittsburgh, PA, USA.

Quantification of chlorine dioxide release over time from EVA package labels was performed using Beckman DU-640 Spectrophotometer, Beckman Coulter, Inc., MA, USA and a spectrophotometric cuvette of 1 cm pathlength.

Table 6. EVA (28% VA) package labels.

Package label	EVA (28% VA)			
Citric acid loading (% , w/w)	7.5 %		15 %	
Thickness (mil)	13.3	26.6	11.9	19.2
Label Number	3	4	5	6

### **Preparation of EVA package labels**

EVA labels used in release kinetics experiment had 10 cm<sup>2</sup> (5 cm x 2 cm) surface area.

### **Sodium Chlorite Solution**

Sodium chlorite solution (3 M) was prepared by dissolving 271.32 g in 1 L of millipore water. The solution was kept in an amber bottle and refrigerated until use. This solution was prepared on fresh basis to prevent sodium chlorite decomposition.

### **Activation Mechanism of EVA Package Labels (spraying and heat-pressing)**

EVA labels were prepared and sprayed with 25 mL of 3 M sodium chlorite solution with

1 % glycerol (wt/v) using the ultrasonic atomizer at 5 mL/min flow rate and 1 foot/min conveyor speed. The labels were then heat-pressed at a temperature of 90°C and pressure of 300 KPa for a duration of time that varied based on label thickness, 10 s for 7.5 % citric acid loading, 13.3 mil and 15 % citric acid loading, 11.9 mil labels and 15 s for 26.6 and 19.2 mil labels of 7.5 and 15 % citric acid loading respectively. The activated package labels were then placed in 40 mL amber glass vials containing 3 glass beads to allow homogenous distribution of  $\text{ClO}_2$  in headspace upon shaking prior to sample withdrawal.

### **UV-VIS Spectrophotometry**

3 mL of chilled millipore water (temperature ranging from 2-12°C) were first drawn into a 10 mL syringe. Prior to sampling, the vial was shaken for about 10 s. The tip of the syringe as well as another syringe needle were inserted into sampling port (septum) of amber glass vials at the same time to form a gas tight seal and 3 mL of headspace were slowly drawn to the 6 mL mark. The extra needle served as a vent to replace the 3 mL sample volume with 3 mL of air in order to minimize pressure change inside the vial. The contents in the syringe were mixed by shaking for ten seconds, placing finger over tip of syringe. The solution in the syringe was then transferred into a 3 mL cuvette of 1cm pathlength. Absorbance was measured for the different samples at 360 nm, wavelength corresponding to maximum absorbance of chlorine dioxide, using UV-Vis spectrophotometer. Quantification of  $\text{ClO}_2$  concentration was measured over time, after 30, 60, 90, 120 min, 3 h, 4 h, 5 h and 6 h. Each sample was run in duplicate in three independent analyses to test repeatability of the experiment. Values reported were the

averages of replicates of each sample.

### **Determination of Gaseous Chlorine Dioxide Concentration**

Gaseous chlorine dioxide released from different EVA labels over time was measured by UV-VIS spectrophotometer at 360 nm wavelength. Based on Beer-Lambert law, aqueous chlorine dioxide concentration was calculated according to the following equation:

$[\text{ClO}_2] \text{ (M)} = (A / l \times \epsilon)$ ; where, A is absorbance of sample read at 360 nm wavelength, l is pathlength of spectrophotometric cell (cuvette) used in the analysis (1 cm in this experiment), and  $\epsilon$  is molar absorptivity of chlorine dioxide (1250 l/M cm).

Chlorine dioxide amount released from the package label with time was expressed in mg  $\text{ClO}_2$ / L air. Molecular weight of  $\text{ClO}_2$  is 67450 mg/mol.

#### **7.2.5. Antimicrobial Effectiveness of EVA Package Labels**

*Salmonella* Montevideo G4639, ATCC BA-710 (a clinical isolate associated with a tomato outbreak), was obtained from the American Type Culture Collection (Manassas, VA, USA). Stock cultures were stored in tryptic soy broth (TSB; BBL, Sparks, MD, USA) containing 20% glycerol at -80°C. Working stocks were maintained on tryptic soy agar (TSA; BBL) slants containing 0.6% yeast extract stored at 4°C for 2-4 weeks. A loop full of culture from a TSA slant was transferred into 10 ml of TSB and allowed to grow for approximately 8 h at 37°C. This culture was then used to inoculate fresh 10 ml of the same medium at 0.01 % (v/v) level, and was allowed to grow overnight to stationary phase at 37°C. This culture was then serially diluted and plated on duplicate sets of 50 mm PALL Sterile Petri Dishes (Fischer Scientific, Pittsburg, PA, USA)

containing TSA. Each inoculated plate was placed uncovered in 250 ml amber jar (Fisher Scientific) with or without activated EVA labels. Jars were covered using caps with Teflon septum and were held for 2, 4 and 6 h at room temperature. TSA plates from jars with and without activated EVA labels were incubated at 37 °C for 24 h, and cell concentration was enumerated. The results reported were the average of two independent analyses performed on duplicates.

## **Approach -II-: Coating**

### **8. Materials and Methods**

#### **8.1. Materials**

Low-methoxyl pectin manufactured from citrus peel and standardized with sugars (Grinsted Pectin LC 950) was purchased from Danisco New Century, KS, USA. Beefhide Gelatin of 250 Bloom and 45 mps viscosity was purchased from Vyse Gelatin Company, Illinois, USA. Glycerol, Certified ACS was purchased from Fisher Scientific, New Jersey, USA. Quantification of chlorine dioxide release over time from the biobased labels was performed using Shimadzu UV-1700 PharmaSpec UV-VIS Spectrophotometer, Shimadzu Scientific Instruments, Inc., Maryland, USA and a spectrophotometric cuvette of 1 cm pathlength.

#### **8.2. Methods**

##### **8.2.1. Biobased Package Label Manufacture**

##### **Pectin Layer**

##### *Sugar Removal from Standardized Low-Methoxyl (LM) Pectin*

Commercially available LM pectin is seldom diluted with sugar or dextrose for standardization purposes (reproducible performance from batch to batch) given that pectin is extracted from a variety of raw materials. The removal of sugars can be performed by dissolving them in water as pectin precipitates out of solution by the addition of water-miscible organic solvents such as methanol, ethanol or isopropanol; pectin being insoluble in organic solvents.



Five grams of the standardized LM pectin (32 % DE and 28 % sucrose) were dissolved in 100 ml of methanol/water solution (70:30, v/v), stirred overnight to dissolve the sugar and precipitate the LM pectin. The mix was then filtered to separate dissolved sugars in solvents from LM pectin and the filtrate was freeze-dried using Vitris Sentry Benchtop 3L Freeze Dryer (SP Scientific, NJ, US) and ground to a fine powder using mortar and a pestle.

#### *Pectin Film Formation*

Pectin films were produced by solution casting technique by adding a slow stream of LM pectin (5 %, w/w wet basis) into a water and glycerol (3%, w/w wet basis) mixture. The mix was heated to 70°C to ensure the dissolution of pectin in solution. Citric acid (2 %, w/w wet basis) was added to pectin slurry and mixed until complete homogenization. Pectin slurry was poured onto rimmed glass frame (200 x 200 mm) and allowed to air-dry at ambient conditions for 36 hours. Pectin film was manually peeled off and cut into 10 cm<sup>2</sup> SA (5cm x 2cm) pieces and stored under ambient conditions until further use.

The thickness (mm) of pieces was measured using Walter Stern metric micrometer caliper (0-25 mm range; 0.01 mm accuracy) from Fisher Scientific (Pittsburgh, PA). The thickness of pectin layer was in the order of 0.2 mm (7.9 mil).

### **Gelatin Layer**

#### *Gelatin Film Formation (Gelatin-based coating)*

Gelatin used in this research is alkaline-processed, type B gelatin derived from beefhide (bovine source) with 250 bloom and 45 mpoise viscosity.

#### *Gelatin Hydrogel with Sodium Chlorite Salt* (Figure 14)

Prewieghed gelatin powder (15 %, w/w wet basis) was added as a slow stream to a highly agitated water and glycerol (3 %, w/w wet basis) mixture heated to temperature of 60-80°C to prevent gelatin clumping. The mixture was let to stir for few minutes to complete gelatin dispersion and ensure dissolution. After complete dissolution of gelatin, the slurry was allowed to cool to 40°C while stirring and sodium chlorite salt (4 %, w/w wet basis) was added to gelatin blend and allowed to homogenize for 5 min. The gelatin mix containing sodium chlorite was poured onto rimmed glass frame (200 x 200 mm) and allowed to air-dry at ambient conditions (room temperature and relative humidity), in the dark for 12 hours. The hydrogel was then manually peeled off and conditioned at 100 % RH and 25°C until further use. Gelatin film was cut into 5 x 2 cm<sup>2</sup> SA pieces and thickness determined using Intelligent L Laser Sensor IL-065 (Keyence, IL, USA). The thickness of the gelatin film was in the order of 0.4 mm (15.7 mil).

#### **Barrier Layer (Gelatin film)**

Gelatin barrier layer was prepared in the same manner as the previous gelatin hydrogel with the exception of the absence of sodium chlorite salt in its formulation. Pre-weighed gelatin powder (15 %, wet basis) was added as a slow stream to a highly agitated water and glycerol (3 %, wet basis) mixture heated to temperature of 60-80°C to prevent gelatin clumping. The mixture was let to stir for few minutes to complete gelatin dispersion and ensure dissolution. After complete dissolution of gelatin, the slurry was poured onto rimmed glass frame (200 x 200 mm) and allowed to air-dry at ambient conditions (room temperature and relative humidity) for 12 hours. The hydrogel was then manually peeled

off and conditioned at 100 % RH and 25°C until further use. Gelatin film was cut into 5 x 2 cm<sup>2</sup> SA pieces and thickness determined using Intelligent L Laser Sensor IL-065 (Keyence, IL, USA). The thickness of the gelatin film was in the order of 0.4 mm (15.7 mil).

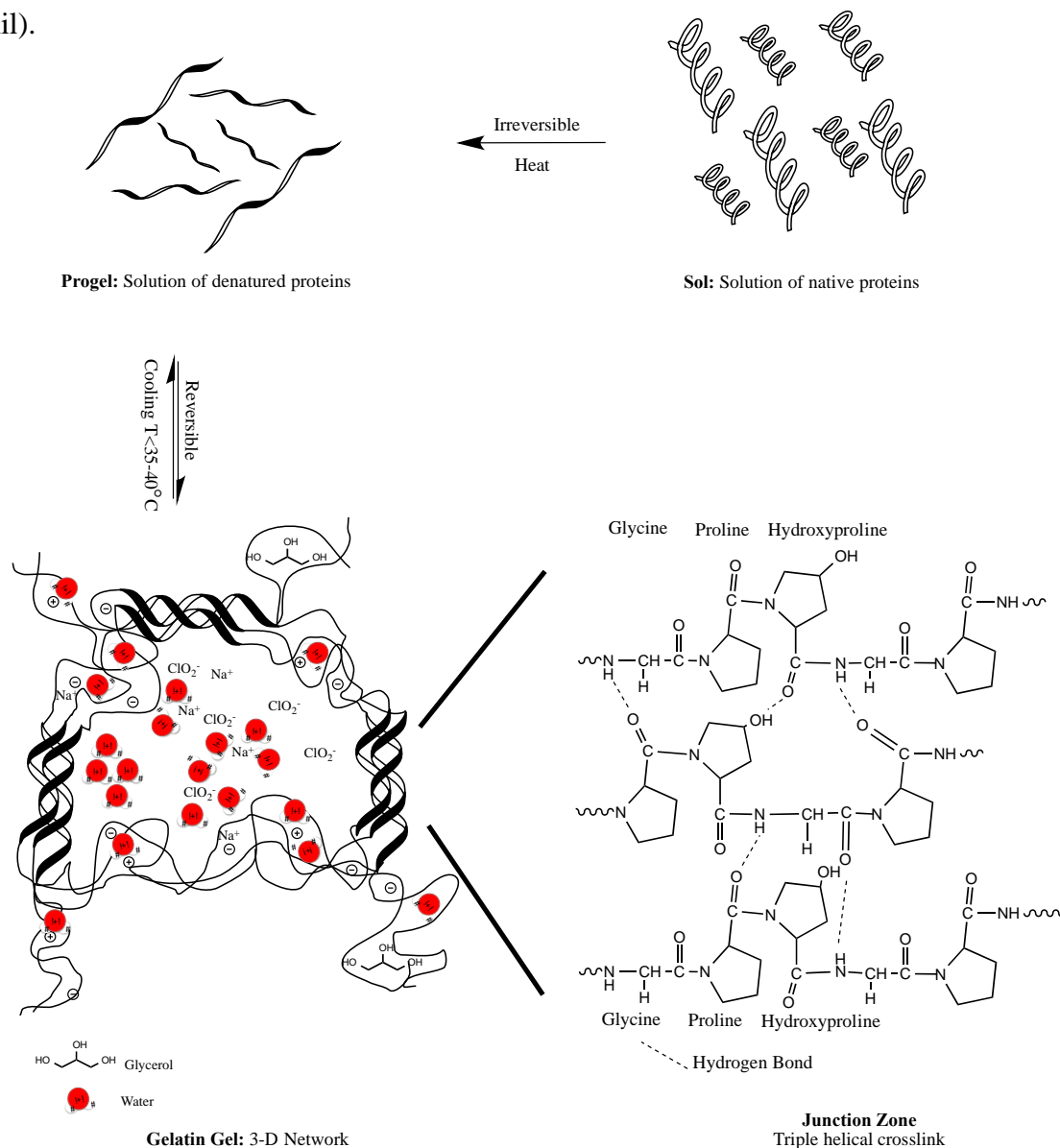


Figure 14. Manufacture of gelatin functional layer incorporated with sodium chlorite. A solution of native proteins (sol) is heated to denature proteins (progel). This step is irreversible. The second step takes place upon cooling of gelatin progel to below 35-40°C leading to formation of a 3-D gel network. The aggregation of three helices through inter-chain hydrogen bonding forms junction zones conferring long-range rigidity to these regions.

Gelatin aqueous solution (40 %) was prepared by adding pre-weighed gelatin to heated water of pH 3.5 (pH was adjusted using citric acid) and stirred slowly until the temperature reached 60-65°C. Heating the glue above 60°C will cause the degradation of the glue by hydrolysis, weakening its adhesive strength. Besides, the maximum adhesive strength of gelatin glue lies in the pH range between 3.5-7.5, exceeding these limits leads to a rapid decrease in its adhesive strength (Bogue, 1922). At pH of 3.5, gelatin glue carries a positive charge promoting electrostatic interactions with the negatively charged gelatin hydrogel containing  $\text{NaClO}_2$  at pH (8.4) > pI (4.8-5) and slightly negatively charged LM pectin containing acid at pH (2.6) < pKa (3.5) (Figure 15). Gelatin glue was applied as a thin and continuous glue film and spread evenly onto pectin layer surface to be bonded, using a paintbrush. Prior to the application of pressure, the film was allowed to become tacky (transition point from liquid to gel). Pressure was applied uniformly to minimize inequalities of glue at the joint making sure complete contact between surfaces to be bonded was achieved.

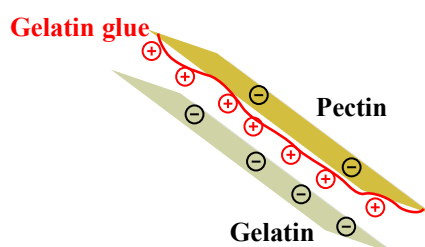


Figure 15. Inter-layer binding of pectin and gelatin layers. Application of the positively charged gelatin glue solution on the surface of slightly negatively charged pectin followed by pressing onto the negatively charged gelatin layer to promote binding and achieve contact between the layers.

### 8.2.2. Multilayer Label Assembly

#### **Multilayer Label Without Barrier Layers (ML w/o BL)**

Multilayer label without barrier layers was composed of five continuous layers of pectin embedded with citric acid (3) and gelatin incorporated with sodium chlorite (2) (Figure 17). Pectin and gelatin layers were assembled at the point of use, before analysis. Gelatin glue was applied as a thin and continuous glue film and spread evenly onto pectin layer surface to be bonded, using a paintbrush. First, a thin and continuous film of glue was applied using a paintbrush at the surface of (pectin + acid) layer to be bonded with the (gelatin + salt) layer. Once the glue film became tacky, the (gelatin + salt) layer was applied. The previous step was repeated, followed by application of the last (pectin + acid) layer. The label obtained had a surface area of 10 cm<sup>2</sup> and 1.4 mm (55 mil) thickness.

#### **Multilayer Label With Barrier Layers (ML w/ BL)**

Multilayer label with barrier layers was composed of nine continuous layers of pectin embedded with citric acid (3) gelatin incorporated with sodium chlorite (2), separated by gelatin barrier layers (4) (Figure 18). Pectin and gelatin layers were assembled at the point of use, before analysis. First, continuous film of glue was applied using a paintbrush at the surface of (pectin + acid) layer to be bonded with the gelatin layer. Once the glue film became tacky, the gelatin barrier layer was applied. The gelatin surface was brushed with thin film of glue and (gelatin + salt) was bonded to it, so on and so forth until the application of the last (pectin + acid) layer. The label obtained had a surface area of 10 cm<sup>2</sup> and 3 mm (118 mil) thickness.

### 8.2.3. Controlled Activation Mechanism of Labels

Once the multilayer label was assembled, chlorine dioxide release was triggered by controlled activation mechanism using pressure only. Pressing of layers was performed using a heat-press (Sencorp Systems, Inc., Hyannis, MA, USA) composed of a top metal bar and a lower bar connected to a compressed air tank. The assembled multilayer label was placed on the surface of the lower bar, upon application of pressure, the upper bar pressed on the label at the predetermined pressure (100 KPa) and time (5s). Pressure was applied uniformly to minimize inequalities of glue at the joint and make sure complete contact between surfaces to be bonded was achieved (Figure 16).

After activation, the labels were transferred into 40 mL EPA, amber glass vials assembled with 24 mm Miniret Valves for screw cap bottles (from Supelco, Bellefonte, PA, USA.) containing 3 mL of distilled water to mimic water vapor released from fresh produce respiration.

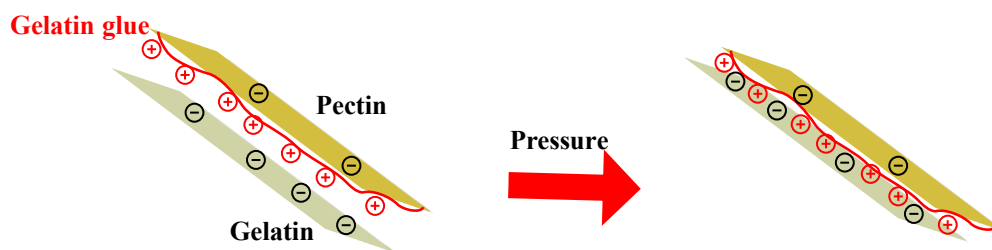


Figure 16. Activation of multilayer label. Application of a continuous film of gelatin glue solution onto the pectin layer followed by label assembly and pressure to promote binding between pectin and gelatin functional layers and trigger  $\text{ClO}_2$  release.

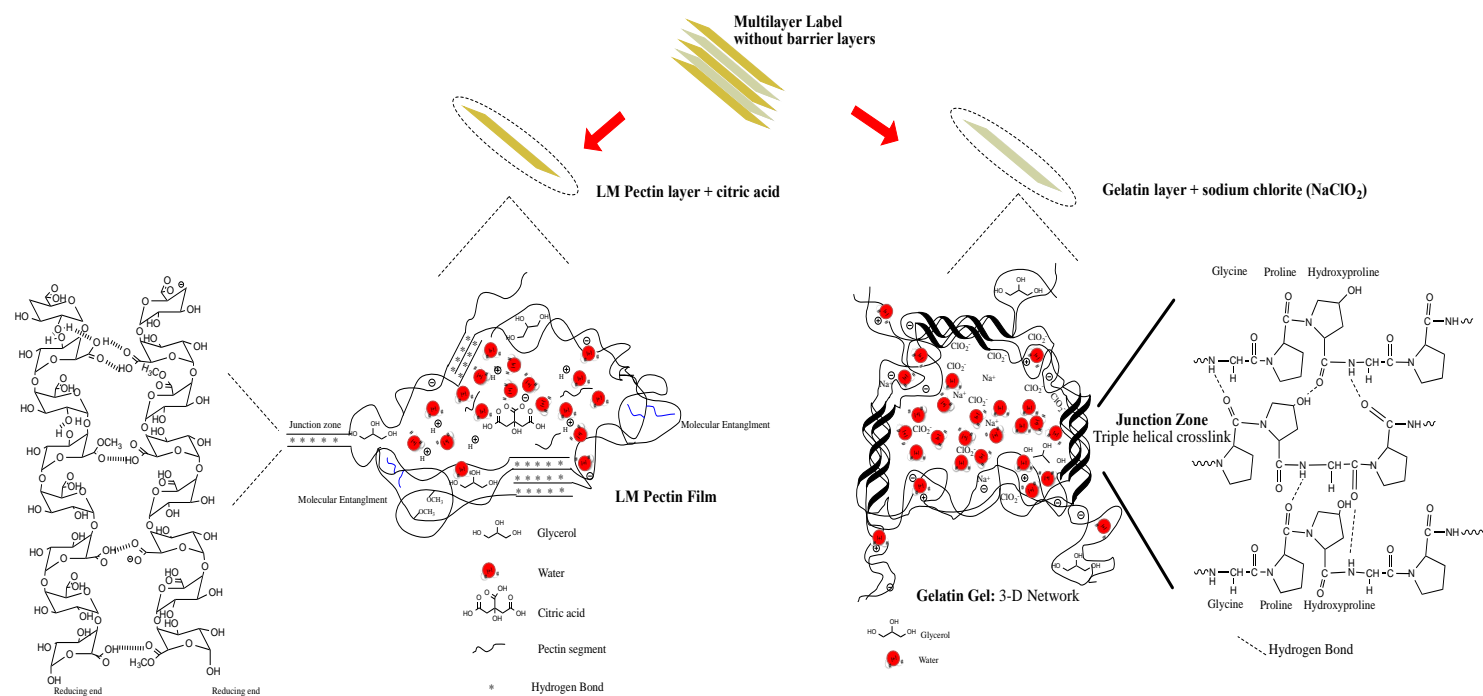


Figure 17. Diagram of multilayer label without gelatin barrier layers showing pectin (left-hand side) and gelatin (right-hand side) functional layer molecular structures as well as junction zones maintained by hydrogen bonding.

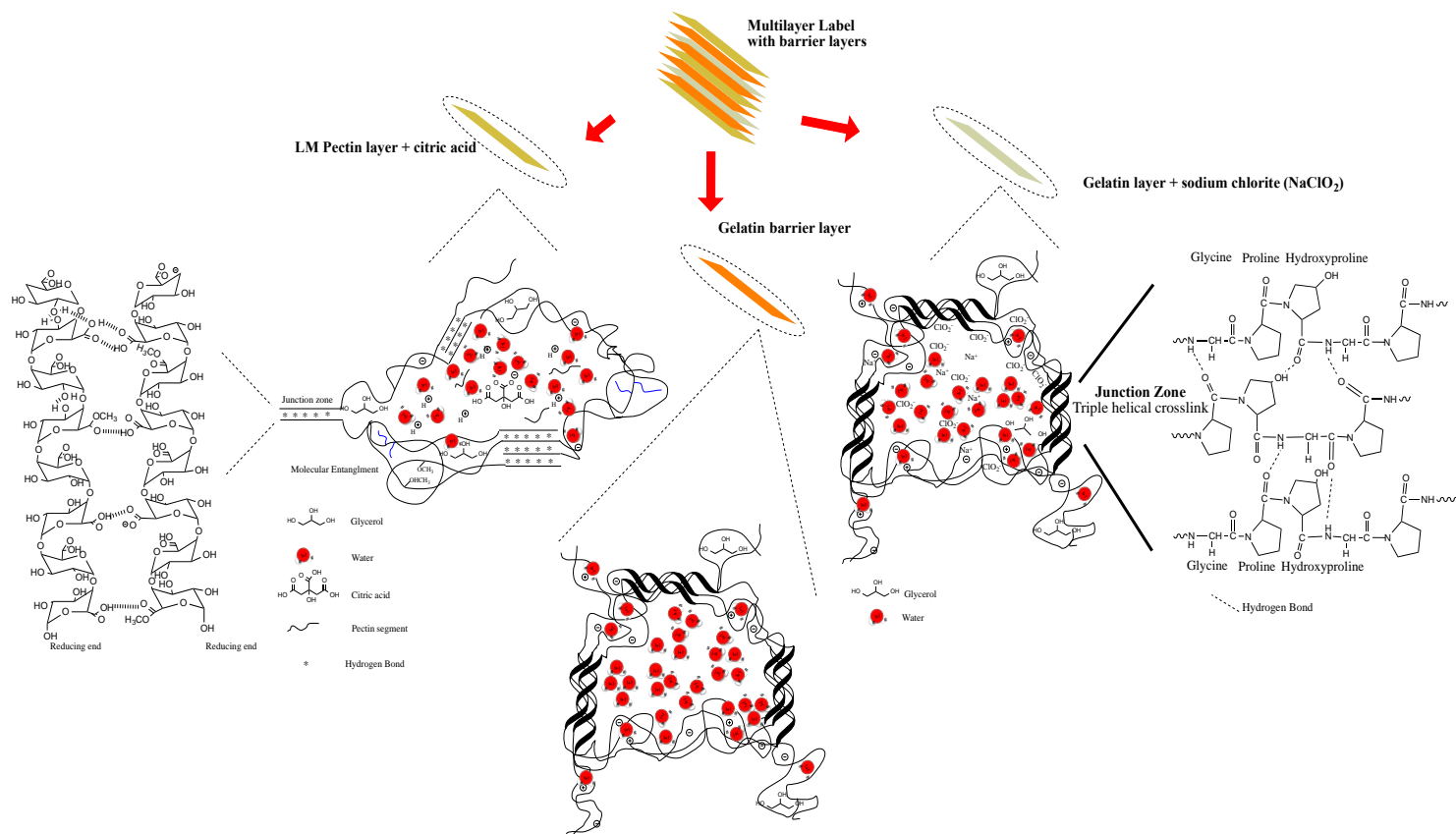


Figure 18. Diagram of multilayer label with gelatin barrier layers showing the pectin functional layer (left-hand side), gelatin barrier layer (center) and gelatin functional layer (right-hand side) molecular structure as well as junction zones maintained by hydrogen bonding.



#### 8.2.4. Quantitative Determination of ClO<sub>2</sub> from Biobased Labels

Quantification of chlorine dioxide release over time from multilayer labels was performed using Shimadzu UV-1700 PharmaSpec UV-VIS Spectrophotometer, Shimadzu Scientific Instruments, Inc., Maryland, USA and a spectrophotometric cuvette of 1 cm pathlength.

##### **UV-VIS Spectrophotometry**

Chlorine dioxide is highly soluble in water, its solubility increases with decrease in temperature (Masschelein, 1979). In order to quantify the amount of gaseous chlorine dioxide released from the labels over time, both headspace and water contained in vial were sampled each time and total chlorine dioxide concentration was the sum of both concentrations at each sampling time. 1.5 mL of chilled distilled water (temperature 7-10°C) were first drawn into a 3 mL syringe. The tip of the syringe was inserted into sampling port of amber glass vials and 0.5 mL of headspace was slowly drawn to the 2 mL mark. The contents in the syringe were mixed by shaking for ten seconds, placing finger over tip of syringe. The solution in the syringe was then transferred into a 1.5 mL cuvette of 1cm pathlength. At the same sampling time, 1.5 mL of water contained in vial were drawn and transferred into a 100 mL amber volumetric flask containing chilled distilled water. The solution was thoroughly mixed to ensure homogenization. Absorbance of both chlorine dioxide in the headspace and chlorine dioxide dissolved in water was measured for the different samples at 360 nm wavelength corresponding to maximum absorbance of chlorine dioxide, using UV-Vis spectrophotometer. Quantification of chlorine dioxide amount (mg) released from both biobased labels was

measured over time for both labels (without and with barrier layers) at room temperature (23°C) and 10°C. Each sample was run in triplicate in three independent analyses to test repeatability of the experiment. Values reported were the average of replicates of each sample.

### **Determination of Gaseous Chlorine Dioxide Concentration**

Gaseous chlorine dioxide released from biobased labels over time was measured by UV-VIS spectrophotometer at 360 nm wavelength. Based on Beer-Lambert law, aqueous chlorine dioxide concentration was calculated according to the following equation:

$[\text{ClO}_2] \text{ (M)} = (A / l \times \epsilon)$ ; where, A is absorbance of sample read at 360 nm wavelength, l is pathlength of spectrophotometric cell (cuvette) used in the analysis (1 cm in this experiment), and  $\epsilon$  is molar absorptivity of chlorine dioxide (1250 l/M cm).

Chlorine dioxide amount released from the package label with time was expressed in mg  $\text{ClO}_2$  and reflected the total amount of  $\text{ClO}_2$  in the headspace and that dissolved in water at each sampling time.

#### **8.2.5. Antimicrobial Effectiveness of Biobased Labels on Media Plates (TSA)**

Antimicrobial effectiveness of multilayer labels without and with barrier layers against microbial growth was performed using 250 ml amber straight side, Fisher Scientific, Pittsburg, PA, USA.

*Salmonella* Montevideo G4639, ATCC BA-710 (a clinical isolate associated with a tomato outbreak), was obtained from the American Type Culture Collection (Manassas, VA, USA). Stock cultures were stored in tryptic soy broth (TSB; BBL, Sparks, MD, USA) containing 20% glycerol at -80°C. Working stocks were maintained on tryptic soy

agar (TSA; BBL) slants containing 0.6% yeast extract stored at 4°C for 2-4 weeks. A loop full of culture from a TSA slant was transferred into 10 ml of TSB and allowed to grow for approximately 8 h at 37°C. This culture was then used to inoculate fresh 10 ml of the same medium at 0.01 % (v/v) level, and was allowed to grow overnight to stationary phase at 37°C. This culture (10 µL) was directly plated without further dilution on duplicate sets of 50 mm PALL Sterile Petri Dishes (Fischer Scientific, Pittsburg, PA, USA) containing TSA. Each inoculated plate was placed uncovered in 250 ml amber jar (Fisher Scientific) without (control) or with activated multilayer labels, in the presence of 3 mL of water contained in vial taped to the side of the jar. Jars were covered using 70-400 closures with bonded liner and were held at room temperature for 2, 4, or 6 h for the multilayer labels without barrier layers and 2, 4 and 8 h for the multilayer labels with barrier layers. TSA plates were removed from the jars at each sampling time and incubated at 37 °C for 24 h, and cell concentrations were enumerated.

#### 8.2.6. Antimicrobial Effectiveness of Biobased Labels on Inoculated Mung Bean Seeds

*Salmonella* Montevideo G4639 was grown overnight in 200 ml of TSB as previously described, spun down, washed once with sterile ddH<sub>2</sub>O, and suspended in 200 ml sterile ddH<sub>2</sub>O to give a final cell concentration of ca. 8 log CFU/ml. This was used as the inoculum to inoculate the seeds. Raw mung bean seeds (*Vigna radiata*), unprocessed and not subjected to any additional cleaning procedure, were obtained from The Sprout House (Lake Katrine, NY, USA). Mung bean seeds were rinsed under running deionized double distilled water (ddH<sub>2</sub>O) till the water ran clear (ca. 5 min) to remove dirt and surface debris. Cleaned mung bean seeds were submerged in 200 ml of inoculum at room

temperature (RT; 21°C) for 20 min with mixing every two minutes for 30 seconds using a spoon. Seeds were placed on an absorbent paper under a laminar biosafety flow hood and allowed to dry for 3 h. Inoculated seeds were then stored at 4°C overnight prior to treatments, to allow for bacterial attachment and/or biofilm formation.

Inoculated seed samples (15 g) were placed in 250 ml amber jar without or with activated multilayer labels and were covered (see above). Jars were covered using 70-400 closures with bonded liner and were held at room temperature for 2, 4, or 6 h for the multilayer labels without barrier layers and 2, 4 and 8 h for the multilayer labels with barrier layers (Figure 19). Seeds were removed from the jars at each sampling time and were analyzed for residual *Salmonella* population.

Seed samples (treated and non-treated) were individually analyzed for residual *Salmonella* populations. Enumeration of *Salmonella* populations on seeds was done with the use of the selective media, xylose lysine Tergitol-4 agar (XLT-4; BBL).

Sterile 1-liter stainless steel blender jars (Waring Products; Torrington, CT) were used for each sample preparation. Each blender jar was sanitized with 400 ppm chlorine solution and rinsed twice with deionized water between every single use. Sanitized jars were then autoclaved for sterilization to ensure that there is no carryover of *Salmonella* cells between each use. Each seed sample (15 g) was placed in the sterile 1-liter stainless steel blender jar, combined with 150 ml of neutralizing buffer (NB; BBL), and blended at medium speed for 2 min with a commercial blender (Waring Blender Model 51BL31). The resulting homogenate was placed in a filter bag (Spiral Biotech, Bethesda, MD, USA) and duplicate 10-ml filtrate samples were transferred to sterile tubes. The filtered homogenates were then serially-diluted in 0.1% peptone water (PW; BBL) as needed and

surface plated on the selective XLT-4 medium. All plates were then incubated at 37°C for 24-48 h, and the resultant colonies were enumerated and were expressed as log CFU/g seed.

**Statistical Analysis.** Single-factor analysis of variance tests and t tests in Microsoft Excel 2007 were used to compare the *Salmonella* populations exposed to ClO<sub>2</sub> gas released from biobased labels at different sampling times on inoculated mung bean seeds at 95% significance level ( $P < 0.05$ ).



Figure 19. Antimicrobial effectiveness of biobased labels on Mung bean seeds – Experimental setup.

## **9. RESULTS AND DISCUSSION**

### **Approach -I-: Extrusion**

#### **9.1. Synthetic Package Label Characterization Results**

The labels used for the purpose of this experiment were composed of polymer matrix of different thickness, using three polymers, polycaprolactone, ethyl vinyl acetate (28% VA) and aromatic polyether-based thermoplastic polyurethane incorporated with various concentrations of citric acid. Label characterization consists of determining physical and structural properties such as tensile, thermal and surface morphology in order to study the effect of citric acid incorporation on label integrity.

##### **9.1.1. Scanning Electron Microscopy (SEM)**

SEM was conducted to determine surface texture and distribution of citric acid crystals on the package labels made of three different polymers, polycaprolactone, ethyl vinyl acetate and aromatic polyether-based thermoplastic polyurethane.

All the tested samples showed a non-uniform dispersion of citric acid crystals on the surface.

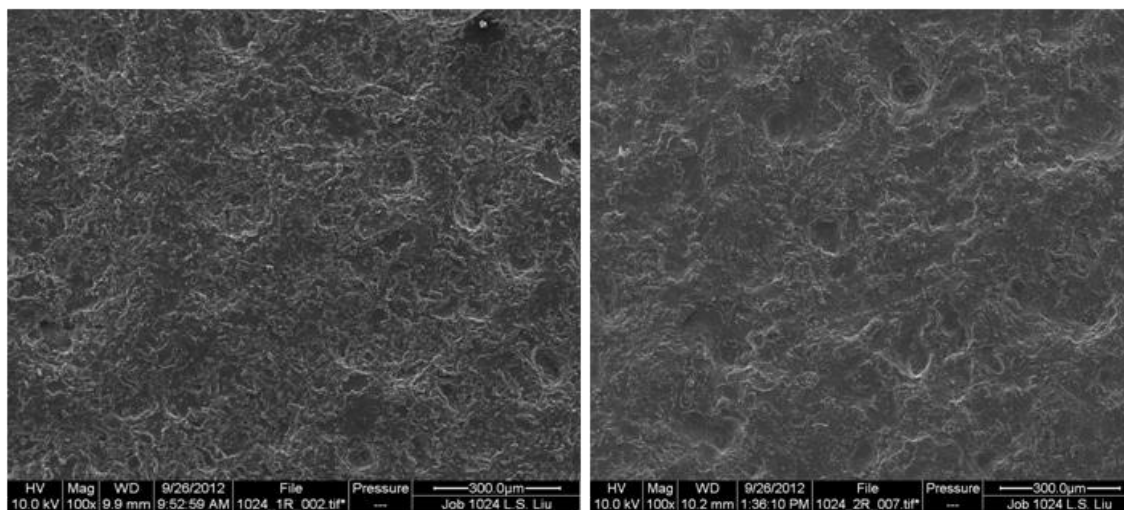
**Label 1****Label 2**

Figure 20. SEM Photomicrograph of labels 1- PCL 8.6% citric acid 13.5 mil and 2- PCL 8.6% citric acid 25 mil thickness at 100X magnification.

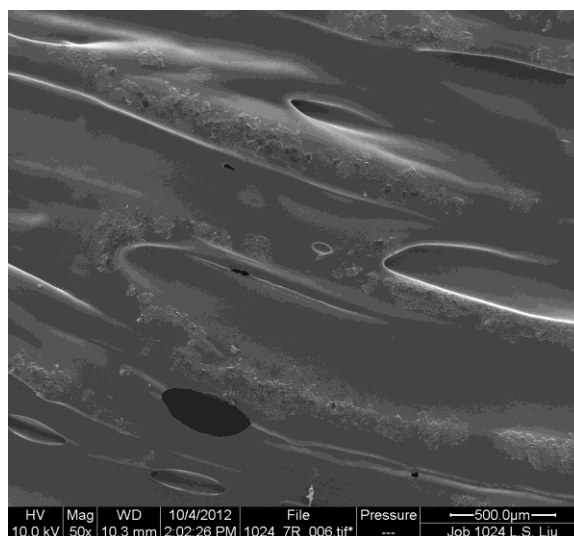
**Label 7**

Figure 21. SEM Photomicrograph of label 7- TPU 15 % citric acid 32.5 mil thickness at 50X magnification.

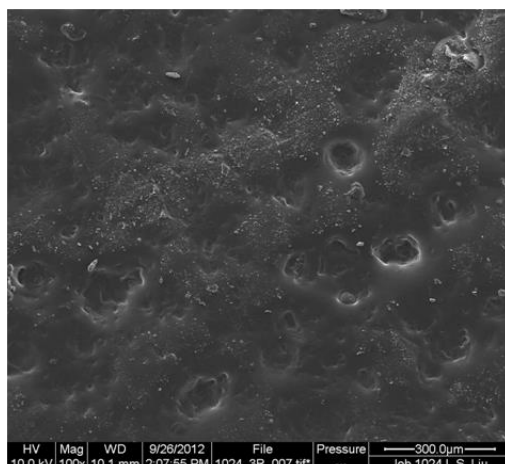
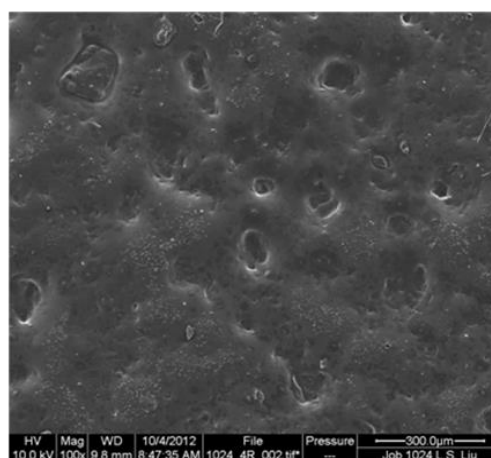
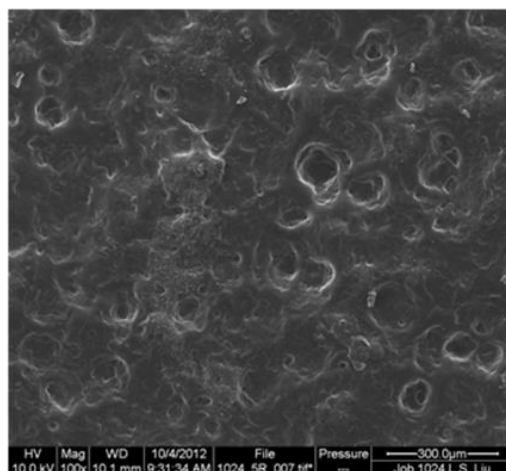
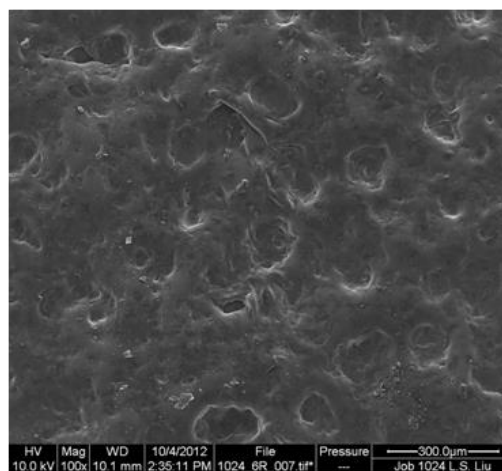
**Label 3****Label 4****Label 5****Label 6**

Figure 22. SEM photomicrographs of EVA (28 % VA) labels at 100 X magnification. Label 3- 7.5% citric acid 13.3 mil; label 4- 7.5% citric acid 26.6 mil; label 5- 15% citric acid 11.9 mil and label 6- 15% citric acid 19.2 mil.

TPU package label was of poor morphological quality, and showed scattered regions of melted and resolidified citric acid unevenly distributed on the TPU surface with presence of holes. This is due to the utilization during extrusion, of processing temperatures higher than citric acid melting point ( $153^{\circ}\text{C}$ ).



SEM analysis of samples revealed information regarding dispersion pattern of citric acid on the surface without covering internal distribution of such compound in the bulk of polymer.

The microstructural difference of the matrices loaded with citric acid, as well as the dispersion pattern of this compound (surface and bulk) would impact release as well as efficiency of ClO<sub>2</sub> delivery from packaging labels.

#### 9.1.2. Tensile Properties

Tensile strength represents the stress needed to break a sample (force per unit area MPa), in other words its ability to withstand external forces without breaking. This property reflects the strength of the specimen being analyzed.

Elongation-to-break refers to strain on a sample when it breaks and measures the extent of deformation of a material before fracture (ratio between the changed length and initial length after sample breaks  $\Delta L/L_0$ , expressed as a percentage).

Young's modulus also called modulus elasticity or tensile modulus is the slope of a tensile stress-strain curve (MPa) and a measure of stiffness of an elastic material.

Fracture energy or toughness is the area under the stress-strain curve that corresponds to the amount of energy a sample can absorb per volume (J/cm<sup>3</sup>) and plastically deform without breaking. The resulting sample is said to be tough or brittle depending on whether it exhibits high or low strain, respectively.

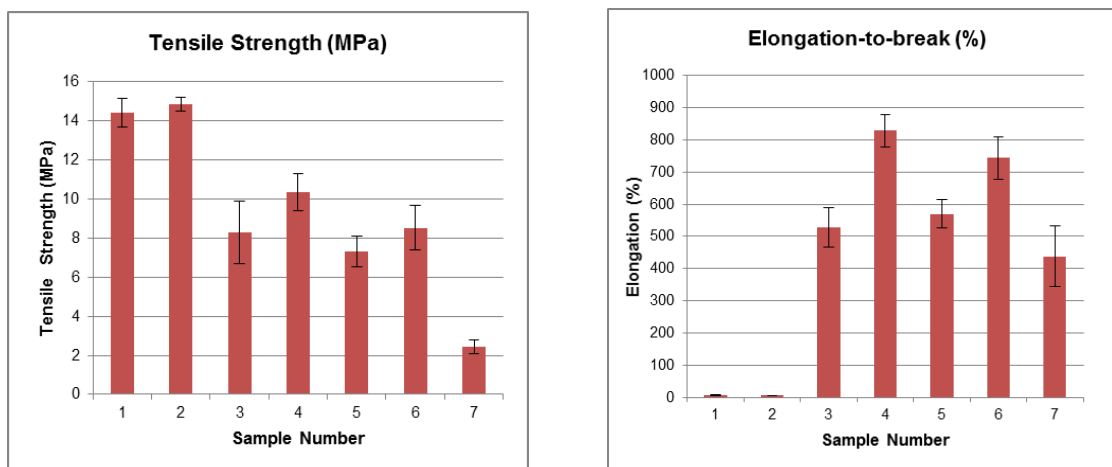


Figure 23. Tensile strength and elongation-to-break of the synthetic package labels.

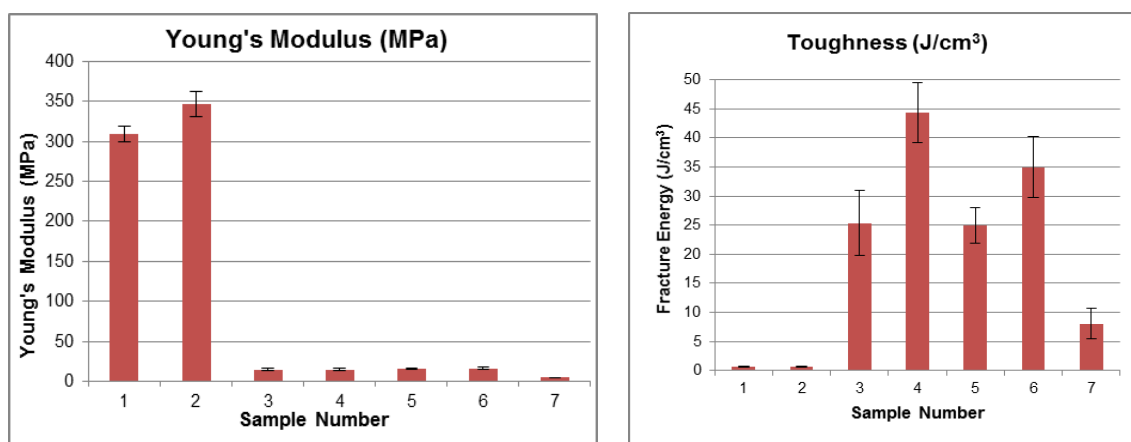


Figure 24. Young's modulus and toughness of the synthetic package labels.

### PCL Package Labels

PCL package labels incorporated with same level of citric acid (8.6 %) but of different thickness showed high tensile strength (~ 14 MPa) and modulus (309-346 MPa) (Figure 23 and Figure 24) due to high crystallinity of this polymer (40-50%) % (Jenkins & Harrison, 2006). However, these labels showed very low elongation-to-break (~ 7%) and

toughness ( $\sim 0.6 \text{ J/cm}^3$ ) (Figure 23 and Figure 24) highlighting the brittleness of these samples from both the crystallinity nature of this polymer and the effect of citric acid incorporation on polymer structure. Citric acid acts as a catalyst speeding up the hydrolysis of ester linkages resulting in the degradation and weakening (brittleness) of polymer structure (Woodruff & Hutmacher, 2010). It is worth noting that doubling the thickness did not affect tensile strength of PCL labels.

### **EVA Package Labels**

EVA package labels (samples 3-6) exhibited comparable tensile strength ranging from 7.3-10.4 MPa, low stiffness (14.6-16 MPa), high elongation-to-break (527.8-828 %), and high toughness ( $25.3\text{-}44.3 \text{ J/cm}^3$ ) (Figure 23 and Figure 24). These values were in agreement with reported data provided by Dupont supplier on EVA extruded films without citric acid (thickness up to 126 mil) with tensile strength ranging from 15-24 MPa, stiffness between 18 and 26 MPa and elongation-to-break ranging between 800-1000 %. Doubling the citric acid loading did not have an observable effect on the tensile properties of the EVA package labels.

### **TPU Package Label**

TPU package labels exhibited poor tensile properties with tensile strength, elongation, toughness and Young's modulus of 2.44 MPa, 438 %,  $8 \text{ J/cm}^3$  and 4.4 MPa, respectively (Figure 23 and Figure 24). Both strength and elongation-to-break were greatly affected

when compared to values of a 30 mil TPU extruded film from supplier (34.5 MPa and 700 %). This is mainly due to the detrimental effect of citric acid (15%) on the molecular structure of TPU. The high temperature during processing of TPU polymer, ( $T > 153^{\circ}\text{C}$ ) caused the cleavage of ether linkages on the accessible polyether segments of TPU chain (soft segments) from increased chemical reactivity of melted citric acid and the breakdown of its molecular structure during extrusion (Oertel & Abele, 1994).

#### 9.1.3. Differential Scanning Calorimetry (DSC)

DSC is conducted to study the effect of citric acid and processing conditions on thermal properties of the different labels namely glass transition temperature, melting temperature and enthalpy of fusion used to determine degree of crystallinity.

DSC results provide valuable information permitting characterization of the different samples through assessment of their material properties and storage conditions (thermal history).

#### **PCL Package Labels**

Samples 1 and 2 showed similar melting dips at temperatures nearing  $53\text{-}54^{\circ}\text{C}$  and  $153\text{-}155^{\circ}\text{C}$  (Figure 25) corresponding to the melting point of PCL (datasheet from supplier) and citric acid, respectively. The melting dips characteristic of the individual components detected on PCL thermograms suggested that PCL and citric acid were not miscible.

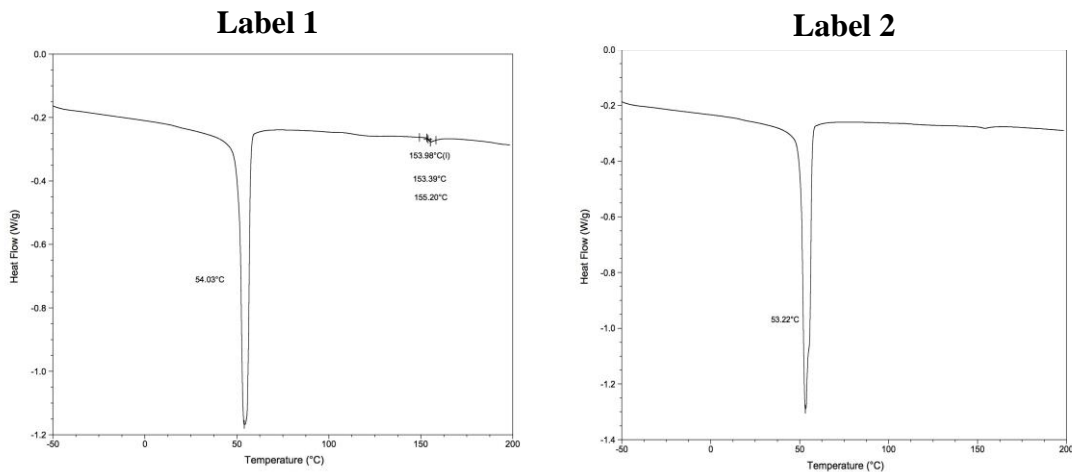


Figure 25. DSC thermograms of PCL labels.

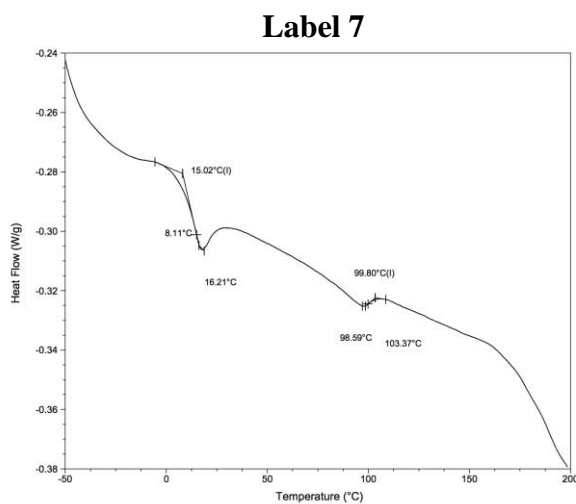


Figure 26. DSC thermogram of TPU label.

### TPU Package Labels

As to TPU package label, melting dips observed on the thermogram fell outside TPU (136°C) and citric acid (154°C) melting temperatures (Figure 26).

Extrusion of TPU and citric acid at processing temperatures above melting point of citric acid (Table 4) caused breakdown of TPU molecular structure, hindering film formation. This problem was not encountered upon TPU compounding with citric acid probably due to lower processing temperatures used in this step (Table 4). During extrusion, the melting of citric acid crystals present at high level (15 %) could have contributed to increased reactivity of citric acid molecules with TPU molecular chains resulting in the formation of new products such as amides and carbon dioxide, with different physical properties (Bosman, 2002). In addition, citric acid, at high temperatures used during extrusion, has the potential to cleave ether linkages of polyether soft segments of TPU resulting in degradation of its molecular structure (Oertel & Abele, 1994). Moreover, exposure of the TPU molecular chains to solar radiation (UV) and oxygen during extrusion caused photooxidative decomposition of aromatic diisocyanate via quinoid path leading to formation of chromophore compounds causing yellowing of packaging film (Thapliyal & Chandra, 1990). As a result, the multitude of chemical reactions, thermal and oxidative decomposition processes taking place during processing of TPU + citric acid blend may have led to a vast array of new products with different thermal properties.

The DSC thermogram of TPU and citric acid blend showed a melting dip at T around 100°C reflecting melting point of smaller hard crystalline segments resulting from scission of TPU larger polyether chains, whereas the other melting dip observed at 16°C could correspond to the melting point of by-products of detrimental chemical reactions and other degradative processes occurring during extrusion (Thapliyal & Chandra, 1990); Oertel & Abele, 1994 and (Bosman, 2002).

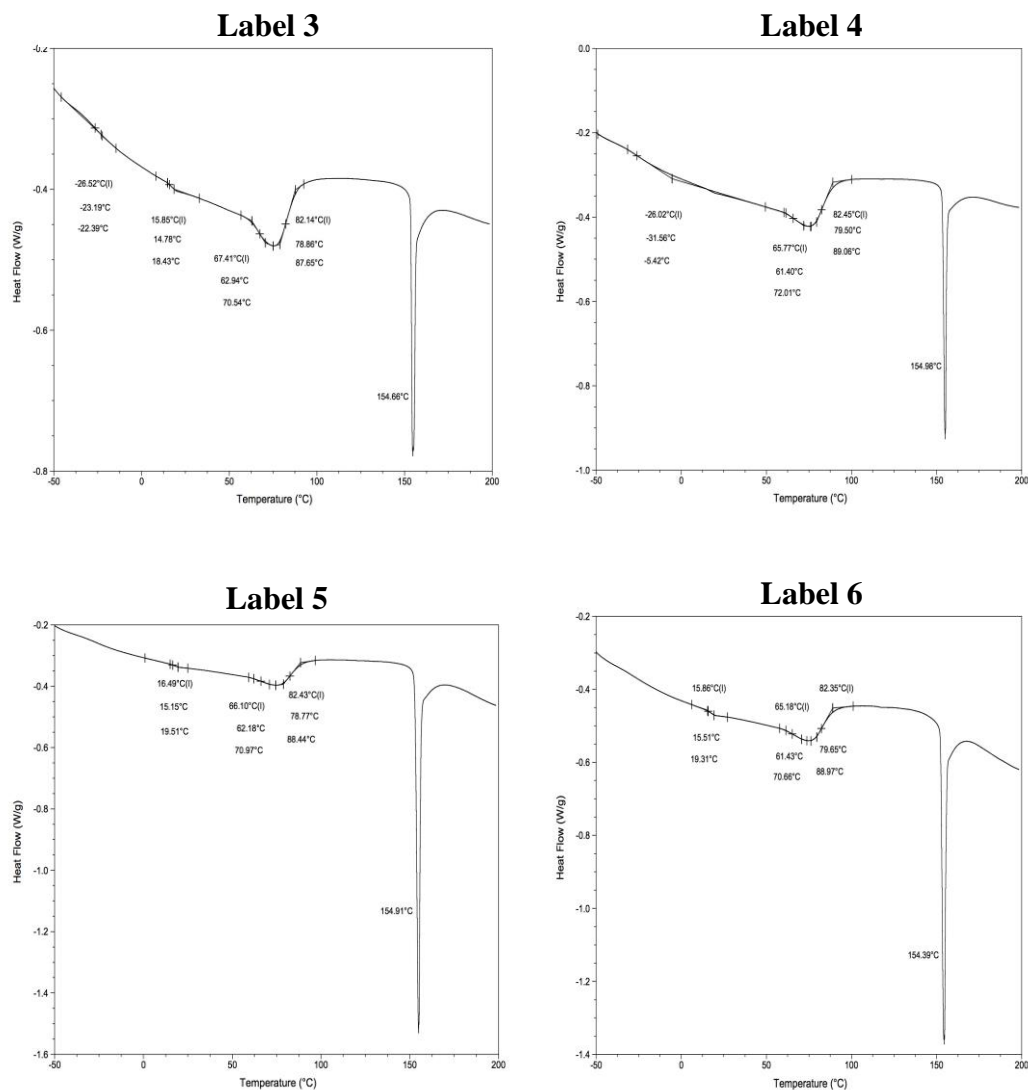


Figure 27. DSC thermograms of EVA labels.

### EVA Package Labels

The four different EVA labels exhibited similar thermograms with a distinct dip around 154°C corresponding to melting point of citric acid seen in all samples (Figure 27). Glass transition temperature of -26°C was detected in samples 3 and 4, a value corresponding to

T<sub>g</sub> of EVA (28% VA) of  $-28.6 \pm 0.5^{\circ}\text{C}$  (Almeida et al., 2011). Glass transition temperature characterizes the amorphous part of the semicrystalline sample. In addition, two melting dips were observed in EVA thermograms, one falling between  $18\text{--}19^{\circ}\text{C}$  in labels 3, 5 and 6 and another one occurring at  $75^{\circ}\text{C}$  in all the 4 EVA samples (Figure 27) which corresponds to EVA (28% VA) melting temperature ( $72.8 \pm 0.9^{\circ}\text{C}$ ) (Almeida et al., 2011). EVA thermograms reflect the immiscibility and heterogeneous nature of the four different EVA (28% VA) and citric acid blends. It is worth noting that there was no noticeable difference among thermograms of EVA samples despite their different thickness (13.3, 26.6, 11.9 and 19.2 mil for samples 3, 4, 5 and 6, respectively). Samples 5 and 6 showed deeper citric acid melting dips due to higher citric acid loading in these labels (15 % in samples 5 and 6 as opposed to 7.5 % in samples 3 and 4).

Overall, incorporating citric acid at different concentrations in both PCL and EVA (28% VA) did not affect thermal properties of such polymers. However, citric acid addition to TPU produced a thermogram with different thermal properties than those of individual compounds in their pure form highlighting the detrimental effect citric acid had on the structure and thermal properties of TPU.

## **9.2. Quantitative Determination of $\text{ClO}_2$ from EVA Package Labels**

Package labels made of three different polymers (polyurethane, polycaprolactone and ethylene vinyl acetate, 28 % VA) behaved differently under different conditions of processing and storage. Polyurethane for instance, failed to form a cohesive film during extrusion process due to the high operating temperature ( $> 153^{\circ}\text{C}$ , the melting point of citric acid) and high citric acid loading in this polymer (15 % citric acid) (Figure 28). At



high temperature encountered during processing of TPU polymer, citric acid melting and consequently its increased chemical reactivity led to degradation of polyether segments of TPU through cleavage of ether linkages, resulting in breakdown of this polymer's molecular structure (Oertel & Abele, 1994). Polycaprolactone became extremely brittle after the first month of storage. Polycaprolactone is a linear polyester whose hydrolysis is autocatalyzed by the presence of acid. The brittleness of PCL package labels may be attributed to high degree of crystallinity of this polymer (40-50 % (Jenkins & Harrison, 2006)). In addition, citric acid embedded within polymer matrix acted as a catalyst accelerating autocatalysis of ester linkages of PCL molecular chains causing degradation, overall reduction in molecular weight and cracking of label (Woodruff & Hutmacher, 2010) (Figure 28). Among the three polymers used in this study, EVA (28 % VA) seemed the best candidate for the purpose of this research since it formed a cohesive film during extrusion process, and did not undergo structural changes during storage. Consequently, chlorine dioxide release kinetics and microbial analyses will only be performed on EVA labels of two citric acid loadings (7.5 % and 15 %) and different thicknesses.

Two methods were used in combination to demonstrate the feasibility of package label with regard to chlorine dioxide generation and release after controlled activation mechanism (spraying with sodium chlorite aqueous solution, followed by heat-pressing). The first method consisted of directly quantifying the concentration of chlorine dioxide released from label with time using UV spectrophotometry. The second method, served as a validation of the first method by testing the effectiveness of activated labels on the growth of microorganisms, in this case, *Salmonella* Montevideo G4639 on trypticase soy agar (TSA) media, and evaluating log reduction of this microorganism as compared to a

control. Total inactivation of *Salmonella* Montevideo G4639 growth on plates would suggest that gaseous chlorine dioxide was generated and released from the package label at adequate concentration, demonstrating therefore the feasibility of this label.



PCL + citric acid upon extrusion



PCL + citric acid after storage



TPU + citric acid  
during extrusion

Figure 28. Effect of extrusion processing conditions and storage on the structural integrity of PCL and TPU incorporated with citric acid.

## UV Spectrophotometry

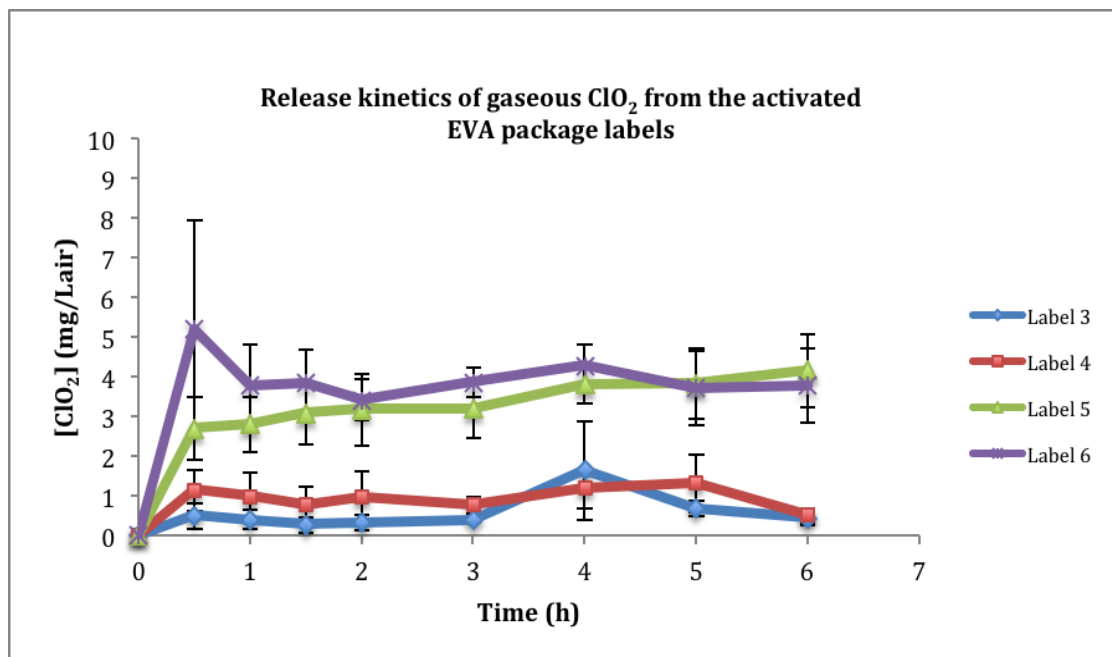


Figure 29. Release of  $\text{ClO}_2$  (mg/L air) from EVA package labels over 6 hours, after controlled activation.

As can be seen from the graph above (Figure 29), all EVA labels generated and released  $\text{ClO}_2$  over time.

There was no effect of thickness on release of  $\text{ClO}_2$  from EVA labels as shown by the comparable  $\text{ClO}_2$  release profile observed for labels with similar citric acid loading.

The release of  $\text{ClO}_2$  from the EVA labels was derived from surface reaction of citric acid molecules encountered on the labels' surface, with those located in the bulk of polymer being inaccessible for reaction given the hydrophobicity nature of EVA.

As can be expected, labels with higher citric acid loading released more  $\text{ClO}_2$  because of

the higher concentration of citric acid on the surface of these labels.

EVA package labels with 7.5 % citric acid released  $\text{ClO}_2$  at sustained levels over time, producing around 0.5 to 1 mg  $\text{ClO}_2$  /L air over 6-hour period for labels 3 and 4 respectively. Similarly, labels with 15 % citric acid released  $\text{ClO}_2$  at a constant rate over 6 hour period with concentrations in the range of 3.8 mg/L air (Figure 29).

The bulk of the research conducted so far on antimicrobial effectiveness of gaseous  $\text{ClO}_2$  on fresh produce has focused on a single exposure to this compound over the predetermined application time.  $\text{ClO}_2$  at concentrations between 0.5-8 mg/L air applied for time periods ranging from 6-720 min on a variety of fresh produce have been effective at reducing *Salmonella* populations (Gómez-López et al., 2009). Prodduk et al. 2014 demonstrated the effectiveness of releasing  $\text{ClO}_2$  at a fixed concentration of 0.5 mg/L air over extended periods of time (15, 30 and 60 min) on the inactivation of *Salmonella* on mung bean sprouts, with or without mechanical mixing (Prodduk et al., 2014). Ray et al. designed a  $\text{ClO}_2$  releasing packaging film capable of releasing  $\text{ClO}_2$  in a controlled manner over time in response to moisture resulting from respiration of grape tomatoes to inhibit the growth of *Salmonella* spp. and *E.coli* O157:H7.  $\text{ClO}_2$  was released at concentrations between 0.3 and 1.8 mg/L air after 5 hours exposure to the film resulting in reductions of these microorganisms population on grape tomatoes (Ray et al., 2013).

Controlled activation mechanism applied onto the labels, through its spraying step allows  $\text{ClO}_2$  precursors and moisture to come together. Heat-pressing step, through its press component, forces reactant molecules and moisture to become spatially close to one another however, heat component, increases kinetic energy of such molecules accelerating therefore the rate of reaction and amount of chlorine dioxide released as

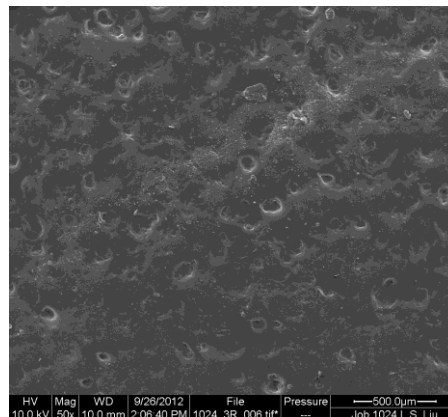
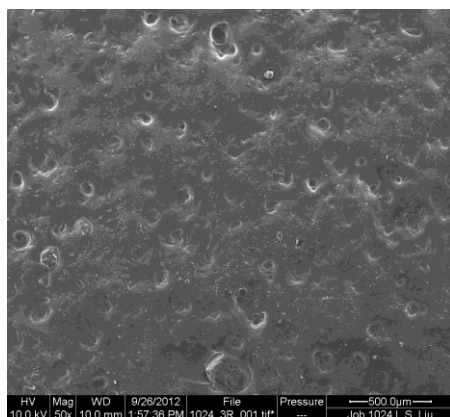
compared to labels activated only by spraying step.

It is worth mentioning that  $\text{ClO}_2$  loss does occur from activation mechanism. Heat-pressing causes thermal degradation of  $\text{ClO}_2$  and moisture evaporation lowering the amount moisture required for  $\text{ClO}_2$  production.

The results showed noticeable sample variability with regard to amount of  $\text{ClO}_2$  released over time. This was supported by SEM analysis performed on EVA labels. As can be seen in the figures (Figure 22, Figure 30 and Figure 31), variability in the citric acid particle size and distribution on the surface of labels was observed between duplicates of the same EVA label as well as between EVA labels of similar citric acid loading but different thickness. It is worth noting that distribution pattern of citric acid (uniform vs. non-uniform) on the surface of EVA package labels as well as the location of such compound on the polymer matrix (surface vs. bulk) influence time-release delivery of  $\text{ClO}_2$  from these labels. A number of factors occurring at different stages of processing may have contributed to varying extents to variability in citric acid particle size and distribution observed on the surface of these labels. Citric acid used in this experiment is in the crystalline form, reducing particle size of this compound could improve its even dispersion in compounded pellets and finished label. Improper compounding may have failed to achieve distributive (spatial rearrangement of citric acid throughout volume) and dispersive (size reduction of agglomerates with cohesive character) mixing resulting in non-uniform citric acid particle size and distribution in the compounded pellets. Improper mixing of the compounded resin with pure polymer resin prior to film extrusion may have also contributed to uneven distribution of citric acid and consequently the variability in the microstructure observed in the finished labels. Finally, variability in the extrusion

process could have affected the spatial distribution of dispersed phase in polymer blend and flow orientation of citric acid molecules resulting in uneven distribution of citric acid in the final microstructure of the labels.

**A, 50X**



**B, 50X**

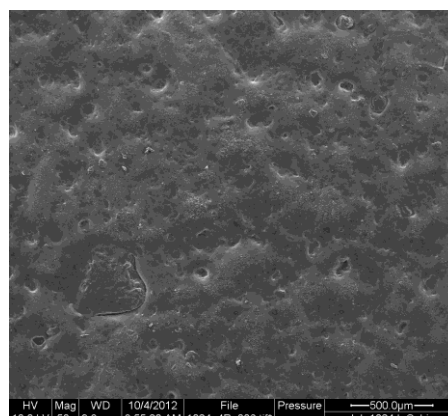
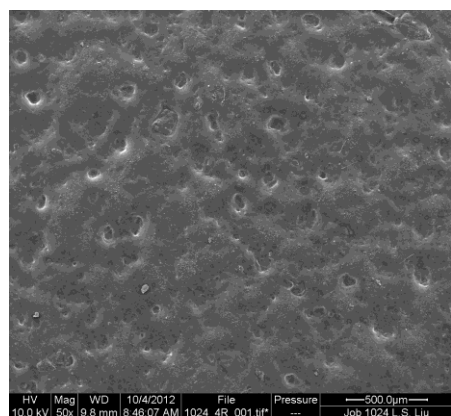


Figure 30. SEM photomicrographs of EVA labels at 50 X magnitude reflecting sample variability within the same label with regard to citric acid particle size and distribution on the surface of these labels. A: Label 3; B: Label 4.

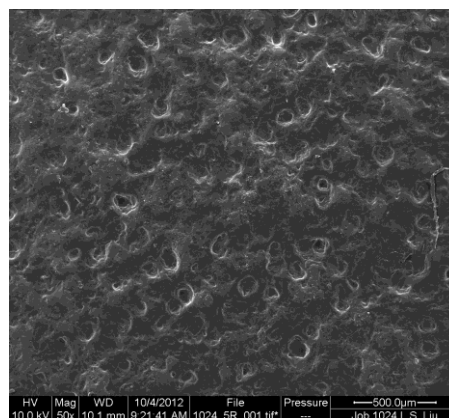
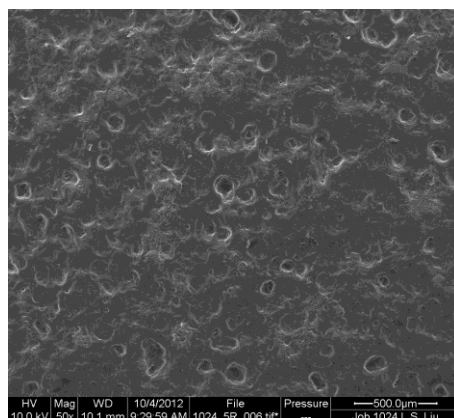
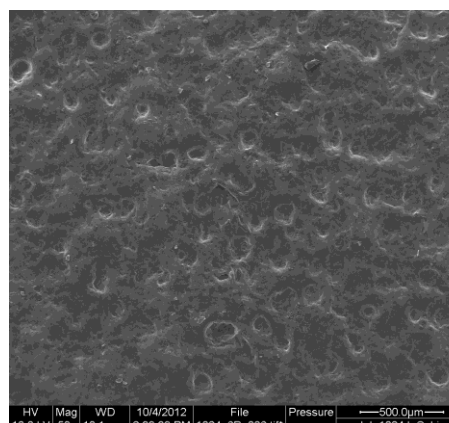
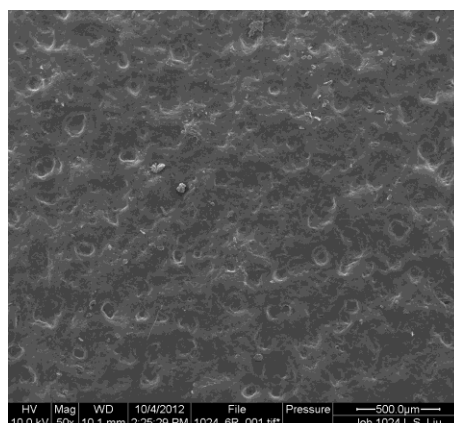
**C, 50X****D, 50X**

Figure 31. SEM photomicrographs of EVA labels at 50 X magnitude reflecting sample variability within the same label with regard to citric acid particle size and distribution on the surface of these labels. C: Label 5 and D: Label 6.

### 9.3. Antimicrobial Effectiveness of EVA Package Labels

The EVA package labels achieved between 0.6 and 2.3 log reduction (log CFU/mL) of *Salmonella* Montevideo G4639 on TSA plates after 2, 4 and 6 h exposure to ClO<sub>2</sub> demonstrating the feasibility of these labels in terms of ClO<sub>2</sub> generation and release at concentrations adequate to inactivate *Salmonella* Montevideo G4639 growth, partially or completely, on TSA plates (Table 7).

Labels 3 and 4 released similar amounts of ClO<sub>2</sub> after 2, 4 and 6 hours achieving partial reduction in the population of *Salmonella* of about 1 log-reduction on TSA plate after 6 hours exposure to these labels (Table 7).

The concentration of ClO<sub>2</sub> produced by labels 5 and 6 was comparable in the 2, 4 and 6 hours exposure and enabled the inactivation of *Salmonella* growth completely on inoculated TSA plates with around 2 log reductions (log CFU/mL) achieved in the first 2 hour exposure to these labels (Table 7).



Table 7. Antimicrobial effectiveness of EVA package labels on the growth of *Salmonella* Montevideo on TSA plates after 2, 4, and 6 hours.

<b>Label</b>	<b>Sampling Time (h)</b>	<b>Concentration of ClO<sub>2</sub> released (mg/L air) ± Standard Deviation</b>	<b>log reduction (log CFU/mL) ± Standard Deviation</b>
<b>Label 3</b>	2	0.2 ± 0.2	1.8 ± 1.7
	4	0.4 ± 0.2	1.8 ± 1.6
	6	0.4 ± 0.2	1.2 ± 0.2
<b>Label 4</b>	2	0.3 ± 0.3	0.9 ± 1.3
	4	0.4 ± 0.4	0.6 ± 0.3
	6	0.5 ± 0.4	1.0 ± 0.7
<b>Label 5</b>	2	1.5 ± 1.0	2.7 ± 0.6
	4	2.1 ± 1.9	2.6 ± 0.6
	6	2.9 ± 2.5	2.7 ± 0.6
<b>Label 6</b>	2	1.9 ± 0.6	2.1 ± 0.0
	4	2.7 ± 0.6	2.3 ± 0.3
	6	3.2 ± 0.7	2.3 ± 0.2

ClO<sub>2</sub> released from EVA labels was generated from surface reaction of citric acid after spraying the label with NaClO<sub>2</sub> aqueous solution with no observable effect of thickness on the ClO<sub>2</sub> release. The variability in the microbial results reported for these labels reflects sample variability in terms of citric acid surface concentration and distribution on the label as well as potential ClO<sub>2</sub> loss occurring from activation step.

As can be expected, labels 5 and 6 released higher ClO<sub>2</sub> concentrations than labels 3 and 4 due to their higher citric acid loading (15%) and the higher concentration of citric acid on the surface of these labels. The concentration of ClO<sub>2</sub> produced from these labels (5 and 6) was comparable after 2, 4 and 6 hours exposure of inoculated TSA plates to these labels resulting in complete inhibition of *Salmonella* on TSA plates after 2 hours (2.1-2.3 log CFU/mL).

Similarly to labels with lower citric acid loading, ClO<sub>2</sub> release from labels 3 and 4 was derived from reaction of citric acid on the surface of the label, with no effect of thickness on the release due to the hydrophobic nature of EVA polymer.

Higher inactivation level of *Salmonella* populations was observed on TSA plates exposed to thinner labels of both citric acid loading due to higher concentration of citric acid at the surface of these labels as compared to their thicker counterparts where citric acid is distributed within the thickness of label with lower concentration on the surface. Not to mention the effect of sample variability and possible losses of ClO<sub>2</sub> during activation process on the variability of the results obtained.

The inactivation level of *Salmonella* achieved from these labels on TSA plates is in agreement with log-reductions of *Salmonella* reported in the literature on fresh produce (1.54 log reduction of *Salmonella* on raspberry (Sy, McWatters, et al., 2005), 1.94 log

reduction of *Salmonella* on onions (Sy, Murray, et al., 2005) and 2.44 log reduction *Salmonella* on blueberry (Sy, McWatters, et al., 2005)).

However, it is important to note the difference in microbial inactivation on media plates as compared to fresh produce. In media plates, microorganisms grow uniformly on the surface offering ideal conditions for a maximized treatment efficacy and inactivation of microorganisms' colonies. Fresh fruits and vegetables however are complex systems due to variability of their surface topography, composition, possibility of physical injuries on the surface from handling or transportation as well as the diversity of microorganism populations surviving on the surface of these products.

Consequently, the effectiveness of the same treatment applied on media plate is reduced significantly when tested on fresh produce.

For the purpose of this research, testing the EVA labels on TSA plates was conducted to validate the technical feasibility of these labels in terms of  $\text{ClO}_2$  generation and release from the labels at concentrations enabling inactivation of microorganisms.

The next step however would consist of optimizing the labels from the design and manufacturing aspects to release  $\text{ClO}_2$  consistently at adequate amounts and to test the antimicrobial effectiveness of these labels on fresh produce safety.

### **Approach –II-: Coating**

Both LM pectin and gelatin used in the manufacture of the multilayer label are hydrophilic polymers that undergo swelling upon exposure to moisture. Upon contact with moisture vapor, the LM pectin and gelatin polymer networks shift from the state where they're dense and mobility of macromolecules is restricted to the state where the network structure is less dense, with higher macromolecular mobility and increased system volume (Figure 32). This process is known as the polymer chain relaxation process, which is dependent on the polymer characteristics such as chemical structure, side chains, and average molecular weight as well as temperature. Water diffusion due to concentration gradient starts by hydrating the most polar and hydrophilic functional groups on the polymer chains causing swelling of the polymer matrix, increasing macromolecular mobility, volume expansion, ClO<sub>2</sub> precursors dissolution and diffusion through the swollen polymeric network down their concentration gradient (Figure 32). Either water diffusion or polymer chain relaxation process, whichever occurs slower than the other, governs water penetration kinetics into the polymer network and the subsequent mobility and diffusion of ClO<sub>2</sub> precursors in the polymer matrix, their reaction and ClO<sub>2</sub> generation and release from the package label.

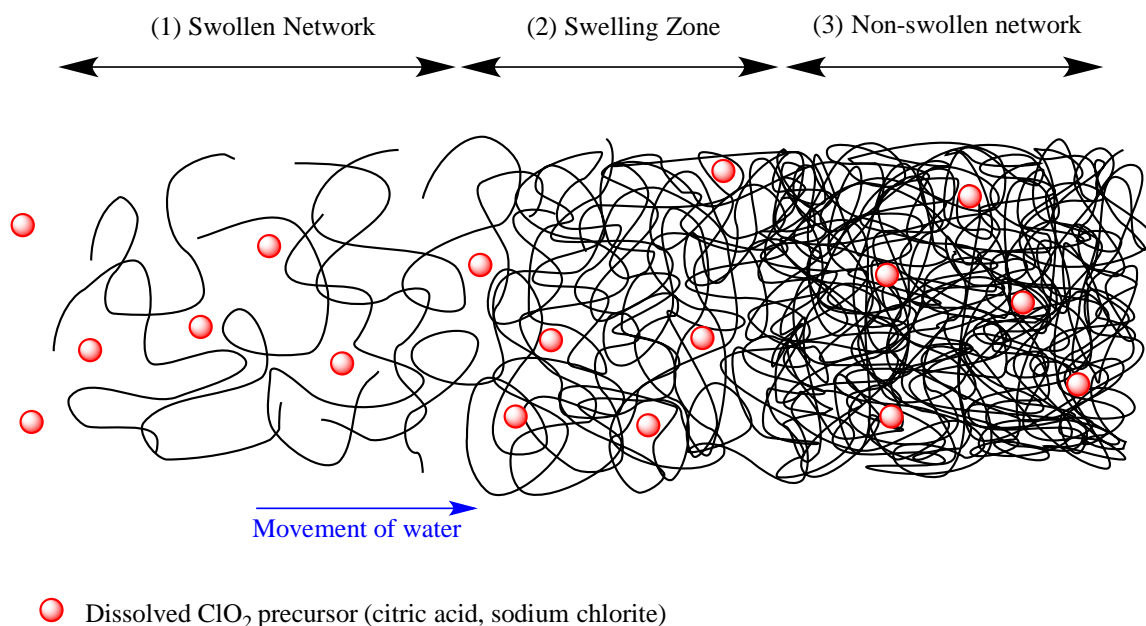


Figure 32. Polymer relaxation-controlled penetration of water into pectin or gelatin polymeric film. One surface of the film is exposed to the moisture (left-hand side). Three different zones can be distinguished inside the film: (1) swollen network (high water content, high mobility of ClO<sub>2</sub> precursors and water molecules); (2) swelling zone (polymer chain relaxation); and (3) non-swollen network (low mobility of ClO<sub>2</sub> precursors, no water).

#### 9.4. Quantitative Determination of ClO<sub>2</sub> from Biobased Labels

In this research, two release profiles of ClO<sub>2</sub> were achieved, a faster profile with multilayer label without barrier layers and a slower one with the multilayer label with barrier layers incorporated between the pectin and gelatin functional layers.

Activation of chemical reaction and ClO<sub>2</sub> release from labels was triggered by inherent moisture of the gelatin barrier and functional layers and maintained by that released from respiration of fresh produce. This allows diffusion of ClO<sub>2</sub> precursors, their chemical reaction and ClO<sub>2</sub> release out of label.

The results of the release kinetics analysis support the main objective of this work, the technical feasibility of the package label in terms of generation and controlled release of  $\text{ClO}_2$  over time after label activation (pressure and moisture).

#### 9.4.1. Multilayer Labels Without Barrier Layers (ML w/o BL)

Upon the assembly of pectin and gelatin layers to form the multilayer label, a highly viscous, hot gelatin glue solution was applied as a thin continuous film onto the surface of pectin layer incorporated with citric acid before its adhesion to sticky gelatin layer embedded with  $\text{NaClO}_2$ . Whereas gelatin is negatively charged at pH (8) above its pI (4.8-5) and LM pectin is slightly negatively charged at pH (2.6) below its pKa (3.5), the gelatin glue solution carries a positive charge promoting electrostatic interaction and adhesion of the functional layers (Figure 16).

Upon the application of glue onto the surface of pectin functional layer, water diffusion causes rapid softening and relaxation of pectin polymer chains. Following label assembly, the multilayer label is pressed at 100 KPa for 5s to ensure proper binding between the functional layers and trigger the generation and release of  $\text{ClO}_2$  from package label (Figure 16).

The pressing step maximizes contact between functional layers and forces protons ( $\text{H}^+$ ) and chlorite ions ( $\text{ClO}_2^-$ ) located at the contact surface of both pectin and gelatin layers to react rapidly (surface reaction) causing the generation and rapid release of  $\text{ClO}_2$  from package label observed in Figure 35.

At the same time, water diffusion from gelatin functional layer (70% moisture content, dry basis and 0.9  $a_w$ ) to pectin functional layers (20 % moisture content, dry basis and 0.5  $a_w$ ) located above and below it occurs, resulting in hydration and swelling of pectin

polymeric network leading to increase in macromolecular mobility and volume expansion Figure 33. The immobilized dissolved citric acid in the non-swollen pectin network becomes mobile in the swollen region. Diffusion of the protons ( $H^+$ ) then takes place down its concentration gradient in the pectin swollen region towards the gelatin layer also, diffusion of chlorite ions ( $ClO_2^-$ ) from the gelatin layer takes place down its concentration gradient towards the top and bottom pectin layers (Figure 33).

Due to the thicker gelatin layer (four times the thickness of pectin layer), diffusion of  $ClO_2^-$  through the gelatin layer is slower than  $H^+$  movement through the pectin polymeric network.

Moisture released from respiration of fresh produce over time is replicated in this experiment by the addition of 3 mL of water into a vial upon quantification of  $ClO_2$  released from the label over time. This constant supply of moisture with time acts further by relaxing the polymer chains increasing the extent of swelling of pectin and gelatin layers and volume expansion, leading to greater mobility of  $ClO_2$  precursors, maximizing chemical reaction,  $ClO_2$  generation and release from package label.

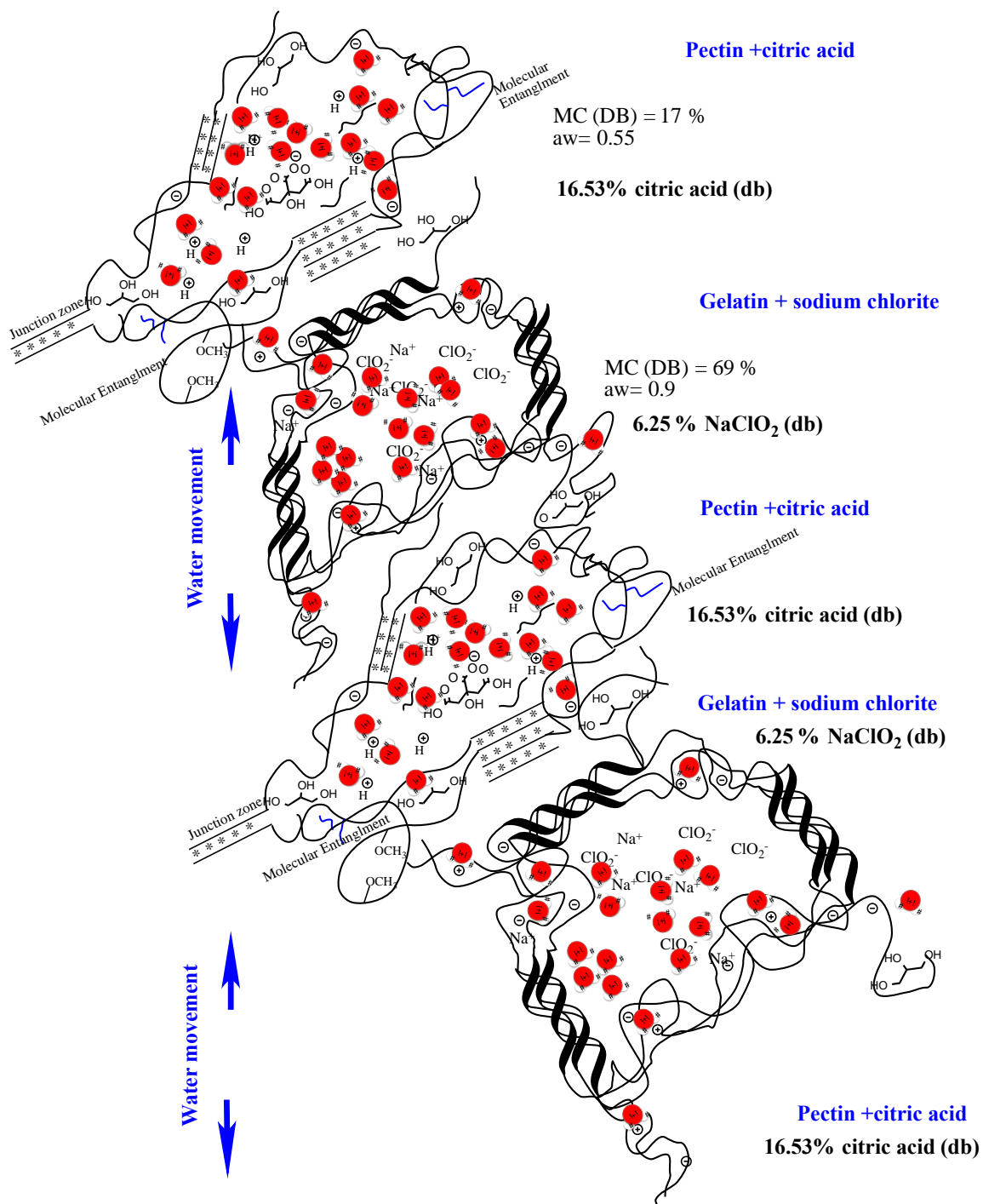


Figure 33. Schematic representation of the multilayer label without barrier layers (5 layers) at the molecular level. The moisture content, water activity ( $a_w$ ) of each layer as well as loading % on dry basis (db) of each layer were shown to indicate the direction of water diffusion as well as diffusion of ClO<sub>2</sub> precursors (H<sup>+</sup> and ClO<sub>2</sub><sup>-</sup>) after controlled activation.



Form the graph below (Figure 35) it can be seen that multilayer label without barrier layers released  $\text{ClO}_2$  at levels up to 20 mg after 7 days (169 hours). This release is due to both surface reaction of the  $\text{ClO}_2$  reactants located at the contact surface and diffusion of those reactants within the bulk of polymer down their concentration gradient throughout the swollen polymer network.

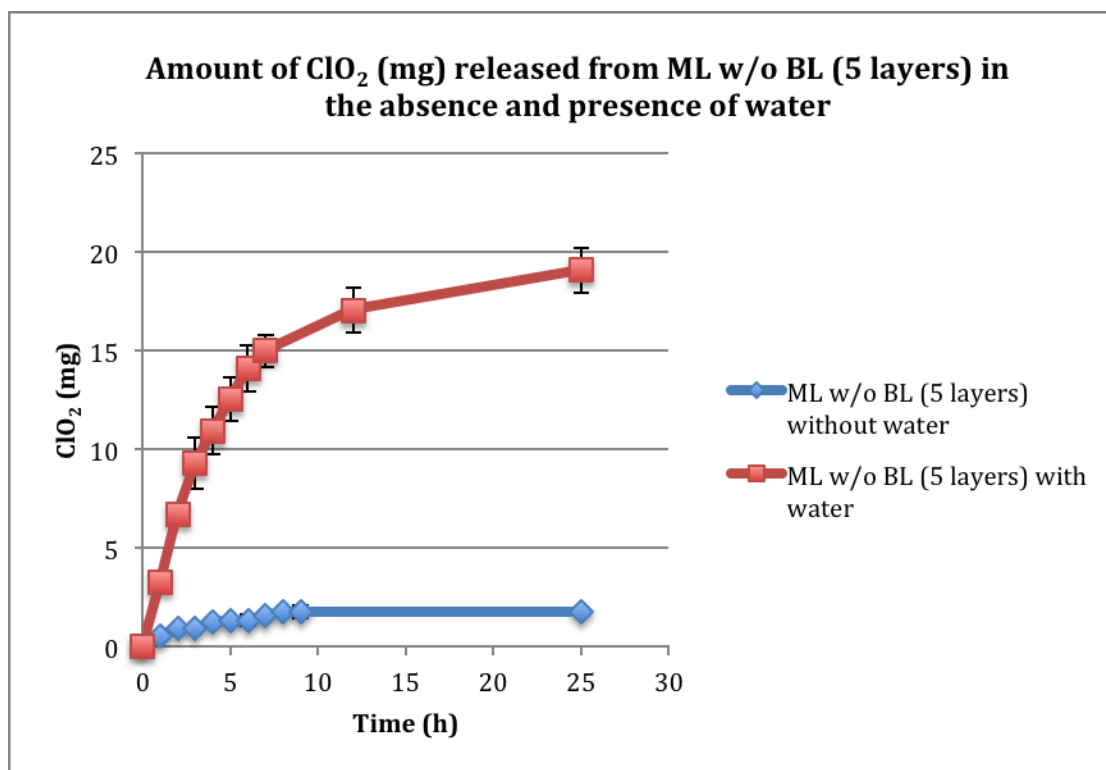


Figure 34. Release of  $\text{ClO}_2$  (mg) from multilayer label without gelatin barrier layers (5 layers) in the absence and presence (high relative humidity condition) of water in the vial, over 25 hours after controlled activation.

The high relative humidity maintained from the presence of water in the vial plays a major role in the release of the bulk of  $\text{ClO}_2$  from this label over time (20 mg of  $\text{ClO}_2$  after 25 hours or 90.8 % of the total  $\text{ClO}_2$  released) (Figure 34). In the absence of water in

the vial, the release of very low amounts of  $\text{ClO}_2$  from the same package label (1.75 mg of  $\text{ClO}_2$  after 25 hours or 9.2 % of the total  $\text{ClO}_2$  released) is attributable to the combined effect of both surface reaction of  $\text{ClO}_2$  precursors from the direct contact between functional layers upon pressing, and the effect of inherent moisture of the gelatin functional layer (Figure 34).

In this package system, the gelatin layer acts as an inherent short-term trigger for  $\text{ClO}_2$  generation and release, due to its high  $a_w$ . Before the moisture release from fresh produce respiration starts taking effect, moisture diffusion from gelatin layer to surrounding pectin layers takes place enabling reaction between  $\text{ClO}_2$  precursors which become more mobile due to swelling of the respective polymeric networks, resulting in  $\text{ClO}_2$  generation and release. If this moisture trigger were the only one acting upon the system,  $\text{ClO}_2$  release from the package label would cease at some point due to inability of all embedded  $\text{ClO}_2$  precursors to diffuse throughout the polymeric network and react together. A sustained source of moisture, supplied by respiration of fresh produce, ensures the continuous replenishment of  $\text{ClO}_2$  at higher concentrations over an extended period of time from the label until the depletion of the reactants from package label.

Controlling the moisture content of gelatin layer by further drying would slow down the generation and release of  $\text{ClO}_2$  from the multilayer package label, the latter being mainly dependent on external moisture source supplied by respiring fresh produce for instance.

There was no noticeable effect of temperature on the release profile of  $\text{ClO}_2$  from multilayer labels stored at 10 and 23°C (Figure 35). Lowering the temperature to 10°C did not affect  $\text{ClO}_2$  generation and release probably due to the low activation energy of the reaction and sufficient mobility of the reactants at this low temperature to collide

effectively and release  $\text{ClO}_2$ .

Gaseous  $\text{ClO}_2$  treatments reported in the literature often times use one-time exposure of this compound at a constant concentration (1-18 mg/L air) maintained over a short period of time (6-120 min) (Gómez-López et al., 2009). This multilayer label however, released  $\text{ClO}_2$  at increasing concentrations over time (controlled release), at concentrations varying from 3.3-20.7 mg/L air over 73 hours considering a 1 L package volume, falling within the range of target  $\text{ClO}_2$  concentration used in the disinfection of fresh produce.

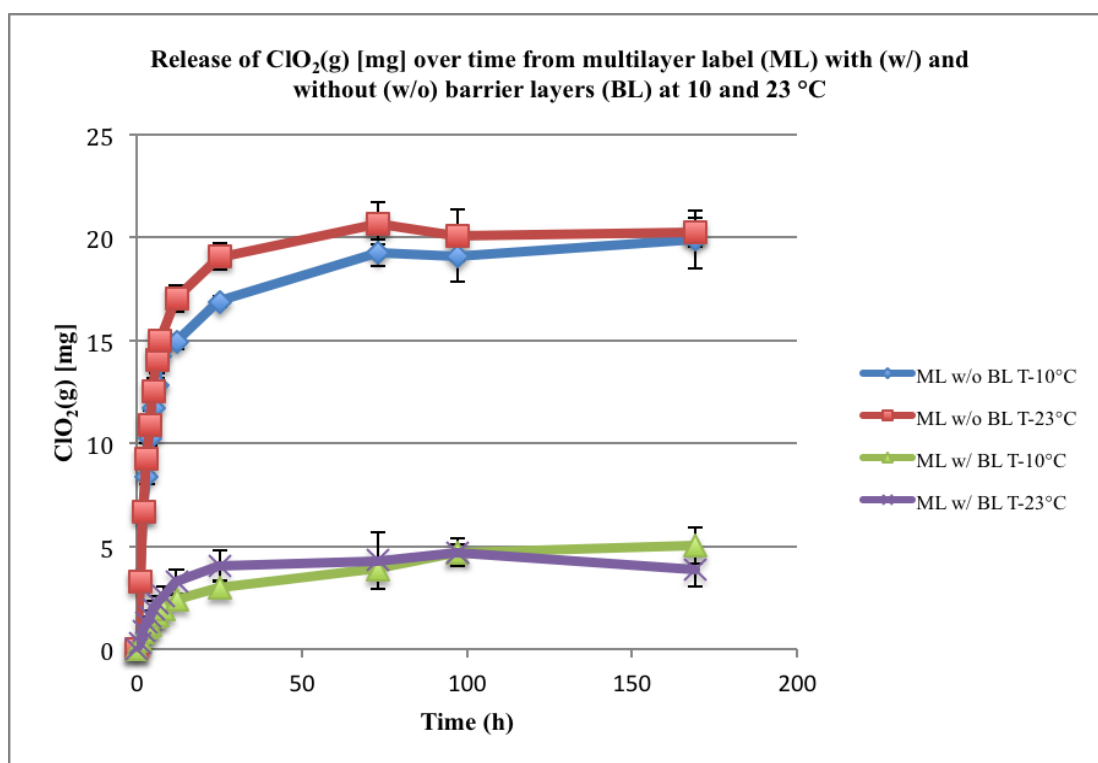


Figure 35. Release of  $\text{ClO}_2$  (mg) from multilayer labels without (5 layers) and with (9 layers) gelatin barrier layers over time after controlled activation at 10 and 23 °C.

#### 9.4.2. Multilayer Labels With Barrier Layers (ML w/ BL)

Despite the introduction of gelatin barrier layer in between the pectin and gelatin

functional layers, the package label still generated and released  $\text{ClO}_2$  with time but at slower rate than label without barrier layers (Figure 35).

$\text{ClO}_2$  generation from this label, unlike in the previous label, was only due to diffusion of  $\text{ClO}_2$  precursors throughout their respective polymeric network, following the swelling of pectin and gelatin matrices from gelatin barrier layer inherent moisture and moisture generated from water in vial.

No surface reaction of  $\text{ClO}_2$  precursors took place in this label due to separation of the functional layers by the gelatin barrier layers.

At first, water diffuses from gelatin barrier layer (70 % moisture content on dry basis and 0.98  $a_w$ ) to upper pectin and lower gelatin functional layers, causing polymer chain relaxation, swelling of these polymeric matrices, and increased mobility of the reactants in these swollen regions.  $\text{H}^+$  diffusion down its concentration gradient towards the underlying gelatin barrier layer and slower  $\text{ClO}_2^-$  down its concentration gradient towards gelatin barrier layers above and below it, enable reaction of these precursors and  $\text{ClO}_2$  generation.

Shortly after, moisture evaporated from water in vial, starts taking effect, causing swelling of pectin functional layer facing the bottom of vial, upward towards the other layers causing further swelling of these hydrophilic matrices.

Once generated,  $\text{ClO}_2$  diffuses through the label and releases out of the label in the headspace of the vial over time.

As can be seen from the graph above (Figure 35), lowering the temperature to  $10^\circ\text{C}$  also did not affect the release profile of  $\text{ClO}_2$ , indicating that even at this lower temperature, there was sufficient thermal energy for many of the reactant molecules to reach the

transition state in a short time and produce  $\text{ClO}_2$ .

Despite the fact that this label had the same loading of citric acid and sodium chlorite (16.5 % and 6.3 % by weight, dry basis respectively) and similar surface area ( $10 \text{ cm}^2$ ) as the multilayer label without barrier layers, the amount of  $\text{ClO}_2$  released (5 mg) was noticeably lower (4 times lower) than that from the label without barrier layers (20 mg of  $\text{ClO}_2$ ), after 169 hours (Figure 35).

This could be attributed to many factors: 1) the introduction of barrier layers adds more organic molecules to the system resulting in higher consumption of  $\text{ClO}_2$  by its reaction with organic molecules; 2) the swollen label entraps higher amount of  $\text{ClO}_2$  which will remain dissolved in water located within spaces of the network preventing it from diffusing throughout the matrix into the headspace, due to its high solubility in water ( $\sim 70 \text{ g/L}$  in water at  $20^\circ\text{C}$  and  $760\text{mmHg}$ ) and its even higher solubility at lower temperature (Henry's law constant and increase in the gas solubility with decreasing temperature) (Mortimer, 1967); 3) poor binding between the different layers constituting the label which becomes more pronounced upon swelling impeding diffusion of the reactants and subsequent generation and release  $\text{ClO}_2$  from this label and; 4) not all of the reactants loaded into the pectin and gelatin functional layers diffuse and react to produce  $\text{ClO}_2$ , for instance, some of the reactants may get involved in side reactions.

Similarly to the multilayer label without barrier layers, this label achieved the controlled release of  $\text{ClO}_2$  at concentrations varying from  $0.3\text{--}4 \text{ mg/L}$  air over 25 hours considering a 1 L package volume, falling within the range of  $\text{ClO}_2$  target concentrations used in the disinfection of fresh produce (Gómez-López et al., 2009).

It is worth noting that the choice of the barrier layer in terms of polymer type (s), polymer

modification (crosslinking), hydrophilicity level, thickness and moisture content play an important role on the release behavior of  $\text{ClO}_2$  from the package label, providing control over the amount and the rate of  $\text{ClO}_2$  released from the label.

The following step consists of evaluating the antimicrobial effectiveness of these labels against *Salmonella* growth to test their efficacy and practical application.

For the sake of simplicity, multilayer label without barrier layers (ML w/o BL) composed of 3 functional layers (pectin: gelatin: pectin) and multilayer label with barrier layers (ML w/ BL) composed of 5 layers: 3 functional layers and 2 barrier layers (pectin: BL: gelatin: BL: pectin) were prepared for the microbial study conducted at 23°C. The new labels released 2.5 times less  $\text{ClO}_2$  amounts, with 8 mg and 2 mg of  $\text{ClO}_2$  released from ML w/o and w / BL respectively, as compared to 20 mg and 5 mg of  $\text{ClO}_2$  released from the original labels, over 100 hours (Figure 36).

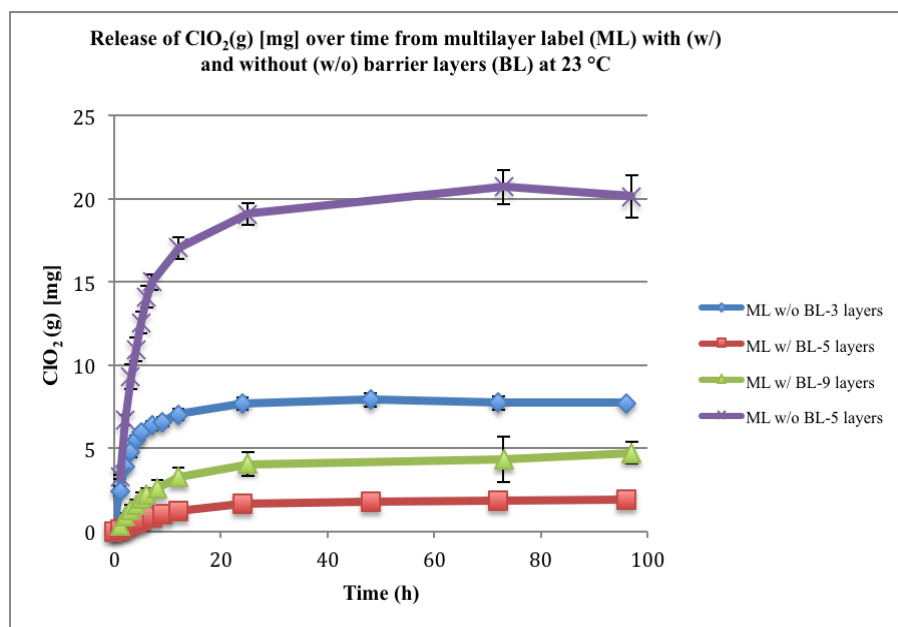


Figure 36. Release of  $\text{ClO}_2$  (mg) from original multilayer labels without (5 layers) and with (9 layers) and new multilayer labels without (3 layers) and with (5 layers) over 96 hours after controlled activation, at 23°C.

## 9.5. Antimicrobial Effectiveness of Biobased Labels

### 9.5.1. Antimicrobial Effectiveness of Labels on *Salmonella* Growth on TSA Plates

Both labels with and without barrier layers inactivated completely *Salmonella* growth on TSA plates regardless of time exposure, demonstrating the antimicrobial effectiveness of these labels in terms of release of ClO<sub>2</sub> at concentrations effective to kill all the microorganisms growing on the TSA plates (Table 8).

Total kill of *Salmonella* was achieved in the first 2-hour exposure of the inoculated TSA plates with ClO<sub>2</sub> releasing labels with and without barrier layers. ClO<sub>2</sub> concentration as low as 1.2 mg/L air was able to achieve 8.1 log reductions of *Salmonella* Montevideo G4639 on TSA plate after 2 hours exposure to the ML w/ BL, indicating the disinfection efficacy of ClO<sub>2</sub> at low concentration (Table 8).

Upon the incubation of *Salmonella* inoculated TSA plates (37°C for 24 hours), TSA offers a nourishing medium for the growth of colonies on the surface of the plate. Upon sustained release of ClO<sub>2</sub> from the label, *Salmonella* cells, spread evenly at the surface of TSA plate, are uniformly exposed to ClO<sub>2</sub> treatment. The lethality of these vegetative bacterial cells is achieved through nonspecific oxidation of their outer membrane resulting in loss of permeability and inhibition of respiration process (Berg et al., 1986).

The results from this experiment constitute a validation of the technical feasibility of the biobased labels in terms of ClO<sub>2</sub> generation and release at concentrations effective to inactivate the growth of *Salmonella* on TSA plates. However, relying on this data to generalize the antimicrobial effectiveness of these labels on fresh produce would be inaccurate since real food product brings an element of complexity to the system that needs to be investigated before general conclusions are made.

Table 8. Effect of ClO<sub>2</sub> concentration released from ML w/o and w/barrier layers on *Salmonella* Montevideo growth (log CFU/mL) on TSA plates.

Label	Sampling Time (h)	Concentration of ClO <sub>2</sub> released (mg/Lair) ± Standard Deviation	log reduction (log CFU/mL) ± Standard Deviation
ML w/o BL	2	15.6 ± 1.2	8.2 ± 0.1
	4	22 ± 1.2	8.2 ± 0.1
	6	24.8 ± 1.2	8.2 ± 0.1
ML w/ BL	2	1.2 ± 0.0	8.1 ± 0.1
	4	2.4 ± 0.4	8.1 ± 0.1
	8	4.0 ± 0.8	8.1 ± 0.1

#### 9.5.2. Antimicrobial Effectiveness of Labels on *Salmonella* Growth on Inoculated Mung Bean Seeds

Whereas the microbial study conducted on TSA plates constituted a validation step of the technical feasibility of the biobased labels with regard to generation and release of ClO<sub>2</sub> in a controlled manner that was quantified by the inactivation level of *Salmonella* on these plates, testing these labels on real food products would shed some light on their practical application in terms of their antimicrobial efficacy and useful application in commercially available primary packages (clamshell, bags, etc.).



The target concentration of  $\text{ClO}_2$  specific to fresh produce commodities is defined as the concentration of  $\text{ClO}_2$  needed to achieve total or partial inhibition of microorganism of interest on a specific fresh produce without compromising its quality attributes.

Target concentration could vary with time (different than single exposure) and is dependent on 1) the release kinetics of labels, a function of label design (polymer type, loading, surface area and thickness), 2) the volume of package, and 3) temperature (Arrhenius kinetics).

In order to relate technical feasibility to practical feasibility aspect of these labels, mung bean seeds were selected as the commodity.

The surface of mung bean seeds was artificially inoculated with *Salmonella* and treated with  $\text{ClO}_2$  releasing labels without and with barrier layers over time to evaluate the disinfection efficacy of these labels on the growth of *Salmonella*.

Whereas the multilayer label without barrier layers achieved 1.6-1.7 log reductions of *Salmonella* on the mung bean seeds after 2, 4 and 6 hour exposure to  $\text{ClO}_2$ , multilayer label with barrier layers achieved between 1.9-2 log reductions of *Salmonella* on these seeds after 2, 4 and 8 hour exposure to  $\text{ClO}_2$ . The only significant difference between both labels with regard to *Salmonella* inactivation was observed at 4-hour exposure to  $\text{ClO}_2$  (1.6-log reductions achieved by ML w/o BL versus 2-log reductions achieved by ML w/ barrier layers) (Table 9).

Table 9. Effect of ClO<sub>2</sub> concentration released from ML w/o and w/barrier layers on *Salmonella* Montevideo Population (log CFU/g) on Mung Bean Seeds.

Label	Sampling Time (h)	Concentration of ClO <sub>2</sub> released (mg/Lair) ± Standard Deviation	log reduction (log CFU/g) ± Standard Deviation
ML w/o BL	2	15.6 ± 1.2	1.7 ± 0.3 <sup>a</sup>
	4	22 ± 1.2	1.6 ± 0.2 <sup>a</sup>
	6	24.8 ± 1.2	1.7 ± 0.3 <sup>a</sup>
ML w/ BL	2	1.2 ± 0.0	1.9 ± 0.2 <sup>a</sup>
	4	2.4 ± 0.4	2.0 ± 0.2 <sup>b</sup>
	8	4.0 ± 0.8	2.0 ± 0.3 <sup>a</sup>

The control (without exposure to package label) had initial count of 5.2 log CFU/g. For every exposure time, data points with different letter superscripts were significantly different ( $p < 0.05$ ).

These results were different from the ones obtained in the previous experiment performed on TSA plates, where 8 log reductions of *Salmonella* were achieved after 2-hour exposure of plates with both ClO<sub>2</sub> releasing labels (Table 8).

Mung bean seeds possess different surface structure and morphology than the smooth surface of TSA medium exposing all *Salmonella* cells growing at the surface to ClO<sub>2</sub> treatment. The surface of these seeds directly exposed to ClO<sub>2</sub> release from labels was mostly affected by the treatment; the other inaccessible surfaces were harder to reach

with minimal lethal effect observed on *Salmonella* populations.

The arrangement of the mung bean seeds in two layers at the bottom of the jar also affected the treatment efficacy due to nonuniform inactivation level of *Salmonella* with higher inactivation occurring at the top layer rather than the bottom one (Figure 19).

Moreover, mung bean seeds act differently than fresh produce in that respiration rate of these seeds at the dormant stage occurs at a very slow rate as compared to germination stage where high-energy needs of germinating beans significantly increase rates of cellular respiration and moisture release (Bewley & Black, 1994). Consequently, the bulk of ClO<sub>2</sub> released from both labels was predominantly derived from inherent moisture content of gelatin layers, the moisture released from respiration of mung bean seeds being negligible. In the previous experiment conducted on TSA plates, the release of ClO<sub>2</sub> from both labels was sustained by constant high relative humidity established in the headspace of the jar maintained by water in the vial.

It is worth noting that mung bean seeds offer larger surface area than that provided by TSA plates, resulting in higher levels of ClO<sub>2</sub> consumption from its reaction with organic molecules of these seeds.

The slightly higher inactivation level of *Salmonella* achieved by ML w/ BL (2 log reductions as compared to 1.7 log reductions after 8 hours exposure) is attributed to the moisture supply provided by the extra gelatin barrier layers sustaining the release of ClO<sub>2</sub> over a relatively longer period of time. The ML w/o BL is expected to generate and release ClO<sub>2</sub> at lower concentrations over a shorter period of time, being dependent only on the moisture of the gelatin functional layer, insufficient to ensure controlled release of ClO<sub>2</sub> especially that the relative humidity condition in the treatment jar is maintained low

by low respiring mung bean seeds.

The results of the microbial study conducted on *Salmonella* Montevideo inoculated mung bean seeds exposed to ML w/o and w/ BL were within the range of inactivation levels of *Salmonella enterica* serotypes achieved by Sy et al. (2005) on blueberries, strawberries and raspberries exposed to TriNova ClO<sub>2</sub> releasing sachet (0 mg/L at 0 min, 4.1 mg/L at 30 min, 6.2 mg/L at 60 min and 8 mg/L at 120 min)(Sy, McWatters, et al., 2005).

Whereas 2 log-reductions of *Salmonella* were achieved after 30-min treatment of blueberries and strawberries to ClO<sub>2</sub> at exposure level of 4 mg/L air, similar reduction in *Salmonella* population was achieved on the surface of mung bean seeds after 2-hour treatment with ML w/ BL generating less than 1.2 mg/ L air. ML w/o BL achieved 1.7 log reduction of *Salmonella* after 2 hours exposure (concentration of ClO<sub>2</sub> much less than 15.6 mg/L) close to 1.5 log reduction achieved on raspberries after 120 min exposure to the sachet releasing 8 mg/L of ClO<sub>2</sub>. Higher reductions in *Salmonella* populations of 3.7 to 4.4 log CFU/g in blueberries and strawberries respectively were achieved after 120 min release of ClO<sub>2</sub> (8mg/L air) from sachet (Sy, McWatters, et al., 2005), whereas in the current releasing system only 2 log CFU/g reduction of *Salmonella* on the surface of mung bean seeds were achieved after 8 hours treatment to ML w/ BL ([ClO<sub>2</sub>] less than 4 mg/L air).

Difference in these results is primarily attributed to fast release of higher concentrations of ClO<sub>2</sub> over time by the sachet system (up to 8 mg/L) as compared to current labels releasing low concentrations of ClO<sub>2</sub> (< 4 mg/L air) over 8 hours. Also, differences in experimental conditions such as placement of fruits in a single layer instead of multiple layers on the shelf to maximize surface exposure to ClO<sub>2</sub>, the use of a cooling fan to

allow more uniform air and ClO<sub>2</sub> circulation inside the treatment cabinet, and constant high relative humidity (75-90%) environment in the cabinet to maintain the release of ClO<sub>2</sub> from the sachet system (Sy, McWatters, et al., 2005) explain lower inactivation level of *Salmonella* reported in this study.

Work conducted by Prodduk et al. 2014 on mung bean sprouts artificially inoculated with a cocktail of *Salmonella enterica* exposed to 0.5 mg/L air of ClO<sub>2</sub> for 15, 30 and 60 min reduced *Salmonella* populations by 3, 3 and 4 log CFU/g without mechanical mixing, respectively (Prodduk et al., 2014). These results were better than the ones reported in this research since a low concentration of ClO<sub>2</sub> was able to achieve a better kill of the microorganisms than the labels under study.

The lower log-reduction of *Salmonella* populations obtained in this research after treatment of mung bean seeds with multilayer labels is mainly due to 1) the very low respiration rate of mung bean seeds with low final relative humidity in the jar's headspace influencing the releasing ability of labels over time, 2) lower concentrations of ClO<sub>2</sub> generated and released from these labels over a shorter period of time than those reported by the release kinetics study, due to low relative humidity condition in the treatment jar, 3) experimental conditions where mung bean seeds stacked in ~2 layers at the bottom of the jar lessened the antimicrobial effectiveness of the treatment with less seed surface exposed to ClO<sub>2</sub> treatment and higher survivability of bacteria hidden and protected from lethality of the treatment, and 4) different fresh produce commodities exhibit various surface structures, morphology and porosity resulting in differences in the protection level of bacterial cells against ClO<sub>2</sub> exposure and therefore difference in overall biocidal efficacy of ClO<sub>2</sub>.

The selection of low respiring mung bean seeds to test the biocidal efficacy of the moisture dependent labels was not suitable for the intended application of these labels and explains the low inhibition levels of *Salmonella* populations obtained after 6-8 hour exposure to ClO<sub>2</sub>, due to lower concentrations of ClO<sub>2</sub> generated and released from these labels over a shorter period of time than those reported by the release kinetics study.

Using respiring fresh produce instead of very slow respiring seeds or beans would provide a better understanding of the antimicrobial effectiveness of these labels, especially that sustained release of ClO<sub>2</sub> from these labels is dependent on moisture produced from fresh produce during respiration. Also, optimizing the label design to release higher concentrations of ClO<sub>2</sub> in the first two hours of treatment could potentiate the biocidal efficacy of these labels as was reported by others in literature (Gómez-López et al., 2009).

## 10. CONCLUSIONS

The technical feasibility of this package label depends on the ability to design and manufacture this label and have it generate and release  $\text{ClO}_2$  in a controlled manner after controlled activation at levels adequate for microbial inactivation. The findings of both the release kinetics and microbial study are in good agreement and support the technical feasibility of package labels (synthetic and biobased) in terms of label design and manufacture,  $\text{ClO}_2$  generation and release after controlled activation, and antimicrobial effectiveness against *Salmonella* growth on TSA plates and in some cases, on mung bean seeds.

Label manufacture from the processing standpoint was feasible for both synthetic and biobased labels, after undergoing several iterations during the conceptualization stage of label design to meet the objective of this research. Among the three polymers selected for synthetic label manufacture, only EVA withstood extrusion processing and storage conditions. Two multilayer biobased labels made with pectin and gelatin, were successfully manufactured, one without gelatin barrier layers and one with gelatin barrier layers to control the release of  $\text{ClO}_2$  over time.

All EVA labels generated and released  $\text{ClO}_2$  over time after controlled activation (spraying of sodium chlorite solution followed by heat-pressing). These labels showed a burst release of  $\text{ClO}_2$  mainly due to surface reaction of citric acid crystals/aggregates located at the surface of these labels. Citric acid crystals/aggregates located within the bulk of the polymer were inaccessible for reaction due to hydrophobic nature of EVA. As to their antimicrobial effectiveness, labels with higher citric acid loading (15%) achieved higher inactivation level of *Salmonella* Montevideo G4639 on TSA plates after 6 hours

(2.1 to 2.3 log reductions). EVA labels with lower citric acid loading (7.5%) achieved between 0.6-1.3 log reductions of *Salmonella* on TSA plates after 6 hours exposure.

The biobased labels, multilayer labels without and with barrier layers generated two release profiles for  $\text{ClO}_2$  after controlled activation (pressure only), a faster release profile observed with labels without barrier layers and a slower release profile obtained from the introduction of barrier layers, at high relative humidity conditions. Whereas both labels achieved around 8 log reductions of *Salmonella* on TSA plates, up to 2 log reductions of *Salmonella* on inoculated mung bean seeds were achieved after 6 hour exposure to  $\text{ClO}_2$  released from multilayer label without barrier layers and 8 hour exposure to  $\text{ClO}_2$  released from multilayer label with barrier layers.

The biobased labels were also feasible from practical aspect. The target concentration of  $\text{ClO}_2$  specific to fresh produce commodities is defined as the concentration of  $\text{ClO}_2$  needed to achieve total or partial inhibition of microorganism of interest on a specific fresh produce without compromising its quality attributes. Target concentration could vary with time (different than single exposure) and is dependent on 1) the release kinetics of labels, a function of label design (polymer type, loading, surface area and thickness), 2) the volume of package, and 3) temperature (Arrhenius kinetics). Both multilayer labels without and with barrier layers generated and released  $\text{ClO}_2$  at concentrations within the target concentration range reported in the literature for the disinfection of fresh produce, mostly applied as a single exposure treatment (0.5-18 mg/L air)(Gómez-López et al., 2009). The antimicrobial efficacy of both labels on *Salmonella* populations (1.7 to 2 log reduction after 6 and 8 hour treatment respectively) on the surface of mung bean seeds was within the *Salmonella* inactivation range reported by



others, 1.54-5.15 log reduction as a function of treatment: one-time application of  $\text{ClO}_2$  spanning treatment time from 6 min to 120 min depending on fresh produce and target microorganism. The advantage of the current labels is the sustained release of  $\text{ClO}_2$  they can achieve over time, as opposed to one-time exposure to this compound. These labels could be further optimized to release the target concentration of  $\text{ClO}_2$  through different release profiles suitable to specific fresh produce and microorganism of interest.

## 11. FUTURE WORK

Optimization of both synthetic and biobased labels should start from determination of target concentration range of  $\text{ClO}_2$  effective to inactivate microorganism of interest (pathogenic or spoilage) on a specific fresh produce to improve its safety without compromising quality parameters.

### 11.1. Synthetic Labels

The hydrophobic nature of polymers used, particle size of citric acid selected (crystalline form) as well as citric acid non-uniform dispersion at the surface and bulk of polymer presented the synthetic labels with limitations as to label manufacture and  $\text{ClO}_2$  release.

Out of the three polymers selected, only EVA polymer withstood extrusion and storage conditions. After activation of the EVA labels incorporated with citric acid (spraying with sodium chlorite concentration followed by heat and pressure),  $\text{ClO}_2$  burst release from rapid surface reaction of citric acid scattered at the surface of the label was observed. There was large sample variability among replicates of the same sample due to uneven dispersion of citric acid at the surface and in the bulk of the polymer.

Possible options to improve the uniform dispersion of citric acid on the surface and in the bulk of the polymer matrix, and releasing ability of these labels would be to use different synthetic polymers (hydrophilic polymers) or a blend of synthetic polymers, reduce citric acid particle size (surface area), modify loading of citric acid and try to achieve uniform distribution of citric acid in polymer through compounding, proper mixing prior to feeding and extrusion.

Most of synthetic hydrophilic polymers possess high melting temperatures. At such high temperatures, citric acid would melt and adversely react with polymers during extrusion causing breakdown of their molecular structure and inability to form a cohesive film. The degradation process being further enhanced by large surface area of citric acid particles (larger surface area available for chemical reaction).

Synthetic labels manufactured in this research were able to generate and release  $\text{ClO}_2$  capable of achieving up to 2.3-log reductions of *Salmonella* on TSA plates.

Optimizing the existing EVA labels to release  $\text{ClO}_2$  at concentrations effective to inactivate target microorganism on fresh produce may be possible by achieving uniform distribution of citric acid on the surface of these labels through proper compounding and extrusion, increasing citric acid concentration at the surface of such labels, using citric acid with larger surface area (fine particles), and reducing the overall thickness since the release from these labels is only due to surface effect. Testing these labels on fresh produce should be performed to evaluate the matching between  $\text{ClO}_2$  release kinetics and microbial kinetics on fresh produce.

Also, optimizing the design and manufacture of synthetic label as well as manipulating the activation mechanism could result in the release of  $\text{ClO}_2$  at concentrations effective to inactivate target microorganisms on specific fresh produce.

Optimization of the label design and manufacture would consist of selecting appropriate polymer (s) (with extrusion processing conditions lower than the melting point of citric acid to prevent polymer degradation), citric acid particle size or surface area (powder versus crystalline) and loading, surface area and thickness of label as well as improving the processing steps (compounding and extrusion) to achieve even

distribution of citric acid on the surface and within the bulk of polymer to obtain the desired release profile.

Activation mechanism can be manipulated to control the release rate of  $\text{ClO}_2$  from labels. Activation could be performed in different ways, by the application of radiation, infrared, heat, pressure or a combination of thereof.

After optimization step, the label could be applied to the inside of a package system and allowed to release  $\text{ClO}_2$  in the headspace to act against microorganisms or used as a coating layer lining a substrate and allowed to release  $\text{ClO}_2$  from the surface, after controlled activation, at concentrations adequate to inactivate microorganisms on the surface of specific fresh produce or other food products.

Testing the antimicrobial effectiveness of the optimized labels on fresh produce would provide useful information on its practical application in commercially available package of existing fresh produce.

## **11.2. Biobased Labels**

- Adhesion of the layers composing the label needs to be improved in order to ensure proper contact between the consecutive layers to maximize  $\text{ClO}_2$  release, especially in the case of the multilayer labels with barrier layers.
- Performing  $\text{ClO}_2$  release kinetics experiment from biobased labels with barrier layers in the absence of water is necessary to determine the contribution of the inherent moisture of the gelatin barrier layers to the total concentration of  $\text{ClO}_2$  released from these labels as compared to that released from the same labels in the presence of water.

- Introduction of barrier layers adds organic molecules to the system resulting in higher consumption of  $\text{ClO}_2$  by its reaction with organic compounds. Performing an experiment to quantify the concentration of  $\text{ClO}_2$  released from labels without and with barrier layers in the absence and presence of barrier layers at the same relative humidity as that achieved by moisture released from respiration of fresh produce is needed to elucidate the effect of barrier layer addition into the system.
- Applying labels on respiring fresh produce instead of low respiring mung bean seeds and performing  $\text{ClO}_2$  release kinetics experiment as well as microbial study; the sustained release of  $\text{ClO}_2$  is maintained by constant high relative humidity from respiration of fresh produce.
- Performing release kinetics analysis of these labels in the absence of water would be helpful to correlate the results of the mung bean seeds microbial study with the actual concentration of  $\text{ClO}_2$  released from the labels over time.
- Conducting a release kinetics experiment with and without fresh produce to quantify the amount of  $\text{ClO}_2$  consumed by fresh produce organic matter would provide useful information on the effective concentration of  $\text{ClO}_2$  and its antimicrobial activity.
- Testing the antimicrobial effectiveness of labels on fresh produce contained in commercially available packages (different package volumes).
- Testing labels structural stability over time to study the effect of  $\text{ClO}_2$  precursors incorporation into the label
- Testing the effect of  $\text{ClO}_2$  released from these labels on the quality attributes (color, texture, taste, etc.) of the target food product

All experiments were conducted in amber vials and jars. Quantifying ClO<sub>2</sub> losses at conditions similar to those used at the industry (exposure to light) would provide information with regard to the effective ClO<sub>2</sub> concentration used to inactivate target microorganism on the specific fresh produce without compromising its quality attributes.

- Developing of biobased labels with practical application based on type of fresh produce, target microorganism, package volume and temperature can be performed through optimization of biobased labels.
- The choice of polymers constituting the different layers of the label, properties of barrier layer (polymer, crosslinking, thickness, porosity, moisture content, etc.), form and loading of ClO<sub>2</sub> precursors, activation mechanism, package label size (surface area and thickness) and design of label could be manipulated to generate an array of ClO<sub>2</sub> release profiles that could be applied to a wider range of fresh produce, and easily tailored for use in different package volumes.

## 12. REFERENCES

- [CFR], & Regulations, C. o. F. (2000c). *Secondary Direct Food Additives Permitted in Food for Human Consumption: Chlorine dioxide.*: Retrieved from <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=173.300>.
- Aieta, E. M., & Berg, J. D. (1986). Review of chlorine dioxide in drinking water treatment. *Journal of the American Water Works Association*, 78(6), 67-72.
- Almeida, A., Possemiers, S., Boone, M. N., De Beer, T., Quinten, T., Van Hoorebeke, L., Remon, J. P., & Vervaet, C. (2011). Ethylene vinyl acetate as matrix for oral sustained release dosage forms produced via hot-melt extrusion. *European Journal of Pharmaceutics and Biopharmaceutics*, 77(2), 297-305. doi: 10.1016/j.ejpb.2010.12.004
- Andrews, R. C., & Ferguson, M. J. (1996). *Disinfection By-Products in Water Treatment: The Chemistry of Their Formation and Control*. Boca Raton, FL: CRC Press, Inc.
- Axelos, M. A. V., & Thibault, J. F. (1991). CHAPTER 6 - The Chemistry of Low-Methoxyl Pectin Gelation. In R. H. Walter (Ed.), *The Chemistry and Technology of Pectin* (pp. 109-118). San Diego: Academic Press.
- Berg, J. D., Roberts, P. V., & Matin, A. (1986). Effect of chlorine dioxide on selected membrane functions of *Escherichia coli*. *Journal of Applied Bacteriology*, 60(3), 213-220. doi: 10.1111/j.1365-2672.1986.tb01075.x
- Bernarde, M. A., Israel, B. M., Olivieri, V. P., & Granstrom, M. L. (1976a). Kinetics and Mechanism of Bacterial Disinfection by Chlorine Dioxide. *J. Appl. Microbiol.*, 15(2), 257.
- Beuchat, L. R. (1992). Surface disinfection of raw produce. *Dairy, Food, and Environmental Sanitation*, 12, 6-9.
- Beuchat, L. R. (1999). Survival of enterohemorrhagic *Escherichia coli* O157:H7 in bovine feces applied to lettuce and the effectiveness of chlorinated water as a disinfectant. *Journal of Food Protection*, 62(8), 845-849.
- Beuchat, L. R., & Ryu, J. H. (1997). Produce Handling and Processing Practices. *Emerging Infectious Diseases.*, 3(4), 459-465.
- Beuchat, L. R., Ward, T. E., & Pettigrew, C. A. (2001). Comparison of Chlorine and a Prototype Produce Wash Product for Effectiveness in Killing *Salmonella* and *Escherichia coli* O157:H7 on Alfalfa Seeds. *Journal of Food Protection*, 64(2), 152-158.
- Bewley, J. D., & Black, M. (1994). *Seeds*: Springer.
- Bogue, R. H. (1922). *The Chemistry and Technology of Gelatin and Glue*: McGraw-Hill book Company, Incorporated.

- Bosman, J. (2002). Outline of polyurethane chemistry. In D. Randall & S. Lee (Eds.), *The polyurethanes book*. (pp. 113-126): Distributed by J. Wiley.
- Caetano-Anollés, G., Favelukes, G., & Bauer, W. D. (1990). Optimization of Surface Sterilization for Legume Seed. *Crop Sci.*, 30(3), 708-712. doi: 10.2135/cropsci1990.0011183X003000030047x
- Cherry, J. P. (1999). Improving the safety of fresh produce with antimicrobials. *Food Technology*, 53, 54-59.
- Choffnes, E. R., Relman, D. A., Olsen, L., Hutton, R., & Mack, A. (2012). *Improving Food Safety Through a One Health Approach: Workshop Summary*. Washington, DC: The National Academies Press.
- Chung, C. I. (2000). *Extrusion of Polymers: Theory and Practice*: Hanser Publishers.
- Damodaran, S., Parkin, K. L., & Fennema, O. R. (2008). Fennema's food chemistry. In S. Damodaran, K. L. Parkin & O. R. Fennema (Eds.), (4th ed. ed., pp. 1144). Boca Raton :: CRC Press/Taylor & Francis.
- Deshwal, B. R., & Lee, H. K. (2005). Manufacture of Chlorine Dioxide from Sodium Chlorite: Process Chemistry. *Journal of Industrial and Engineering Chemistry*, 11(1), 125-136.
- The Dietary Guidelines for Americans. (2010): United States Department of Agriculture (USDA).
- Eckenfelder, W. W., Bowers, A. R., & Roth, J. A. (1992). *Chemical Oxidation-Technologies for the Nineties*. Lancaster, Pennsylvania 17064 U.S.A.: Technomic Publishing Company, Inc.
- Engineers, N. B. C. (2007). *The Complete Book on Adhesives, Glues & Resins Technology*: NIIR Project Consultancy Services.
- EPA. (1999). *Alternative disinfectants and oxidants guidance manual*: U.S. Environmental Protection Agency, Office of Water.
- Escudero, M. E., Velazquez, L., Di Genaro, M. S., & de Guzman, A. M. S. (1999). Effectiveness of various disinfectants in the elimination of *Yersinia enterocolitica* on fresh lettuce. *Journal of Food Protection*, 62(6), 665-669.
- Farris, S., Schaich, K. M., Cooke, P., Liu, L., Piergiovanni, L., & Yam, K. (2011). Gelatin-pectin composite films from polyion complex hydrogels. *Food hydrocolloids*, 25(1), 61 - 70. doi: doi:10.1016/j.foodhyd.2010.05.006
- FDA. (2001). *Agency Response Letter GRAS Notice No. GRN 000062*.
- FDA. (2005). Secondary Direct Food Additives Permitted in Food for Human Consumption. Retrieved August 10, 2012, from <http://www.fda.gov/ohrms/dockets/98fr/05-2808.htm>
- Feng, P. (1997). A summary of background information and foodborne illness associated with the consumption of sprouts.: U. S. Food and Drug Administration. Center for Food Safety and Applied Nutrition.



- Ghandbari, E. H., & al., e. (1983). Reactions of Chlorine and Chlorine Dioxide with Free Fatty Acids, Fatty Acid Esters, and Triglycerides. In e. a. R. L. Jolley (Ed.), *Water Chlorination: Environmental Impact and Health Effects*. Lewis, Chelsea, MI.
- Gilsenan, P. M., Richardson, R. K., & Morris, E. R. (2000). Thermally reversible acid-induced gelation of low-methoxy pectin. *Carbohydrate Polymers*, 41(4), 339-349. doi: [http://dx.doi.org/10.1016/S0144-8617\(99\)00119-8](http://dx.doi.org/10.1016/S0144-8617(99)00119-8)
- Gomez-Lopez, V., Ragaert, P., Debevere, J., & Devlieghere, F. (2008). Decontamination Methods to Prolong the Shelf-life of Minimally Processed Vegetables, State-of-the-art. *Critical Reviews in Food Science and Nutrition*, 48(6), 487-495. doi: 10.1080/10408390701638878
- Gómez-López, V. M., Rajkovic, A., Ragaert, P., Smigic, N., & Devlieghere, F. (2009). Chlorine dioxide for minimally processed produce preservation: a review. *Trends in Food Science & Technology*, 20(1), 17-26. doi: 10.1016/j.tifs.2008.09.005
- Gordon, G., Kieffer, R. G., & Rosenblatt, D. H. (1972). The Chemistry of Chlorine Dioxide *Progress in Inorganic Chemistry* (pp. 201-286): John Wiley & Sons, Inc.
- Han, Y., Floros, J. D., Linton, R. H., Nielsen, S. S., & Nelson, P. E. (2001). Response surface modeling for the inactivation of escherichia coli O157:H7 on green peppers (*Capsicum annuum* L.) by chlorine dioxide gas treatments. *Journal of Food Protection*, 64(8), 1128-1133.
- Han, Y., Sherman, D. M., Linton, R. H., Nielsen, S. S., & Nelson, P. E. (2000). The effects of washing and chlorine dioxide gas on survival and attachment of *Escherichia coli* O157: H7 to green pepper surfaces. *Food Microbiology*, 17(5), 521-533. doi: <http://dx.doi.org/10.1006/fmic.2000.0343>
- Harris, L. J., Farber, J. N., Beuchat, L. R., Parish, M. E., Suslow, T. V., Garrett, E. H., & Busta, F. F. (2003). Outbreaks Associated with Fresh Produce: Incidence, Growth, and Survival of Pathogens in Fresh and Fresh-Cut Produce. *Comprehensive Reviews in Food Science and Food Safety*, 2, 78-141. doi: 10.1111/j.1541-4337.2003.tb00031.x
- Harris, P. (1990). *Food Gels*: Elsevier Applied Science.
- Henderson, A. M. (1993). Ethylene-vinyl acetate (EVA) copolymers: a general review. *Electrical Insulation Magazine, IEEE*, 9(1), 30-38. doi: 10.1109/57.249923
- Hoigné, J., & Bader, H. (1994). Kinetics of reactions of chlorine dioxide (OCIO) in water—I. Rate constants for inorganic and organic compounds. *Water Research*, 28(1), 45-55. doi: 10.1016/0043-1354(94)90118-x
- Jeng, D. K., & G., W. A. (1990a). Chlorine dioxide sterilization under square-wave conditions. *Appl Environ Microbiol*, 56, 514-519.
- Jenkins, M. J., & Harrison, K. L. (2006). The effect of molecular weight on the crystallization kinetics of polycaprolactone. *Polymers for Advanced Technologies*, 17(6), 474-478. doi: 10.1002/pat.733

- Jin, H. H., & Lee, S. Y. (2007). Combined effect of aqueous chlorine dioxide and modified atmosphere packaging on inhibiting *Salmonella typhimurium* and *Listeria monocytogenes* in mungbean sprouts. *Journal of Food Science*, 72(9), M441-M445.
- Kazcur, J. J., & Cawfield, D. W. (1992). Chlorine oxygen acids and salts. *Kirk-Othmer encyclopedia of chemical technology* (4th ed., Vol. 5, pp. 968-997). New York: John Wiley and Sons.
- Kieffer, R. G., & Gordon, G. (1968). Disproportionation of chlorous acid. II. Kinetics. *Inorganic Chemistry*, 7(2), 239-244. doi: 10.1021/ic50060a014
- LeChevallier, M. W., Norton, W. D., & Atherholt, T. B. (1997). Protozoa in open reservoirs. *J.Am. Water Works Assoc.*, 89(9), 84-96.
- Ledward, D. A. (2000). Gelatin In G. O. Phillips & P. A. Williams (Eds.), *Handbook of Hydrocolloids* (pp. 67-86): CRC Press.
- Lee, S. (1998). *Thermoplastic Polyurethane Markets in the EU: Production, Technology, Applications and Trends : a Report from Rapra's Industry Analysis and Publishing Group*: Rapra Technology.
- Leifheit, D., & Hutchings, R. (1992). U.S. Patent No. 5126070.
- Li, G.-H., Shi, Y.-H., Liu, H., & Le, G.-W. (2006). Antihypertensive effect of alcalase generated mung bean protein hydrolysates in spontaneously hypertensive rats. *European Food Research and Technology*, 222(5-6), 733-736. doi: 10.1007/s00217-005-0147-2
- Mackey, P. (2002). Introduction to elastomers. In D. Randall & S. Lee (Eds.), *The polyurethanes book*. (pp. 285-299): Distributed by J. Wiley.
- Mahovic, M. J., Tenney, J. D., & Bartz, J. A. (2007). Applications of chlorine dioxide gas for control of bacterial soft rot in tomatoes. *Plant Disease*, 91(10), 1316-1320.
- Marzouk, Y., & Gutman, H. (1995). Israel Patent No. 5399288.
- Masschelein, W. J. (1979). *Chlorine Dioxide-Chemistry and Environmental Impact of Oxychlorine Compounds* (R. G. Rice Ed.). 230 Collingwood, P.O. Box 1425, Ann Arbor, Michigan 48106: Ann Arbor Science Publishers, Inc.
- Mortimer, C. E. (1967). *Chemistry-A Conceptual Approach* Reinhold Publishing Corporation.
- Nielson, A. H., & Woltz, P. J. H. (1952). Infrared spectrum of chlorine dioxide. *Journal of Physical Chemistry*, 20, 1879-1883.
- Oertel, G., & Abele, L. (1994). *Polyurethane Handbook: Chemistry, Raw Materials, Processing, Application, Properties*: Hanser.
- Olivieri, V. P., & al., e. (1985). Mode of Action of Chlorine Dioxide on Selected Viruses. In e. a. R. L. Jolley (Ed.), *Water Chlorination: Environmental Impact and Health Effects*. Lewis, Chelsea, MI.

- OSHA. (1996). Occupational Safety and Health Guideline for Chlorine Dioxide. Retrieved 12 September, 2012, from <http://www.osha.gov/SLTC/healthguidelines/chlorinedioxide/recognition.html>
- Painter, J. A., Hoekstra, R. M., Ayers, T., Tauxe, R. V., Braden, C. R., Angulo, F. J., & Griffin, P. M. (2013). Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998-2008. *Emerging Infectious Diseases.*, 19(3), 407-415.
- Pao, S., Kelsey, D. F., Khalid, M. F., & Ettinger, M. R. (2007). Using aqueous chlorine dioxide to prevent contamination of tomatoes with *Salmonella enterica* and *Erwinia carotovora* during fruit washing. *Journal of Food Protection*, 70(3), 629-634.
- Parish, M. E., Beuchat, L. R., Suslow, T. V., Harris, L. J., Garrett, E. H., Farber, J. N., & Busta, F. F. (2003). Methods to Reduce/Eliminate Pathogens from Fresh and Fresh-Cut Produce. *Comprehensive Reviews in Food Science and Food Safety*, 2, 161-173. doi: 10.1111/j.1541-4337.2003.tb00033.x
- Peñas, E., Gómez, R., Frías, J., & Vidal-Valverde, C. (2010). Effects of combined treatments of high pressure, temperature and antimicrobial products on germination of mung bean seeds and microbial quality of sprouts. *Food Control*, 21(1), 82-88. doi: <http://dx.doi.org/10.1016/j.foodcont.2009.04.008>
- Pitt, C. G. (1990). Poly-ε-Caprolactone and Its Copolymers. In M. Chasin (Ed.), *Biodegradable Polymers as Drug Delivery Systems* (Vol. 45, pp. 71-120): Taylor and Francis.
- Pitt, C. G., Chasalow, F. I., Hibionada, Y. M., Klimas, D. M., & Schindler, A. (1981). Aliphatic polyesters. I. The degradation of poly(ε-caprolactone) in vivo. *Journal of Applied Polymer Science*, 26(11), 3779-3787. doi: 10.1002/app.1981.070261124
- Prodduk, V., Annous, B. A., Liu, L., & Yam, K. L. (2014). Evaluation of Chlorine Dioxide Gas Treatment To Inactivate *Salmonella enterica* on Mungbean Sprouts. *Journal of Food Protection*, 77(11), 1876-1881.
- Ray, S., Jin, T., Fan, X., Liu, L., & Yam, K. L. (2013). Development of Chlorine Dioxide Releasing Film and Its Application in Decontaminating Fresh Produce. *Journal of Food Science*, 78(2), M276-M284. doi: 10.1111/1750-3841.12010
- Reina, L. D., Fleming, H. P., & Humphries, E. G. Microbiological Control of Cucumber Hydrocooling Water with Chlorine Dioxide. *Journal of Food Protection*, 58(5), 541-546.
- Rempel, N. (1955). Germany Patent No. 2717237.
- Rulis, A. M. (2001). Agency response letter GRAS notice no. GRN 0 00062
- Sanderson, W. D. (2010). US Patent No. 7666384B2.
- Shibata, T., & Abe, K. (2009). Japan Patent No.

- Siemann, U. (2005). Solvent cast technology – a versatile tool for thin film production *Scattering Methods and the Properties of Polymer Materials* (Vol. 130, pp. 1-14).
- Singh, N., Singh, R. K., Bhunia, A. K., & Stroshine, R. L. (2002). Effect of inoculation and washing methods on the efficacy of different sanitizers against *Escherichia coli* O157:H7 on lettuce. *Food Microbiology*, 19(2-3), 183-193.
- Sy, K. V., McWatters, K. H., & Beuchat, L. R. (2005). Efficacy of gaseous chlorine dioxide as a sanitizer for killing *Salmonella*, yeasts, and molds on blueberries, strawberries, and raspberries. *Journal of Food Protection*, 68(6), 1165-1175.
- Sy, K. V., Murray, M. B., Harrison, M. D., & Beuchat, L. R. (2005). Evaluation of gaseous chlorine dioxide as a sanitizer for killing *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and yeasts and molds on fresh and fresh-cut produce. *Journal of Food Protection*, 68(6), 1176-1187.
- Tarbet, B. (2005). U.S. Patent No.
- Thakur, B. R., Singh, R. K., & Handa, A. K. (1997). Chemistry and uses of pectin--a review. *Crit Rev Food Sci Nutr*, 37(1), 47-73.
- Thapliyal, B. P., & Chandra, R. (1990). Advances in photodegradation and stabilization of polyurethanes. *Progress in Polymer Science*, 15(5), 735-750. doi: 10.1016/0079-6700(90)90010-x
- Thayer, D. W., Rajkowski, K. T., Boyd, G., Cooke, P. H., & Soroka, D. S. (2003). Inactivation of *Escherichia coli* O157:H7 and *Salmonella* by gamma irradiation of alfalfa seed intended for production of food sprouts. *Journal of Food Protection*, 66(2), 175-181.
- Thomas, R. (1979). *Use of ClO<sub>2</sub> in Water Treatment of Fruits and Vegetables*. (GRAS petition 3G0020).
- Weavers, L. K., & Wickramanayake, G. B. (2001). Kinetics of the Inactivation of Microorganisms In S. S. Block (Ed.), *Disinfection, Sterilization, and Preservation* (Fifth Edition ed., pp. 65-78). Philadelphia, PA Lippincott Williams & Wilkins
- Wei, C. I., Huang, T. S., Kim, J. M., Lin, W. F., Tamplin, M. L., & Bartz, J. A. (1995). GROWTH AND SURVIVAL OF *SALMONELLA*-MONTEVIDEO ON TOMATOES AND DISINFECTION WITH CHLORINATED WATER. *Journal of Food Protection*, 58(8), 829-836.
- Weiss, A., & Hammes, W. P. (2003). Thermal Seed Treatment to Improve the Food Safety Status of Sprouts. *Journal of Applied Botany*, 77(5-6), 152-155.
- Wellington, S. T. (1994). 5360609.
- Woodruff, M. A., & Hutmacher, D. W. (2010). The return of a forgotten polymer—Polycaprolactone in the 21st century. *Progress in Polymer Science*, 35(10), 1217-1256. doi: 10.1016/j.progpolymsci.2010.04.002
- Zhang, S., & Farber, J. M. (1996). The effects of various disinfectants against *Listeria monocytogenes* on fresh-cut vegetables. *Food Microbiology*, 13(4), 311-321. doi: <http://dx.doi.org/10.1006/fmic.1996.0037>

Zhu, X. (2008). *Development of Target Release Rate Concept For Controlled Release Packaging*. (Ph.D.), Rutgers University.