TECHNICAL FEASIBILITY STUDY FOR DEVELOPMENT OF CHLORINE DIOXIDE RELEASING PACKAGING SYSTEM AND ITS APPLICATION IN DECONTAMINATING FRESH PRODUCE

By

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ABSTRACT OF THE THESIS

Technical Feasibility Study for Development of Chlorine Dioxide Releasing Packaging System and Its Application in Decontaminating Fresh Produce

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A feasibility study was conducted to develop chlorine dioxide (ClO_2) releasing packaging films for decontaminating fresh produce. Sodium chlorite and citric acid powder were incorporated into polylactic acid (PLA) polymer. Films made with different amount of PLA (100 and 300 mg), percentage of reactant (5-60%), and ratios of sodium chlorite to citric acid (1:2 or 2:1) were prepared using a solvent casting method. The release of ClO_2 from the resultant films was activated by moisture. Increase of reactants in the films produced more ClO_2 while higher PLA content in the films resulted in less release of ClO_2. The ratio of sodium chlorite to citric acid and activation temperature (22°C vs. 10°C) didn't affect the ClO_2 release from the films. Antimicrobial efficacy of ClO_2 released from the films was evaluated using grape tomato as a model food. The results indicate that the films were activated by moisture from tomatoes in the package and the released ClO_2 reduced *Salmonella* spp. and *E. coli* O157:H7 inoculated on the tomatoes to undetectable levels (< 5 CFU/tomato), achieving more than 3 log reduction. The film-treated tomatoes did not show significant changes in color and texture as compared to controls during storage at 10°C for 21 days. This study demonstrated the technical

feasibility for development of gaseous chlorine dioxide releasing packaging system to enhance microbial safety and extend shelf life of fresh produce.

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1. Introduction

1.1 Chlorine dioxide

1.1.1 General overview

In recent years chlorine dioxide has attracted significant attention as a unique oxy-chlorine species that has oxidation and biocidal properties. It is a small, volatile, neutral water soluble gas molecule that dissolves in water and do not hydrolyze. Although this compound has “chlorine” in its name, but it does not chlorinate; instead it works through oxidation mechanism. Its biocidal property is due to one electron transfer mechanism. Unlike chlorine it does not form carcinogenic by-products such as, chloramines, trichloromethane with organic compounds.

1.1.2 Chemistry of chlorine dioxide

Chlorine dioxide gas exists as a free radical and is very unstable in pure state, it can decompose to chlorine and oxygen with heat and irradiation. Presence of chlorine dioxide over 10% in the air is explosive and may cause health hazards, therefore it is generated onsite. The gas is greenish yellow in color and when dissolved in water the solution also has a greenish-yellow color. Its odor is similar to chlorine. Other chemical characteristics of chlorine dioxide gas are shown in Table 1.

Table 1: Chemical characteristics of chlorine dioxide gas*

Chemical formula	ClO_2
Molecular weight	67.5g/mole
Boiling point	11^oC
Solubility in water	3.01g/L at 25^oC and 34.5 Hg mm (partial pressure) 8.0g/L at 15^oC
Melting point	-59^oC
volatile	100%
Vapor density	2.4
Decomposition product	chlorine, oxygen, chlorite and chlorate

*summarized from MSDS Resonant Bioscience,

http://www.puremash.com/pdfs/MaterialDataSheetCIO2.pdf

Chlorine dioxide does not hydrolyze in water and remain as dissolved gas below 11^oC. Above this temperature chlorine dioxide remain as gas in headspace. When exposed to light, it photolytically decomposes in to chlorate ion (ClO_3^-), therefore it should be stored in dark glass bottles to prevent from light. Chlorine dioxide is stable over a wide range of pH. In solution it does not hydrolyze between pH 2-10. Over pH 10, it decomposes into chlorite (ClO_2^-) and chlorite (ClO_3^-) ions.

$$2ClO_2 + 2OH^- = ClO_2^- + ClO_3^- + H_2O$$

Chlorine dioxide is explosive under pressure and that is why it can neither be stored nor transported and needs to be generate onsite prior to its use. It is explosive at a concentration 10g/L or greater.

Unlike chlorine, chlorine dioxide oxidizes and does not chlorinate. It behaves as a selective oxidant through one electron transfer mechanism –

$$ClO_2 + e \rightarrow ClO_2^-$$

1.1.3 Application of chlorine dioxide

Application of chlorine dioxide is versatile. It has very good disinfecting property as well as off-flavor and off-odor elimination capacity. The disinfection efficiency depends on concentration of the gas and its contact time with surface or product that needs to be disinfected. Gaseous chlorine dioxide is more effective compared to liquid and useful for those areas such as stems of fruits and vegetables, hard to reach food contact surfaces and pipelines which are inaccessible by liquid solution due to surface tension of the solution. Studies have shown that chlorine dioxide not only has bactericidal property but it is also effective against virus, yeast, molds etc. It has been shown that 1g/L to 5g/L chlorine dioxide is effective against *E.coli* O157:H7, *Salmonella* and *Shiegella.* Disinfection capacity of chlorine dioxide is equal to or greater than chlorine and that is why washing of fresh produce with chlorine dioxide solution instead of traditional chlorine water are becoming more popular in recent days.

Another common application of chlorine dioxide is elimination of odor and taste caused by algae in drinking water. It is effective against destroying vegetative cells and the phenolic compounds responsible for off odor and taste in drinking water. It can also oxidize iron and manganese present in the water and forms sediments that can be removed by filtration.

1.2 Antimicrobial packaging

1.2.1 Concept of antimicrobial packaging

Active packaging is an innovative packaging system where the package interacts with the food positively in addition to protect it from external environment and physical damage, which is the conventional purpose of a package. Active packaging can be of different types, antimicrobial packaging being most common of them. Antimicrobial packaging is one of the most promising approach to prevent contamination from the pathogens as well as growth of spoilage microorganism on the food surface. Due to USDA and FDA regulations, only a certain amount of antimicrobial agent are permitted to add directly in food formulation which in many cases is not sufficient to inhibit the growth of microorganisms. Moreover, addition of excess antimicrobial compounds in food may affect the taste and flavor of the product negatively. In some cases, direct addition of antimicrobials to food formulation or application on food surface may not be sufficient as these compounds are partially absorbed or inactivated by the food [1]. To overcome these limitations discussed above antimicrobial packaging is a very promising option as it releases the compound from the package onto food surface/package headspace over time at a targeted rate and also compensate for the loss due to partial absorption or inactivation by food [2]. It contains antimicrobial compounds as an integral part of the package. This antimicrobials release from the package to delay/inhibit the growth of spoilage or pathogenic microorganism present in the packaged food [3]. The antimicrobial activity is either due to release of antimicrobial incorporated in the polymer or sachet or due to the antimicrobial property of the polymer itself [4].

1.2.2 Types of antimicrobial packaging

Antimicrobial packaging for food application can be different types based on incorporation methods and antimicrobial mechanism.

Based on the method of incorporation, it can be divided in five categories:

a) Sachet or pads containing volatile antimicrobial agents

Addition of sachet or pads containing volatile antimicrobial agents is most common and widely used. Oxygen, moisture and ethylene absorbers are used very frequently. Although these are not antimicrobial agent but removal of oxygen from package headspace creates stress on survival of aerobic microorganisms. Similarly moisture absorbers prevent water condensation on food surface which facilitates growth of microbes. Removal of excess ethylene from package headspace delays the ripening process as well as growth of microorganism. Other antimicrobial agents such as allyl isothiocyanate, chlorine dioxide, sulfur dioxide and essential oils can also be incorporated.

b) Direct incorporation of volatile and non-volatile antimicrobial agent into polymer used as packaging film

Incorporation of antimicrobial agent directly into polymer is comparatively new approach for food application although it has been commercialized for drug delivery and biomedical applications. Direct incorporation can be done in two ways – 1) addition of antimicrobial agent in the polymer during extrusion or 2) addition of antimicrobial agent in the polymer solution during solution casting method. In both the cases, nature of the antimicrobial agent and its chemical stability towards heat and water needs to be considered.

c) Coating of antimicrobial agents on polymer surface

Thermal stability of antimicrobial agents is a controlling factor for method of incorporation. Antimicrobials which are sensitive to high heat cannot be added during extrusion, instead they are coated on the surface of the polymer after film is formed or sometimes film is dipped into a solution of antimicrobial agent. For example, sorbic acid coating on polyvinyl acetate film [5].

d) Immobilization of antimicrobial into polymer

Immobilization of antimicrobials within the polymer can be achieved when both the antimicrobial agents and polymer have functional groups. Antimicrobials such as peptides, enzymes, organic acids have functional groups and they can be used for immobilization with polymers like nylon, ethyl vinyl alcohol (EVA) and polystyrene (PS).

e) Use of polymers those are inherently antimicrobial

Some polymers are inherently antimicrobials such as chitosan and lysine. These polymers interact with negative charge on bacterial cell membrane. Recently chitosan has been approved by FDA as an ingredient.

Based on mode of action antimicrobial packaging system can be of two different types –

a) Migration/Release

In this case antimicrobial compounds inhibit the growth of microorganism by migrating from the packaging material onto the food surface and/or package headspace.

Direct contact with food

For non-volatile antimicrobials incorporated in the polymer, food has to be in contact with packaging material. Generally liquid and semi-solid food without package headspace is preferred for this system as the compound diffuse directly from packaging material into food matrix. For non-volatile compounds food–package contact should be excellent so that antimicrobial compound released from the package can easily be dissolved within the food matrix. A good example of such antimicrobial is potassium sorbate.

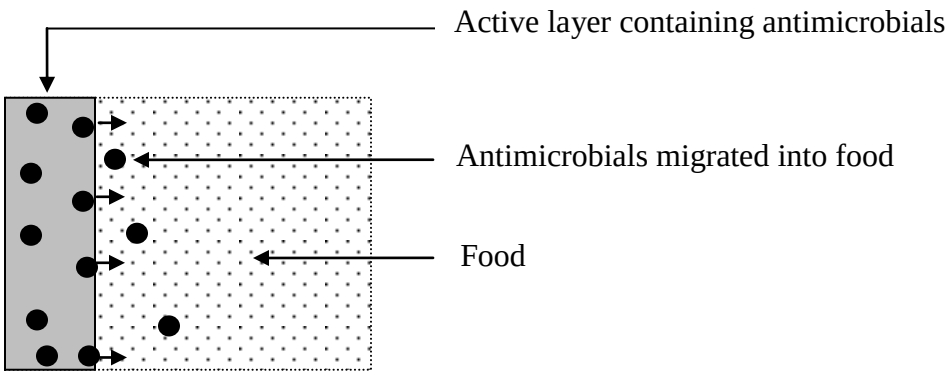


Figure 1: Mode of action of non-volatile antimicrobial agent

Indirect contact with food

In this case the antimicrobial compound has to be volatile in nature. Volatile antimicrobial will first migrate from packaging film to the package headspace and then condense on food surface. Volatile compounds are preferred for foods with irregular shapes or with package headspace where there is not sufficient contact between food and packaging material. Allyl isothiocyanate, chlorine dioxide, sulfur dioxide are examples of volatile antimicrobials.

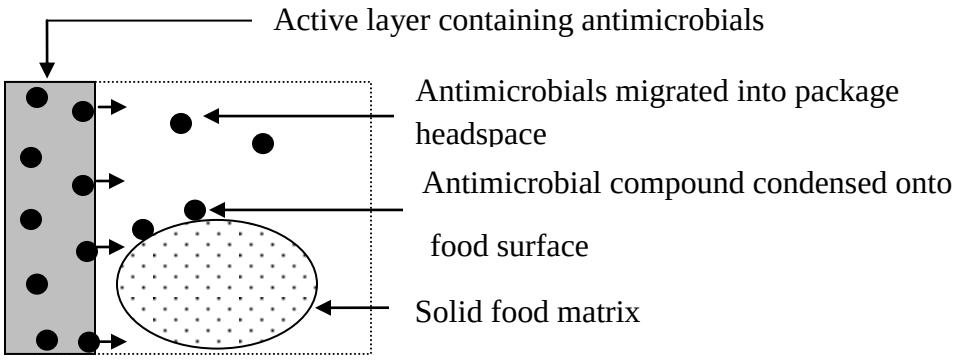


Figure 2: Mode of action of volatile antimicrobial agent

b) Immobilization

Sometimes non-food grade antimicrobial compound is immobilized on the food contact surface and it inhibits the microbial growth without migrating from the packaging material. This system requires very good contact between food and packaging material.

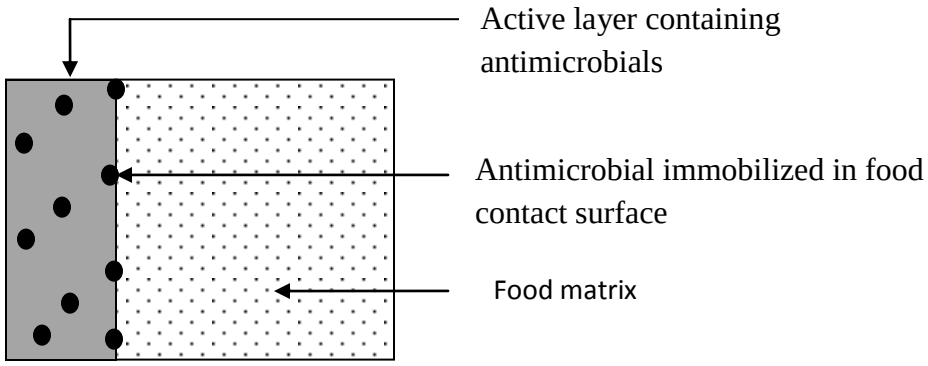


Figure 3: Mode of action of antimicrobials immobilized in food contact Surface of packaging material

1.2.3 Biopolymer based antimicrobial packaging

Increased use of petrochemical based synthetic packaging material in last few years has posed a serious threat to the environment. There is a great concern of waste disposal as these synthetic materials are non-biodegradable and cannot be broken down by natural flora present in soil. In spite of these limitations use of synthetic packaging material has increased in an alarming rate because of their availability, low price and mechanical property suitable for commercial application. However with a growing awareness of environmental sustainability, there is a greater demand for use of bio-based packaging material[6]. Therefore, both food and packaging industry are working hands-in hands focusing more on bio-based polymer as an alternative to synthetic packaging material.

Bio-polymer based packaging is defined as packaging material made from renewable agricultural (poly-lactic acid) or marine sources (chitosan). Based on their origin bio-polymers can be categorized into three categories –

Natural – polymers extracted from natural raw materials such as rice, corn, fruits etc. Examples of such polymers are starch, pectin and many more.

Chemically synthesized - polymers those are chemically synthesized from natural raw materials. For example, poly-lactic acid synthesized from corn.

Microbiologically synthesized – biopolymers naturally produced from microorganism. For example, polysaccharides, polyesters and polyamides.

Bio-polymer based antimicrobial packaging is not novel. Waxy coating are being used for centuries to retain moisture in fresh produce, edible coating to reduce moisture loss in meat was developed in sixteenth century. Later on with further advancement in this field, films with inherent antimicrobial properties were seen and then films

incorporated with chemical or natural antimicrobial compounds became increasingly popular and probably the most common form of antimicrobial packaging.

1.3 Literature review

1.3.1 Chlorine dioxide

1.3.1.1 Antimicrobial effect of chlorine dioxide

Chlorine dioxide is an oxidizing agent that has strong antimicrobial property. It is effective over a wide range of pH (pH 3 – 8) and has strong biocidal activity against a broad spectrum of microorganisms including bacteria, fungi, yeast and mold. It is 3.5 times more powerful than chlorine or chlorinated water [7]. Its biocidal effect is due to oxidative attack on cell membrane proteins and enzyme [7]. It penetrates the cell membrane and inhibits the respiration by altering ionic gradient. Damage of cell membrane also results in spore inactivation [8, 9]. Either gaseous or aqueous chlorine dioxide can be used for disinfecting fresh fruits and vegetables [10]. Studies have shown that more than 5 log reduction of *E.coli* O157:H7 on apple skin was achieved when treated with 3.3mg/L chlorine dioxide for 20 min or 7.2mg/L chlorine dioxide for 10 min [11]. Another study showed that 4.3-4.7 log reduction of *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella enterica* was achieved in strawberries when treated with 5.5mg/L chlorine dioxide gas for 10 min [12]. More over its effectiveness is not lessened by presence of soil and/or other organic matter and it does not form carcinogenic compounds like chloroamines and trihalomethanes because of its inability to react with ammonia which is very common byproducts for chlorine or chlorinated water treatment [13, 14].

Table 2: Antimicrobial effects of liquid chlorine dioxide on fresh fruits and vegetables

Produce	**Microorganism**	**[ClO_2] (mg/L)**	**Time (min)**	**Log reduction**	**Reference**
Lettuce	*Listeria monocytogenes*	5	10	0.8	[15]
	Escherichia coli O157:H7	10	5	1.2	[16]
		20	15	1.7	[17]
	Enterobacter sakazakii	100	1	4.05	[18]
Blueberry	*Listeria monocytogenes*	15	120	4.88	[19]
	Pseudomonas aeruginosa		120	4.48	
	Salmonella typhimurium		20	3.32	
	Staphylococcus aureus		30	4.56	
	Yersinia enterocolitica		60	3.69	
Cabbage	*Listeria monocytogenes*	5	10	0.8	[15]
Green bell pepper	*Listeria monocytogenes*	3	10	3.7	[20]
Baby carrot	*Escherichia coli* O157:H7	20	15	2.5	[17]
Apple	*Enterobacter sakazakii*	100	1	≥4.49	[18]
	Salmonella	5	10	≈2	[21]

	Escherichia coli O157:H7			≈1	
Tomato	*Salmonella enterica* *Erwinia carotovora*	20 10	1 1	5 5	[22]
Mungbean sprout	*Salmonella Typhimurium* *Listeria monocytogenes*	100	5	3 1.5	[23]

Table 3: Antimicrobial effects of chlorine dioxide gas on fresh fruits and vegetables

Produce	**Microorganism**	**[ClO2] (mg/L)**	**Time (min)**	**RH %**	**Log reduction**	**Reference**
Apple	*Escherichia coli* O157:H7 *Listeria monocytogenes* *Salmonella* *Allicyclobacillus acidoterrestris*	18.0 4.0 4.1 4.32	10 10 25 60	90-95% 90%	3.8-7 3.2-5.5 4.2 >5	[11] [24] [25] [26]
Blueberry	*Salmonella* *Salmonella* *Listeria monocytogenes* *Escherichia coli* O157:H7	8.0 4	120 720	99.9	2.4-3.67 3.62 3.94 4.25	[27] [28]
Baby carrot	*Escherichia coli* O157:H7	1.0	15	80	3.08	[17]

Carrot	*Salmonella* *Escherichia coli* O157:H7 *Listeria monocytogenes*	4.1	30.8 20.5 29.3		5.15 5.62 5.88	[25]
Cabbage	*Salmonella* *Escherichia coli* O157:H7 *Listeria monocytogenes*	4.1	30.8 20.5 29.3		4.42 3.13 3.60	[25]
Green bell pepper	*Escherichia coli* O157:H7 *Listeria monocytogenes*	1.24 1.2 3.0	30 30 10	90-95 90-95 90-95	6.45 >8.04 >6	[29] [30] [20]
Lettuce	*Escherichia coli* O157:H7 *Salmonella* *Escherichia coli* O157:H7 *Listeria monocytogenes* *Escherichia coli* O157:H7 *Salmonella enterica*	1.0 4.1 5	15 30.8 20.5 29.3 10	80 90-95	2.31 1.58 1.57 1.53 3.9 2.8	[17] [25] [31]
Onion	*Salmonella*	4.1	20		1.94	[25]
Peach	*Salmonella*	4.1	20		3.23	[25]
Raspberry	*Salmonella*	8.0	120		1.54	[27]
Strawberry	*Salmonella* *Escherichia coli* O157:H7	8.0 5.0	120 10	 90-95	3.76-4.41 4.6	[27] [12]

	Listeria monocytogenes *Salmonella enterica*				4.7 4.3	
Tomato	*Salmonella*	4.1	25		4.33	[25]

1.3.1.2 Environmental factors affecting antimicrobial efficacy of chlorine dioxide

Studies have been conducted to evaluate the effect of different environmental factors such as temperature, humidity, pH, presence of light on the antimicrobial activity of chlorine dioxide. Presence of suspended particle in the solution and surface integrity of the produce plays a role in case of liquid chlorine dioxide application. However antimicrobial efficacy of gaseous chlorine dioxide does not affected by organic materials or surface integrity. Concentration of chlorine dioxide and contact time with produce is the most important factors determining the antimicrobial effectiveness.

Study conducted by Han. et al [20] on inactivation of *E.coli* O157:H7 inoculated on green bell pepper has shown that gas concentration, temperature, humidity and contact time are four most important factors affecting the antimicrobial efficacy of chlorine dioxide. Gas concentration (0.1-0.5 mg/l) being the most important followed by time (7-135 min), relative humidity (55-95%) and temperature (5-25^{o}C) is the least important factor [30]. Moreover a synergistic effect was observed between chlorine dioxide gas concentration and relative humidity. This observation was in agreement with the results from another research conducted by Han et al. [32] to study the sterilization efficacy of chlorine dioxide gas for storage of aseptically processed juice. It was noticed that same level of chlorine dioxide gas concentration (10mg/l) showed a higher kill of *Lactobacillus buchneri* when relative humidity was increased from 70% to 90% [32].

Woodworth and Jeng also reported similar trend of higher killing effect of chlorine dioxide gas with humidification for *Bacillus subtilis* spores. [13]. Westphal et al reported that increase in relative humidity increases the killing of *Bacillus thuringiensis* spores as the spores swell in response to high relative humidity and shrink to their original size when exposed to dry air. Swelling of spores at high relative humidity increases the channel for chlorine dioxide gas to better penetrate and this may account for higher killing efficiency of spores [33].

Temperature is another important factor for antimicrobial efficacy of chlorine dioxide. Similar to chlorine, the effectiveness of chlorine dioxide decreases with decrease in temperature. Study by Han et al (1999) showed that decrease in temperature hindered the disinfection effect of chlorine dioxide [32].

Another important factor affecting the effectiveness of chlorine dioxide gas is the amount of produce exposed to amount of chlorine dioxide gas applied. Efficacy of particular amount of gas also depends on the gas concentration, size of treatment chamber and contact time [34]. Suspended matter and pathogen aggregation affect the disinfection efficiency of chlorine dioxide. Presence of organic matter results in faster degradation of chlorine dioxide. At a certain concentration, chamber with high amount of samples will degrade chlorine dioxide faster compared to chamber with lower amount of samples. Faster degradation will result in less contact time and consequently less killing efficiency.

Surface morphology of fresh produce and location of microorganisms are also very important factors determining the antimicrobial effectiveness of chlorine dioxide. A smooth surface requires less contact time compared to uneven surface with fine channels

and pores. For uneven surfaces gaseous chlorine dioxide is a better option than liquid. Liquid chlorine dioxide solution may not be able to reach the interiors of the pores and channels where microorganism is located due to surface tension of the liquid. Contact time is again a critical factor in this situation. If the produce is exposed for a longer contact time, there is a better chance for chlorine dioxide solution to reach inside the pores. Study by Han et al showed that higher log reduction of *E.coli* O157:H7 was achieved on uninjured bell pepper surface compared to that of injured surface [30]. Du et al reported a higher reduction of *Listeria monocytogenes* inoculated on apple skin compared to that of calyx and stem cavities when treated with 8mg/l chlorine dioxide gas for 30 minutes [24].

For chlorine dioxide solution, pH of the solution affects the inactivation efficiency but to a lesser extent compared to chlorine. The optimum range of pH is in between 6-8.5. Different studies were conducted for inactivation of *E.coli* by chlorine dioxide but the results are inconclusive. Bernarde et al. reported that increase in pH from 6.5 to 8.5 increased the killing rate of *E.coli* by chlorine dioxide[7]. However an earlier study showed that bactericidal efficacy of chlorine dioxide is not affected within the pH range of 6-10 [35]. More research is needed to further understand the impact of pH on inactivation effectiveness of chlorine dioxide.

1.3.1.3 Current generation method of chlorine dioxide and its limitation

Chlorine dioxide can be generated by various methods. Most generators use sodium chlorite ($NaClO_2$) as precursor chemical to generate chlorine dioxide. Recently production of chlorine dioxide from sodium chlorate ($NaClO_3$) has been introduced where sodium chlorate is reduced by hydrogen peroxide (H_2O_2) and concentrated

sulfuric acid. However this method is generally used in paper industry. In food industry and for treating drinking water the most traditional one is reaction of sodium chlorite with strong inorganic acids. Table 4 summarizes various generation methods and some of them are discussed below in detail.

Acid-chlorite system – This is the oldest and most common method of chlorine dioxide production. In this method chlorine dioxide is produced by reaction of sodium chlorite ($NaClO_2$) solution with sulfuric (H_2SO_4) or hydrochloric acid (HCl). The chemical reactions are shown below.

$$5NaClO_2 + 4HCl = 4ClO_2 + 5NaCl + H_2O$$

$$4NaClO_2 + 2H_2SO_4 = 2Na_2SO_4 + 2ClO_2 + HCl + HClO_3 + H_2O$$

and/or

$$10NaClO_2 + 5H_2SO_4 = 5Na_2SO_4 + 8ClO_2 + 2HCl + 4H_2O$$

Generally HCl is preferred over H_2SO_4, as it provides higher chlorine dioxide production yield. The maximum yield that could be achieved is 80%.

Aqueous chlorine-chlorite solution - In this system chlorite ions generated from dissolved sodium chlorite will react with hypochlorous acid to form chlorine dioxide.

$$2ClO_2^- + HOCl \rightarrow 2ClO_2 + Cl^- + OH^-$$

Under ideal conditions, the resulting pH of the effluent will be around 7. The reaction will move towards completion if pH is lowered and for that often time additional chlorine is used. Early generators used 200-300% more aqueous chlorine to improve the yield. The drawback of this system is the reaction rate is much slower than most of the

commercial generation methods except the acid-chlorite system. This system produces around 1000 lb/day.

Another aqueous chlorine based chlorine dioxide generation system is “French Loop”. In this system chlorine gas is directly injected in a continuous water stream thereby eliminating the need for feeding excess chlorine to the reactor. This chlorine solution then reacts with sodium chlorite to form chlorine dioxide.

Gaseous chlorine-chlorite solution - In this system vaporized sodium chlorite solution reacts with molecular chlorine under vacuum. This process utilizes undiluted reactants and much more rapid and easy to scale up.

$$2NaClO_2 + Cl_2 \rightarrow ClO_2 + 2NaCl$$

In this process yield is very high, nearly 95-99% with only 2% of excess chlorine.

Table 4: Various chlorine dioxide generation methods

Type of generator	**Chemical reaction**	**Comments**
ACID-CHLORITE (Direct Acid System)	$4HCl + 5NaClO_2 \rightarrow$ $4ClO_2(aq) + ClO_3^-$	• Slow reaction rates • Production limit ~ 25-30 lb/day. • Maximum yield at ~80% efficiency.
AQUEOUS CHLORINE-CHLORITE	$Cl2 + H2O \rightarrow$ [HOCl / HCl] $[HOCl/HCl] + NaClO_2 \rightarrow$	• Relatively slow reaction rates • Excess Cl_2 or acid

	$ClO_2(g) + HOCl + NaOH + ClO_3^-$	to neutralize NaOH. • Production rates limited to ~ 1000 lb/day. • High conversion but yield only 80-92% • More corrosive effluent due to low pH (~2.8-3.5).
FRENCH LOOP	$2HOCl + 2NaClO_2 \rightarrow 2ClO_2 + Cl_2 + 2NaOH$	• Production rate limited to ~ 1000 lb/day. • Yield of 92-98% • Highly corrosive to pumps
GASEOUS CHLORINE SOLIDS CHLORITE MATRIX	$Cl_2(g) + NaClO_2(s) \rightarrow ClO_2(g) + NaCl$	• Rapid reaction rate • Yield > 99% • Production rate > 10,000 lb/day
GASEOUS CHLORINECHLORITE	$Cl_2(g) + NaClO_2(aq) \rightarrow ClO_2(aq)$	• Rapid reaction • Production rates 5-120,000 lb/day. • No excess Cl_2 (<

		2%) • Yield of 95-99%.
ELECTROCHEMICAL	$NaClO_2(aq) \rightarrow ClO_2(aq) + e^-$	• New technology
ACID/PEROXIDE/CHLORIDE.	$2NaClO_3 + H_2O_2 + H_2SO_4 \rightarrow 2ClO_2 + O_2 + Na_2SO4 + H_2O$	• Uses concentrated H_2O_2 and H_2SO_4

Since chlorine dioxide is very unstable and explosive in nature, it needs to be prepared prior to application and cannot be stored for future use. This requires setting up of chlorine dioxide generators onsite which adds extra operational cost and manufacturing steps to the existing system and this is one of the major limitation for use of chlorine dioxide. In spite of an excellent antimicrobial agent and its commercial viability, difficulty in storage and transportation makes this compound unfit for regular use. All the current methods of chlorine dioxide production have this challenge and therefore inspite of being high yield generation method their use is very limited.

To overcome this limitation, current study focuses on developing a self-generating chlorine dioxide packaging system that can generate chlorine dioxide onsite without any complex instrumentation and additional manufacturing steps. In this research, a feasibility study has been conducted in order to see whether or not it is possible to develop chlorine dioxide releasing films that will release chlorine dioxide over time with moisture trigger. The release profile of the gas from the films should be controlled by varying the reactants and their concentration, polymer and method of processing.

1.3.1.4 Controlled production of chlorine dioxide

As mentioned above, the most traditional method of chlorine dioxide production is reaction of sodium chlorite with hydrochloric or sulfuric acid. However studies have been conducted to produce chlorine dioxide from organic acids. For purpose of this research, hydrochloric or sulfuric acid was replaced by organic acids. This is necessary as the packaging material will be in contact with food, compounds incorporated in it should be GRAS. Moreover hydrochloric or sulfuric acid is so concentrated that even after incorporating in the polymer they will give rise to off odor.

Acetic acid, citric acid and lactic acid were used in this study to replace inorganic acid. There acids were chosen as they are frequently used in food formulations and safe for human consumption. Moreover organic acids have low dissociation constant which means the reaction will be much slower compared to inorganic acids therefore facilitating slow generation of chlorine dioxide over a longer period of time. Another advantage of using these acids is synergistic antimicrobial effect. Ryu et al reported the lethal activity of citric, lactic, acetic and malic acid against *E.coli* O157:H7 [36]. Similar result was observed with *Salmonella typhimuriam* inoculated on liquid egg products [37]. However this is only effective for organic acid based chlorine dioxide solution and not for gaseous chlorine dioxide generated from the film.

Production of chlorine dioxide from sodium chlorite using these acids can be varied by varying the amount of reactant and type of organic acids. Kim et al. reported that with an increase in concentration of sodium chlorite there is an increase in amount of chlorine dioxide produced, regardless of the pH and type of acid used [38]. At a given

concentration of sodium chlorite, chlorine dioxide produced from acetic acid, citric acid and lactic acid decreased with an increase in pH from 3 to 6 due reduction of hydrogen ion concentration with increase in pH. However at a higher pH, stability of chlorine dioxide production increased for all three acids.

1.3.2 Antimicrobial packaging

Antimicrobial packaging is specially designed to control the growth of microorganism and they can be achieved by various method. Selected references of different synthetic and bio-polymer based antimicrobial packaging are listed in table below.

Table 5: Synthetic and bio-polymer based antimicrobial packaging

Packaging material	Antimicrobial compound	Food	Reference
Low density poly-ethylene	Potassium sorbate Imazil Grapefruit seed extract	Culture media Bell pepper Lettuce, Soybean sprout	[39, 40] [41, 42]
Carrageenan Sodium alginate	Nisin Lysozyme GFSE	Agar	[43]
Methyl cellulose/Chitosan	Potassium sorbate Sodium benzoate	Culture medium	[44]
Yam starch Chitosan		Carrots Culture	[45]

	Chitosan	medium	
Poly-ethylene	Sorbic acid anhydride	Culture medium	[46]
Alginate Apple puree	Essential oils	Agar	[47]
Chitosan	Garlic oil Potassium sorbate Nisin Acetic acid Propionic acid Lauric acid	Agar	[48] [1]
Alginate	Garlic oil Potassium sorbate	Agar Potassium sorbate solution	[48] [49]
Whey protein isolate	Potassium sorbate p-amino benzoic acid sorbic acid Oregano oil Rosemary oil Garlic oil	Water-glycerol Agar	[50] [51] [52]
Poly-lactic acid-nanocomposite films	Montmorillonite	Culture medium	[53]

Poly-lactic acid	Nissin	Culture medium Orange juice Liquid egg white	[54]
Oxygen absorber sachet	Iron compound	Bread	[55]

1.4 Chlorine dioxide – poly-lactic acid: A Promising combination for antimicrobial packaging

Chlorine dioxide has been known for its disinfection property for long time. Back in 20th century, it was first used for water disinfection in a spa in Belgium [56]. It is most commonly used in water treatment plant to make drinking water safer. Recently it has attracted significant attention for decontamination fruits and vegetables. It can either be used as liquid solution or gas, but in either case it has to be generated onsite. Different easy to use sachet containing precursor compounds are available commercially for generation of chlorine dioxide. The precursor compounds have to be mixed before the treatment to generate chlorine dioxide [27]. Application of gaseous chlorine dioxide is advantageous over liquid as gas has better penetration ability, therefore it could reach microorganisms located inside the pores or channels where liquid cannot reach due to surface tension, which results in higher killing effect [57]. However, existing methods of chlorine dioxide are costly and requires additional manufacturing steps. Moreover, in these methods production of chlorine dioxide cannot be controlled or extended for a longer period of time to ensure slow release of chlorine dioxide at a desired level. Recently Leung et al reported sustained release of chlorine dioxide for 28 days from a

polymer-encapsulated chlorine dioxide coated surface and its antimicrobial properties [58]. This indicates that chlorine dioxide releasing packaging material is a promising approach for sustained release of chlorine dioxide over longer period of time.

There has been an increased concern of environmental sustainability in recent years which led to search for bio-polymers from agricultural sources to replace traditional petrochemical based synthetic packaging materials such as, poly-ethylene (PE), low density poly-ethylene (LDPE). Like other bio-polymer poly-lactic acid is a bio-polymer derived from renewable sources such as corn and other agricultural sources is gaining popularity as a replacement of synthetic polymers in food packaging applications. It has already been used in drug delivery, tissue culture and other surgical applications. In Europe and North America this polymer is already in use for bottled water, juice containers, etc. Some of its characteristics makes it suitable for food application such as, it is GRAS, eco-friendly, biocompatible, better thermal processability compared to other bio-polymers [54, 59] .

An antimicrobial packaging system based on poly lactic acid with sustained release of chlorine dioxide would be a novel approach. Very minimum information is available on chlorine dioxide releasing packaging system. Therefore combination of poly-lactic acid and chlorine dioxide is definitely something worth exploring.

1.5 Research gap and opportunities

Based on the information obtained from the literature review of antimicrobial property of chlorine dioxide, its intended application and use of poly-lactic acid as a biodegradable food packaging material, some research gaps are identified.

- Most of the researches conducted so far have focused on antimicrobial efficacy of chlorine dioxide, either liquid or gaseous form. None of these researches have focused on sustained generation and release of chlorine dioxide throughout the treatment. Not many attempts are made except a recent research [58] to develop a self-generating chlorine dioxide system without additional manufacturing steps and instrumentations.
- In most cases chlorine dioxide has been generated using hydrochloric or sulfuric acid to get a higher yield. There are very few studies that shows production of chlorine dioxide from organic acids [38]
- There are many researches on development of antimicrobial films with various antimicrobial compounds. However very minimal information is available on system that incorporates the precursor compounds and releases a different compound which is the reaction product of the precursor compounds.
- No information is available that compares the production rate of chlorine dioxide from the precursor compounds outside a polymer matrix versus when they are incorporated within a polymer matrix.
- Potential of poly-lactic acid, a bio-polymer, extensively studied in different medical and surgical application, controlled drug delivery and tissue culture. But so far it has not been tapped in the field of antimicrobial food packaging.
- Literature review of poly-lactic acid indicates that there has not been any research conducted with poly-lactic acid-chlorine dioxide combination, either in form of coating or antimicrobial film.

- Antimicrobial efficacy of chlorine dioxide has mostly been evaluated for single exposure short time treatment maximum up to 2hrs. However, except one research, not many attempts were made to continue the treatment for 24hrs or longer.
- Since there is not much information available on chlorine dioxide releasing packaging material, so it would be beneficial to investigate different factors affecting the release profile of chlorine dioxide from the film as this information in turn could be useful for designing controlled release chlorine dioxide packaging film which is the advanced stage of antimicrobial packaging.
- Since chlorine dioxide is a strong oxidizing agent and also has been reported to induce discoloration in some produce, it is important to monitor the quality attributes such as color, texture of the produce when exposed for a longer period of time.

1.6 Objectives

1.6.1 Overall objective

The overall objective of this research is to develop chlorine dioxide releasing packaging film that can release chlorine dioxide from the film directly to the food surface/package headspace, under certain circumstances, to enhance safety and shelf life of food products where microbial spoilage is the primary shelf life limiting factor. To generate chlorine dioxide directly from the film, precursor compounds will be incorporated in polymer matrix. The polymer containing precursor compounds can be in different forms, such as, single layer film, multiple layer film with barrier layer on the outer side, double layer film containing precursor compounds in different layers or even a coating on another

polymer. After development of the film, it has to be tested on fresh produce for its antimicrobial efficacy and effects on quality attributes of the produce as well.

1.6.2 Specific objective

To achieve the overall objective, it was divided into several sub-objectives.

- Since there is not much information available on chlorine dioxide releasing films, primary sub-objective is to develop a chlorine dioxide releasing film by incorporating precursor compounds and evaluate the effect of different variables on release profile.
- Second sub-objective is to test the antimicrobial efficacy of the films in culture medium as well as on real food.
- Finally, quality attributes such as color and texture of the produce will be of the monitored when they are exposed to the treatment for an extended period of time to ensure that the film developed in this research does not have any adverse effect on quality of the produce.

1.6.3 Scope of the research

Based on the literature review on antimicrobial effect of chlorine dioxide and current mode of application, it is well-understood that chlorine dioxide is a promising disinfectant for washing and sanitizing the food product. However so far its use is very limited because of instability of the compound for which it cannot stored or transported but has to be generated onsite prior to treatment which requires additional manufacturing

steps and instrumentation thereby adding cost to the overall operation. Moreover current practice is one time treatment where the food product is exposed to chlorine dioxide treatment before packaging thereby leaving a chance of cross contamination anytime after that and throughout the supply chain. Therefore availability of a self-generating chlorine dioxide packaging system that can generate chlorine dioxide over a longer period of time would be beneficial to address the current limitation.

Since very minimum information is available on this, as first step, this research will focus on development of a single layer film by incorporating sodium chlorite and organic acid. Primary objective of this research is to test whether or not it is technically feasible to prepare a film that will release chlorine dioxide gas directly from the packaging film onto food surface/package headspace over time in such a concentration that is sufficient to reduce microorganisms to a safe level without affecting the quality of the product negatively. Upon successful completion, this research work will provide valuable insight for further designing of other form of sustained release system such as multi-layer film or gel for coating and in future controlled release packaging system by simple variation of the independent variables. This research will also provide a database of film formulations having different release profiles and antimicrobial efficacy which could be used as a platform to produce chlorine dioxide releasing CRP film from poly-lactic acid polymer with a desired release property with further modification.

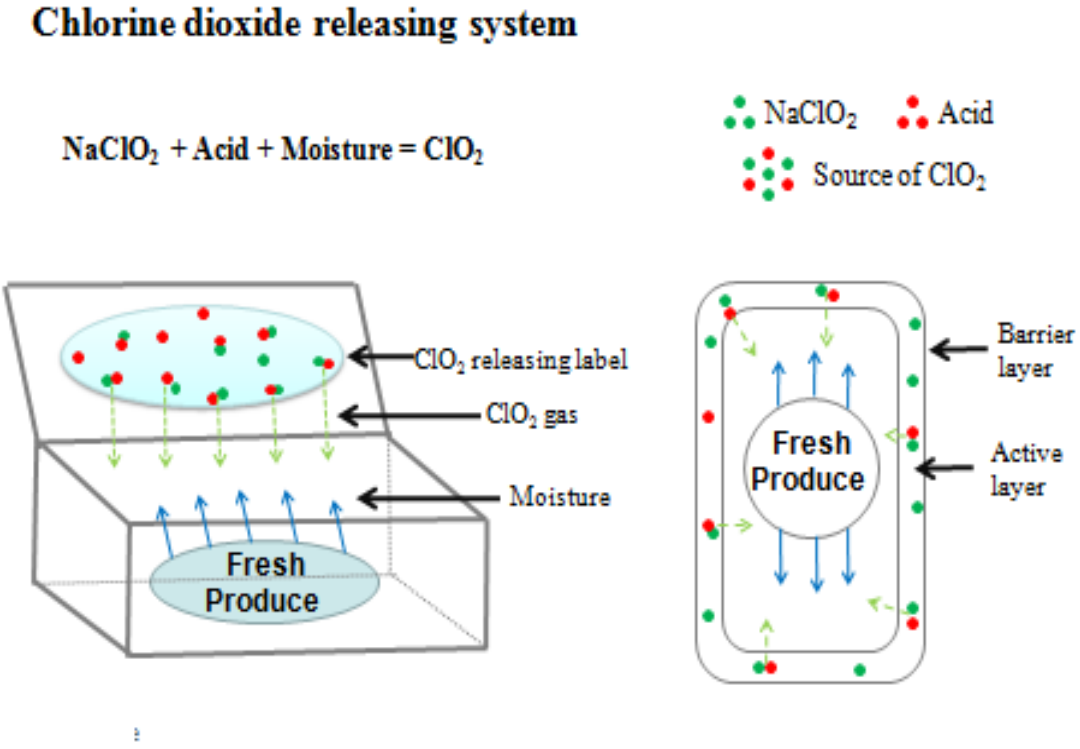


Figure 4. Concept and mechanism of self-generating chlorine dioxide packaging system

1.6.4 Challenges in the development of chlorine dioxide releasing films

- The reaction involved in production of chlorine dioxide is highly moisture sensitive. In presence of moisture sodium chlorite will react spontaneously with acid to form chlorine dioxide gas and this is first challenge that needs to overcome to prepare the film. Therefore sodium chlorite and organic acid have to be mixed in a way that they do not get exposed to moisture and trigger the reaction.
- For film preparation in laboratory, solution casting is the only feasible method. Hence to select the polymer, it has to be soluble in organic solvent or other inert

solvent except water. Poly-lactic acid is soluble in organic solvents such as chloroform, methylene chloride which makes it suitable for this research.

- Drying of the film is another important aspect. After casting the film containing sodium chlorite and organic acid, it cannot be dried in environmental air. Moisture content of the air is sufficient to trigger the reaction and in that situation chlorine dioxide gas will generate and release before the film is dried. To keep the films away from moisture they have to be dried under dry air flow or under the flow of inert gas. A close chamber devoid of atmospheric moisture is not a good option as it will become saturated with the solvent and film will not dry.
- Storage of the film is another challenge. Films have to be stored in airtight containers to avoid direct contact with air.
- There is no direct method available for quantification of chlorine dioxide gas except chlorine dioxide gas detecting tubes and probes which are not very accurate and reliable at low concentration. These tubes and probes can detect the presence of chlorine dioxide. Some of them response only at higher concentration, however do not provide accurate concentration that is required for this research. Since chlorine dioxide is strong oxidizing agent it cannot be directly injected in to GC, or else the column will be choked.

1.7 Research approach

The research approach to achieve the overall objective is illustrated below. Independent and dependent variables are identified and listed in Table 6

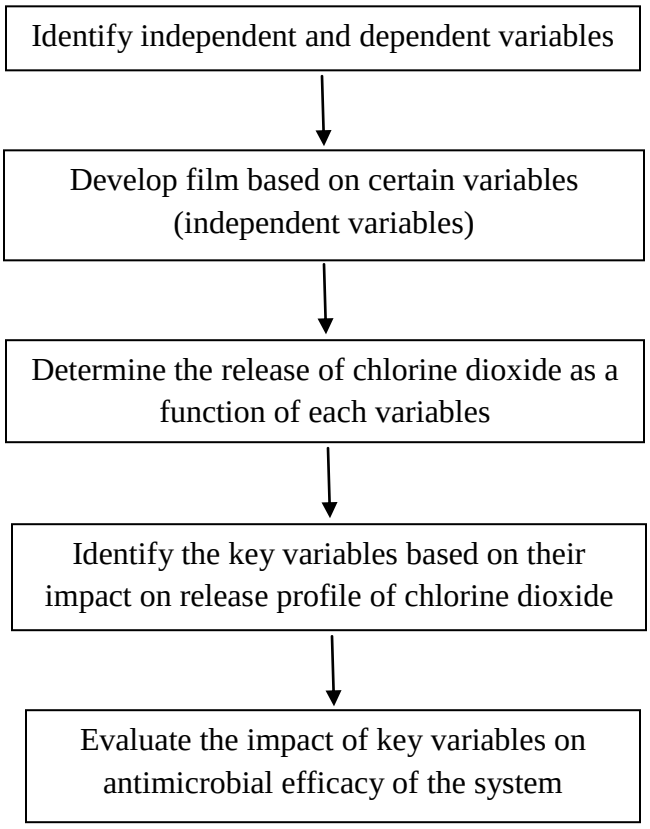


Figure 5. Research approach to identify and establish key variables for development of chlorine dioxide releasing antimicrobial film

Table 6. Dependent and independent variables affecting the generation of chlorine dioxide

Independent variables	Dependent variables
• Combination and ratio of precursor compounds (sodium chlorite and organic acid)	• Reaction kinetics
• Concentration and ratio of precursor compounds in the film • Film thickness/ amount of polymer used • Temperature	• Release profile of chlorine dioxide

Based on the table above the independent variables listed are described below:

- Combination and ratio of precursor compounds – Sodium chlorite is one of the precursor compound and source of chlorine dioxide. Among three organic acid, acetic acid, lactic acid and citric acid, one will be selected based on the reaction kinetics with sodium chlorite. The combination and ratio of sodium chlorite and organic acid will have impact on the production trend and quantity of chlorine dioxide generated.
- Concentration of precursor compounds in the film – Like any other active compound, concentration of precursor compounds incorporated in the film will impact the release profile and quantity of chlorine dioxide released from the film.
- Film thickness – Thickness of the film is directly related to the amount of polymer in the films. Film thickness impacts the diffusion of the gas through the

film and its release. Additionally, thickness might also have impact on the distribution of precursor compounds within the polymer matrix and hence the production of chlorine dioxide.

- Temperature – According to Arrhenius equation, temperature is a critical factor to any reaction. Since the production of chlorine dioxide is dependent on the reaction of precursor compounds, so temperature might affect the production and release of chlorine dioxide depending on temperature range used in this research.

1.7.1 Proof of concept

Based on the above research approach first step was to establish a proof of concept with any one of the precursor compounds combination – ratio, concentration and film thickness. As a first step sodium chlorite and acetic acid were mixed together and exposed at 100% RH to test the production of chlorine dioxide from these reactant mixtures. Next step was to prepare a poly-lactic acid film by incorporating sodium chlorite and acetic acid (1:1 by weight). The film was exposed to 100% RH and headspace was checked using chlorine dioxide detecting tubes to confirm the production and release of chlorine dioxide from the film.

Once proof of concept was established, the research was conducted as per design of experiment by identifying key variables and studying its impact on release profile of chlorine dioxide. Design of experiment is discussed in later section of this thesis.

1.7.2 Research approach to develop chlorine dioxide releasing films

To achieve the overall objective of developing a chlorine dioxide film that releases chlorine dioxide over time to inhibit microbial growth in fresh produce without affecting its quality negatively, a design of experiment was established and followed. This design of experiment helped us to approach in a systemic manner by completing each sub-objective and proceed to the next.

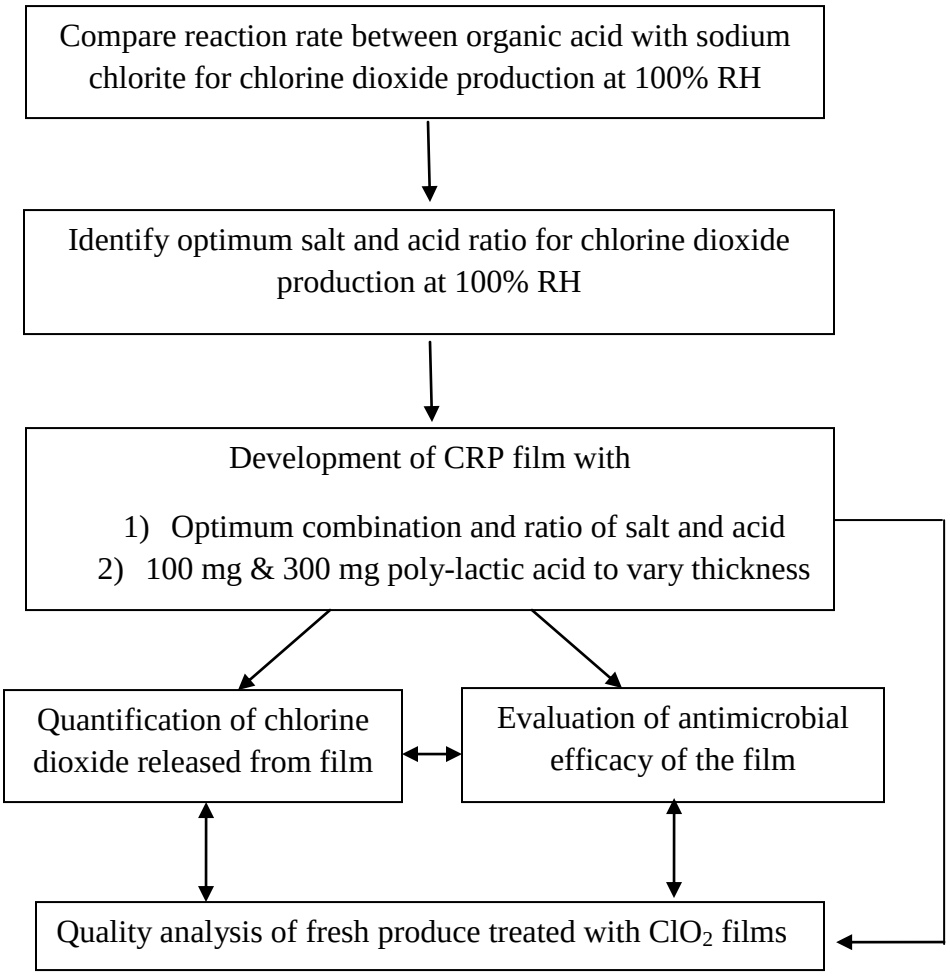


Figure 6. Design of experiment to develop chlorine dioxide releasing antimicrobial film

- In this research, since hydrochloric or sulfuric acid is replaced by organic acids, first step would be to identify the best acid suitable for this application. For that reaction of sodium chlorite with each of the three different acids such as, acetic acid, lactic acid and citric has to be monitored. Based on the experimental result, optimum combination of sodium chlorite and organic acid will be decided for future experiments.
- Once the optimum reactant combination is decided, next step will be to try out different ratios of sodium chlorite to acid to get the best ratio for maximum production of chlorine dioxide.
- Taking the optimum combination and ratio of reactants from the above two experiments, reactants will be incorporated in the polymer matrix to develop the chlorine dioxide releasing film. Films with different reactant concentration, salt-acid ratio, thickness will be made.
- After development of the films, next step will be quantification of chlorine dioxide gas from the films at several temperatures to monitor the effect of different variables on the release profile of chlorine dioxide.
- Antimicrobial property of the films will be evaluated on the microorganisms of food safety concern.
- Quality analysis of the produce will be done after treatment with these films for an extended period of time.

2 Experiments

2.1 Materials

2.1.1 Selection of reactants for generation of chlorine dioxide

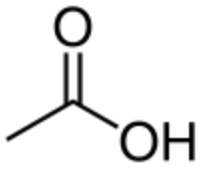
Sodium chlorite

The most traditional method of production of chlorine dioxide involves reaction of sodium chlorite with acid. In this research it was used as precursor of chlorine dioxide, not only due to its extensive use for production of chlorine dioxide but also because it is generally recognized as safe (GRAS). Technical grade sodium chlorite was obtained from Ricca Chemical Company, Arlington, TX, USA.

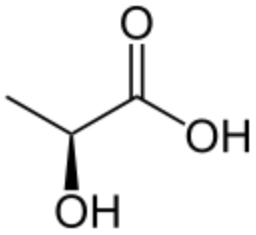
$$5NaClO_2 + 4HCl = 4ClO_2 + 5NaCl + 2H_2O$$

Organic acids

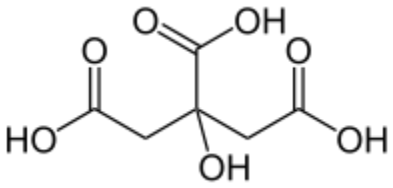
Industrially chlorine dioxide is produced by the reaction of sodium chlorite with strong inorganic acid like HCl or H_2SO_4. However in this research, these cannot be used as they are strong inorganic acids and not food grade. Organic acids like acetic acid, citric acid and lactic acid were investigated as they are all food grade acids and GRAS. Acetic acid and lactic acid used were in liquid form whereas citric acid used was in powder form. Glacial acetic acid and lactic acid were obtained from Fisher chemicals, NJ, USA and technical grade 99% pure anhydrous citric acid was obtained from Acros organics, NJ, USA.



Acetic acid



Lactic acid



Citric acid

Figure 7. Structure of organic acids

2.1.2 Selection of polymer

A variety of polymers were tested initially to make a good film that releases chlorine dioxide gas upon moisture trigger. All the trials were done by solution casting method. Since the reaction of sodium chlorite and acid is extremely moisture sensitive, this put a restriction on the polymers to be tested and also on the solvents to be used. Different polymers those were soluble in solvents other than water were investigated. Table 7 summarizes the polymers those were tested and their outcomes.

Table 7. Solubility of different polymers in organic solvent

Polymer	Solvent	Observation
Ethyl vinyl alcohol (EVOH)	Di-methyl sulfoxide (DMSO)	DMSO did not evaporate even after 72 hrs
	DMSO (10%) + Acetone (90%)	Acetone evaporated but DMSO did not
Polycaprolactone (PCL)	Toluene	Formed opaque film
Low density poly ethylene (LDPE)	Tolune + mild heat	Brittle layer was formed
Nylon 6,6	Isopropyl alcohol	Did not dissolve
	Isopropyl alcohol + heat	Did not dissolve
	Toluene	Brittle layer
Poly-lactic acid	Methylene chloride	Transparent film

Based on the above trials poly-lactic polymer was selected for this research as this one dissolved in organic solvent (methylene chloride) and formed a transparent film that was easy to peel. As a next step, sodium chlorite and organic acids were incorporated and resultant film was still transparent and suitable for this study. For experimental purpose sample of poly-lactic acid polymer resins of grade 4060D was sourced from Natureworks, Minnetonka, MN, USA.

2.1.3 Chlorine dioxide detection aids

Chlorine dioxide detection tubes

For detection of chlorine dioxide released from the films chlorine dioxide detecting tubes were purchased from Gastec Corporation. These tubes were able to detect the gas in the range of 0.5 to 10 ppm. They were mainly used for qualitative detection purpose at the beginning of release study to confirm the release of chlorine dioxide and in quality analysis study to check the amount of chlorine dioxide gas retained inside the package after certain time intervals.

Figure 8. Gastec pump fitted with chlorine dioxide detecting tubes

Figure 9. Chlorine dioxide detecting tube – it turns white to orange-yellow after absorbing chlorine dioxide

Rhodamin B

Rhodamin B was used for quantitative measurement of chlorine dioxide with UV-Visible spectrophotometer. It is a dye, extensively used for spectrophometry, fluorescence microscopy to detect compounds in an inexpensive manner. It is a violet powder and often known as pigment violet or basic violet. The dye has a molecular weight of 479 and its chemical structure is shown below

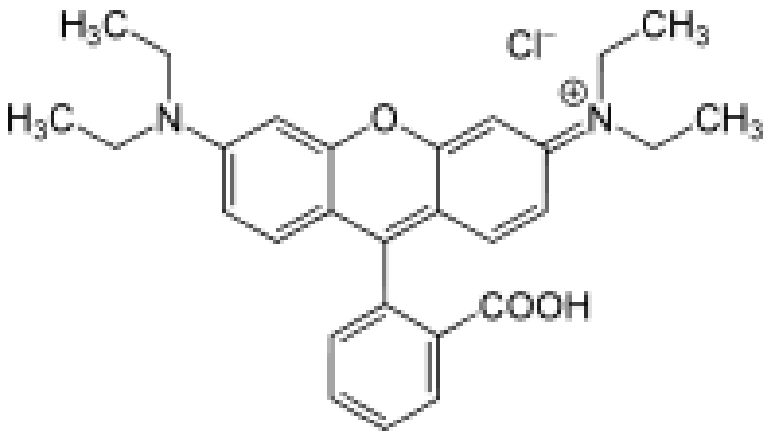
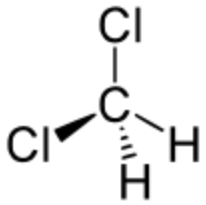


Figure 10. Structure of Rhodamin B (proteomics.dynalias.org)

2.1.4 Chemicals used for film preparation and release study of chlorine dioxide from films

Solvent for PLA

HPLC grade methylene chloride (CH_2Cl_2) purchased from Fisher Chemicals, NJ, USA was used for solution casting of poly-lactic acid polymer.



Methylene chloride

Figure 11. Structure of methylene chloride

Preparation of chlorine dioxide stock solution

Sulfuric acid (H_2SO_4) was obtained from Fisher Chemicals, NJ, USA. 20% v/v sulfuric acid was reacted with sodium chlorite solution for preparation of chlorine dioxide stock solution.

Buffer for spectrophotometric determination of chlorine dioxide

Ammonia buffer (pH 10) was sourced from Ricca Chemicals, NJ,USA to use with rhodamin B for detection of chlorine dioxide in UV-Visible spectrophotometer at 553nm.

2.1.5 Media preparation for microbial study

MacConkey Sorbitol agar (CT-SMAC), a selective medium for the isolation and differentiation of *E.coli* O157:H7 was used. For *Salmonella*, TSA with 0.1% sodium pyruvate and 100 ppm nalidixic acid (TSAPN) was used.

2.1.6 Bacterial culture for microbial study

An overnight culture of *E. coli* O157:H7 and a cocktail of *Salmonella* resistant to nalidixic acid were used in this study. *E. coli* O157:H7 ATCC 43894, *Salmonella*

Panama 19454, *Salmonella* Poona 953, and *Salmonella* Stanley H0558 were taken from the culture collection of the U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center.

2.1.7 Real food selection for antimicrobial study and quality analysis

Grape tomatoes bought from local supermarket were used as model system to test the antimicrobial effect of the films as well as study the effects of the treatment on quality attributes such as color and texture of the fruit. Grape tomato was selected because it is one of highest consumed produce in USA and often consumed raw in salad or as snack. Moreover, since chlorine dioxide has been reported to induce bleaching or burning of tissue in some fruits and vegetables, so tomato with its bright red color would be a good indication for that as well.

2.2 Methods

2.2.1 Optimization of reactant combination and ratio for chlorine dioxide

Pre-determined weight of sodium chlorite and organic acid were mixed together in 50 ml glass vial and the vial was kept inside jar (2 L) containing 300 ml Mili-Q distilled water. Jar was sealed tightly and kept under dark at ambient temperature ($22\pm1^{o}C$). 100% RH was maintained inside the jar. Chlorine dioxide gas produced from salt-acid mixture was dissolved in water and absorbance of the solution was measured in every hour using UV-Visible spectrophotometer (PharmaSpec UV-1700 UV-Visible spectrophotometer, Shimadzu Corporation, Japan) at 360nm. Concentration of chlorine dioxide was calculated from its absorbance using molar absorption coefficient ($1250\ M^{-1}cm^{-1}$) of chlorine dioxide at 360nm [60, 61].

To finalize the optimum reactant combination, experiments were conducted with 50 mg of sodium chlorite and 50 mg of each of the three acids, therefore maintaining a sodium chlorite –acid ratio of 1:1. Based on the observation from this set of experiments, next step was to optimize the reactants ratio. For that, three different sodium chlorite – acid ratios, such as 1:1, 1:2 and 2:1 were tested. All combinations that were tested summarized in table 8 and these experiments were carried out under same experimental conditions mentioned above.

Table 8. Salt-acid combinations and ratios used for production of chlorine dioxide

$NaClO_2$-acid combination	$NaClO_2$ (mg)	Acid (mg)	Salt-acid ratio
$NaClO_2$ – Acetic acid	50	50	1:1
$NaClO_2$ – Citric acid	50	50	1:1
	50	100	1:2
	100	50	2:1
$NaClO_2$ – Lactic acid	50	50	1:1

2.2.2 Chlorine dioxide releasing film preparation

Chlorine dioxide releasing poly-lactic films were prepared by solution casting method by dissolving PLA /$NaClO_2$/acid mixtures in 10 ml methylene chloride. Two different thickness of films were prepared by dissolving either 100 mg or 300 mg PLA resin in 10 ml methylene chloride. When PLA resins were completely dissolved in solvent, pre-determined weight of sodium chlorite was first added to PLA solution and stirred for 15

minutes. After that, citric acid was added with continuous stirring for another 15 minutes to facilitate homogeneous mixing. Mixture was then casted on Teflon petridish (11cm diameter) and dried in a closed chamber with continuous dry air flow at ambient temperature ($22\pm1^{o}C$). Casting was done as quickly as possible to avoid contact with atmospheric moisture. Films were stored in ziplock bags, one in each bag to avoid direct contact with environment. Nine types of films were prepared with different weight of PLA (100 mg & 300 mg), reactant percentage (5%, 20%, 25%, 30%, 60% wt/wt) and ratio (salt:acid- 2:1/1:2) of reactants as shown in Table 9.

Table 9. List of nine different types of chlorine dioxide releasing films

Film	PLA content (% wt/v)	Reactant content (% wt/wt)	Salt-acid ratio
1A	1	20	2:1
1B	1	20	1:2
1C	1	60	2:1
3A	3	20	2:1
3B	3	20	1:2
3C	3	5	2:1
3D	3	30	2:1
3E	3	30	1:2
3F	3	25	2:1

2.2.3 Release study

2.2.3.1 Preparation and standardization of chlorine dioxide stock solution

Chlorine dioxide stock solution was prepared by dissolving 10g sodium chlorite in 500 ml water in gas generating glass bottle. Sulfuric acid (20% (v/v)) was added intermittently through a separating funnel fitted with gas generating bottle. Chlorine dioxide gas produced was passed through a saturated solution of sodium chlorite using air flow to remove any acid vapor mixed with chlorine dioxide gas. Finally stock solution was prepared by bubbling the chlorine dioxide gas through distilled water. This solution was collected in dark glass bottle and stored at 4^{o}C. Stock solution was standardized by measuring absorbance at 360nm.

Dilute standard solutions were prepared from the stock solution and a standard curve for chlorine dioxide was generated using Rhodamine B [62]. 0.8 ml ammonia buffer at pH-10 and 0.8 ml Rhodamin B were added to 4 ml of chlorine dioxide solution and volume was made up to 10 ml in volumetric flask with reagent water. Absorbance was measured at 553 nm using Rhodamin B as indicator.

Figure 12. Preparation of chlorine dioxide stock solution

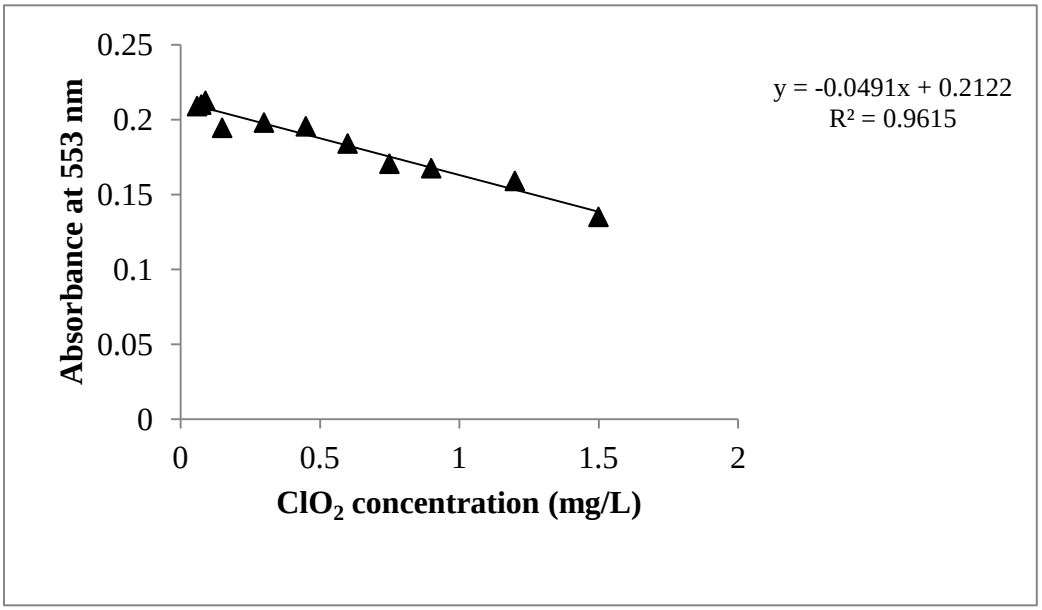


Figure 13. Standard curve for chlorine dioxide solution with Rhodamin B

2.2.3.2 Quantification of chlorine dioxide gas released from film

Chlorine dioxide releasing film was stuck inside wall of a tightly closed 250 ml mason jar maintained at 100% RH. Headspace (2 ml) was withdrawn at predetermined interval

with a gas tight syringe through the septum fitted on the lid and dissolved in reagent water in 4.5 ml amber glass vial fitted with Teflon screw top caps [60]. Absorbance of chlorine dioxide solution in the vial was measured by UV-Visible spectrophotometer at 553nm using Rhodamin B. Concentration of chlorine dioxide in solution was determined from the standard curve and from that concentration of chlorine dioxide gas in headspace was calculated. Average concentration was used for all calculations. Release study was conducted at ambient temperature (22±1^{o}C) as well as at 10^{o}C to study the effect of temperature on release profile of chlorine dioxide gas from the film.

2.2.4 Microbial assay for chlorine dioxide activity

2.2.4.1 Inoculum preparation

E. coli O157:H7 and a cocktail of *Salmonella* resistant to nalidixic acid were used in this study. *E. coli* O157:H7 ATCC 43894, *Salmonella* Panama 19454, *Salmonella* Poona 953, and *Salmonella* Stanley H0558 were taken from the culture collection of the U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center. The strains were maintained in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) at 2ºC and transferred bimonthly. Each fresh culture (10 ml) was grown overnight (18 h) in TSB at 37 ºC for use in experiments. The cell concentration was approximately 10^{9} CFU/ml, as determined by serial dilution in 0.1% peptone water, and 100-µl aliquots of various dilutions were spread plated onto tryptic soy agar (TSA; Difco, Becton Dickinson) and then incubated for 24 h at 37ºC.

2.2.4.2 Film screening for antimicrobial activity

Nine different types of films were tested for antimicrobial effectiveness against cocktail strain of pathogenic *Salmonella*. Inoculum of 10^{-6} dilution was spread plated and each plate was kept inside a clam shell box. One film per box was attached to the inside wall of the box and the boxes were incubated at 37^{o}C overnight. All the treatments as well as control were done in duplicate. Colony was counted after 24hrs of incubation. Based on the observation, five films were selected to test on grape tomato inoculated with *Salmonella* and *E.coli* O157:H7.

2.2.4.3 Sample preparation, inoculation and treatment with films

Grape tomatoes bought from local supermarkets were washed and rinsed with 70% ethanol to eliminate any possible background microorganisms. Samples were inoculated under biohood either by dipping the tomatoes in 50 ml inoculum for 2 minutes or spot-inoculation with 40 µl inoculum Inoculated samples were put in a clam shell box (24 oz) with the film (11 cm^2) stuck on the lid of the box and sealed. Treatment was given for 24hrs at 22^{o}C and 10^{o}C.

2.2.4.4 Microbiological analysis

Inoculated and treated samples were placed in an individual Whirl-Pak bag containing 10 ml of D/E Neutralizing Broth (BD), and hand-massaged for 1 min. Each homogenate was serially diluted in 0.1% peptone water (pH 6.9) (0.1 ml in duplicate) and surface plated on CT-SMAC for *E. coli* O157:H7 or TSA with 0.1% sodium pyruvate and 100

ppm nalidixic acid (TSAPN) for *Salmonella*. All plates were incubated at 37°C for 24 h before counting plates.

2.2.5 Quality analysis of fresh produce

2.2.5.1 Sample preparation

Grape tomatoes bought from local supermarket were washed with 100ppm chlorine solution and dried before experiment. A single layer of tomatoes (175g-180g) was placed in each clam shell box. Chlorine dioxide releasing film was stuck on lid of the box and closed. Samples were stored at 10^{o}C to mimic commercial practice. Treated samples and controls were withdrawn after 2, 7, 14 and 21 days to measure color and texture. O_2/CO_2 and ClO_2 concentrations inside the package were also measured prior to quality analysis of the fruits. The whole experiment was done in triplicates.

2.2.5.2. Color measurement

Color of tomato skin was evaluated instrumentally using Hunter UltraScan® VIS colorimeter (Hunter Associates Lab, Reston, VA., USA) using 1.3 cm measuring aperture to monitor any change in external color due to treatment by measuring lightness (L*), redness (a*) and yellowness(b*). Hue (ATAN (b*/a*)x 57.3) and chroma ($\sqrt{a^{*2}+b^{*2}}$) values were calculated from L*,a*,and b* values.

2.2.5.3 Texture measurement

Firmness of treated and untreated tomatoes was evaluated with a TA-XT2i Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, U.S.A.). A 3 mm diameter probe

was used to penetrate the fruit at 10 mm/s. Texture was reported in terms of firmness or softness and expressed as force (g) required for puncturing the tomatoes.

2.2.5.4 Oxygen/Carbon dioxide concentration

Oxygen and carbon dioxide concentration inside package was measured for each treatment on day 2, 7, 14 and 21 before opening the package. Measurement was done using a gas analyzer (DuralTrak 902 D, Quantek Instruments, Grafton, Mass., U.S.A) and O_2/CO_2 concentration was expressed as %.

2.2.5.5 Chlorine dioxide concentration

Concentration of residual chlorine dioxide inside the package was measured similarly as O_2/CO_2 using chlorine dioxide detecting tubes (Gastek, model 23M). Fifty milliliter of package headspace was withdrawn with the pump and passed through the tube fitted with pump and hold for 1min. Concentration of chlorine dioxide obtained from tube reading was multiplied by 2 to normalize for 100 ml air.

2.2.6 Statistical analysis

Results were analyzed by analysis of variance (ANOVA) by using SAS (SAS Inst. Inc., Cary, NC, USA). Any result with no letter in common are significantly different at p=0.05 significance level by Bonferroni LSD technique.

3 Results and Discussion

3.1 Optimization of reactants combination and ratio for production of chlorine dioxide

The most traditional method of chlorine dioxide production is reaction between sodium chlorite and inorganic acid.

$$5NaClO_2 + 4HCl = 4ClO_2 + 5NaCl + 2H_2O$$

In this research, HCl was replaced by food grade organic acids such as acetic acid, citric acid and lactic acid. Reaction of sodium chlorite with acetic acid, citric acid and lactic acid was monitored for 9 hrs at room temperature. Maximum amount of chlorine dioxide produced from these three acids was within the range of 26.6 ppm to 27.1 ppm (in solution). Therefore reaction of sodium chlorite with these three acids (salt-acid ratio 1:1) was quite comparable in terms of reaction kinetics and chlorine dioxide production (fig 14). For this set of experiment salt-acid ratio was maintained 1:1. Purpose of these experiments was to select the acid that generates maximum chlorine dioxide. Since reaction rate was quite similar for each acid, citric acid was selected for future experiments as it is one of the most commonly found acid in many fruits and vegetables.

To finalize the best salt-acid ratio, experiments were conducted with three different ratios of sodium chlorite and citric acid. Reaction of sodium chlorite with citric acid in different ratios produced different amount of chlorine dioxide, 26.1-34.8 ppm, although this difference was not too large but amount of chlorine dioxide produced and production trend from sodium chlorite – citric acid ratio 2:1 was different compared to that of 1:1 and 1:2. (fig 15). Similar observation was reported by Kim et al. for

production of chlorine dioxide from the reaction of sodium chlorite with acetic acid, citric acid and lactic acid in solution and results showed that chlorine dioxide production was significantly different only when sodium chlorite concentration was much higher[38].

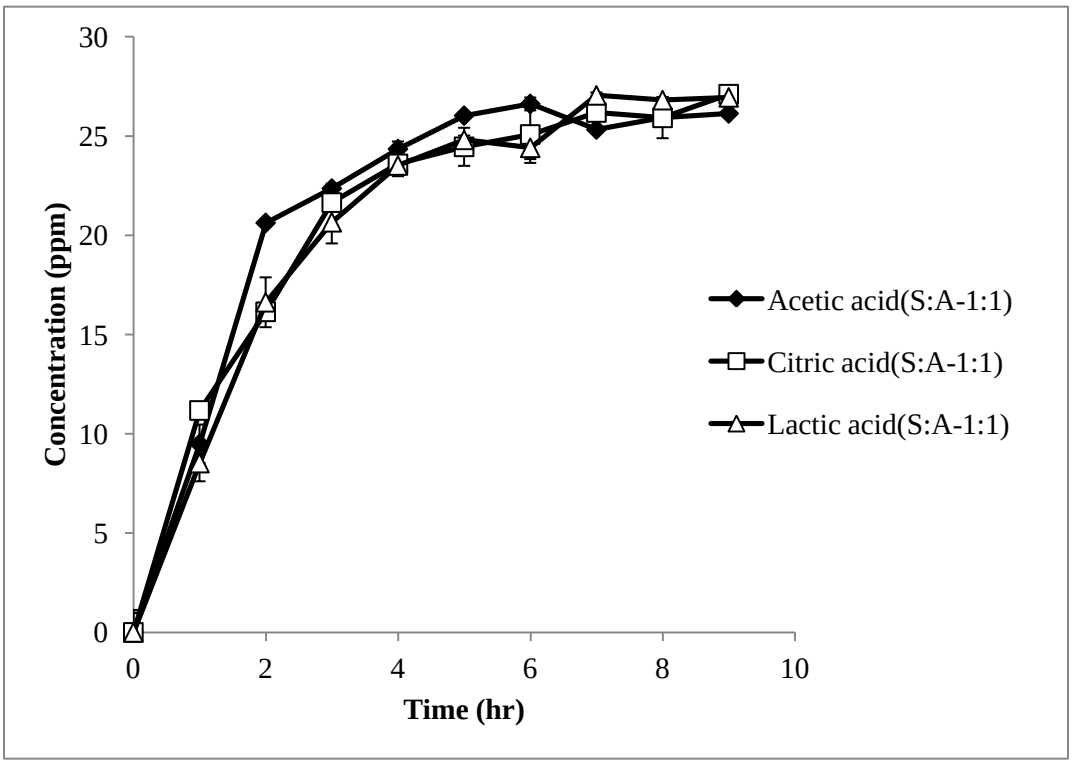


Figure 14. Production of chlorine dioxide from reaction of sodium chlorite with different organic acids (n=2)

However, salt-acid ratio 1:2 was also considered for film development to study whether the reaction trend remains same when incorporated in the polymer.

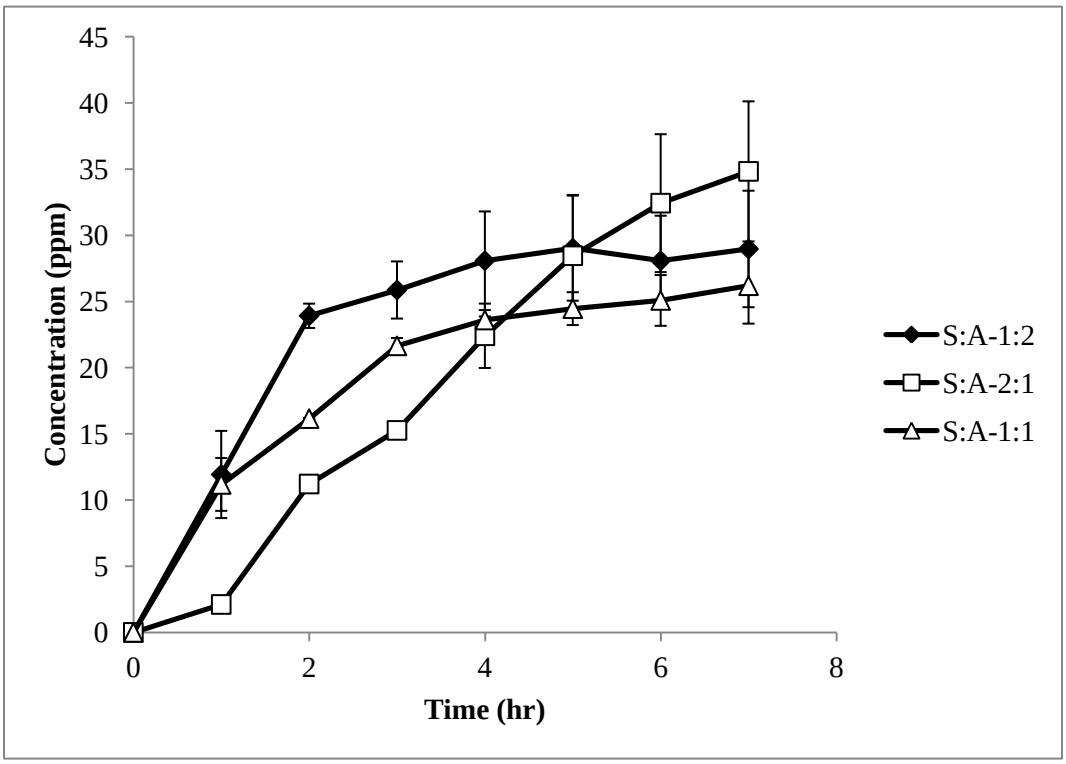


Figure 15. Production of chlorine dioxide from different ratios sodium chlorite and citric acid (n=2)

3.2 Effect of variables on release profile of chlorine dioxide

3.2.1 Effect of salt-acid ratio in the film

ClO_2 released from films with same reactant percentage but at different salt-acid ratio was determined. Chlorine dioxide released from film made with 300 mg PLA and 30% reactants at salt-acid ratio of 2:1 (3D) was not very different from that at salt-acid ratio of 1:2 (3E) during 5 h exposure to moisture (Figure 16). Also, maximum ClO_2 released from both films was not significantly different from each other which were achieved at 4 and 5 h, respectively. Similar trend was observed for the films made with 100 mg PLA and 20% reactants (Figure 17). The results match those obtained in our preliminary

study when the reactants were directly mixed together without incorporating in PLA. However, the ClO_2 released from films was less than that from direct mixture when the same amount of reactants was used. This may be because when salt and acid molecules are distributed within PLA matrix, there is some additional barrier between these molecules and not all of them are in direct contact, which is one of the key factors for production of chlorine dioxide. Ratio of salt to acid didn't affect the quantity of ClO_2 released from films, suggesting salt to acid ratio is not a critical parameter for controlling ClO_2 release.

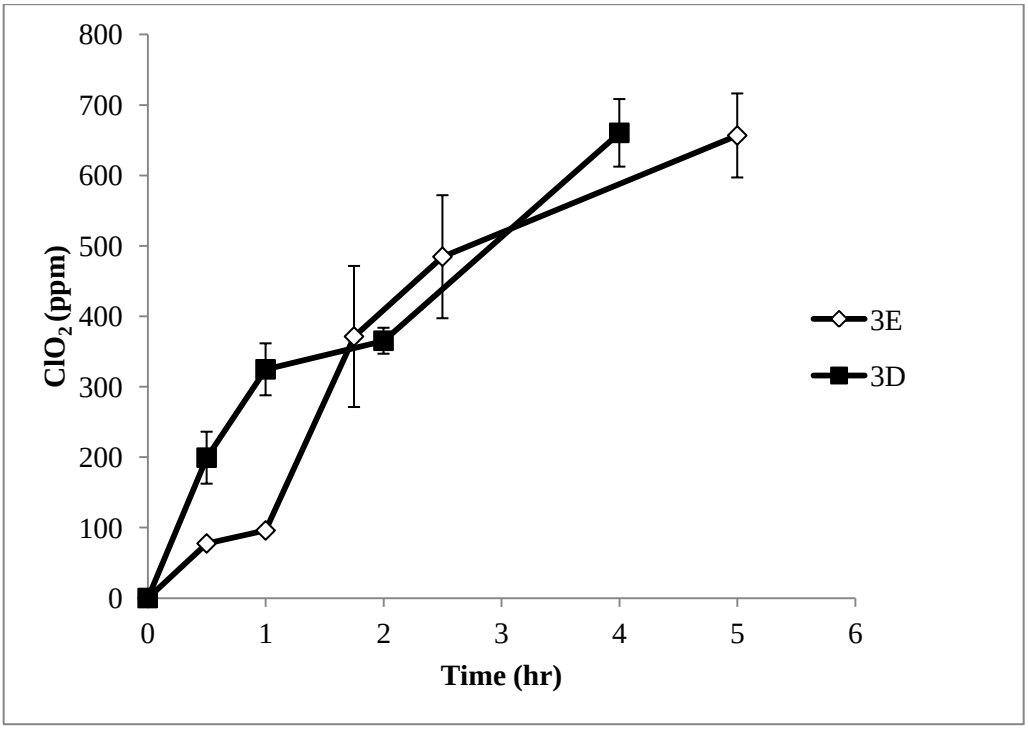


Figure 16 . Effect of salt-acid on release of chlorine dioxide from film made with 300 mg PLA (n=3)

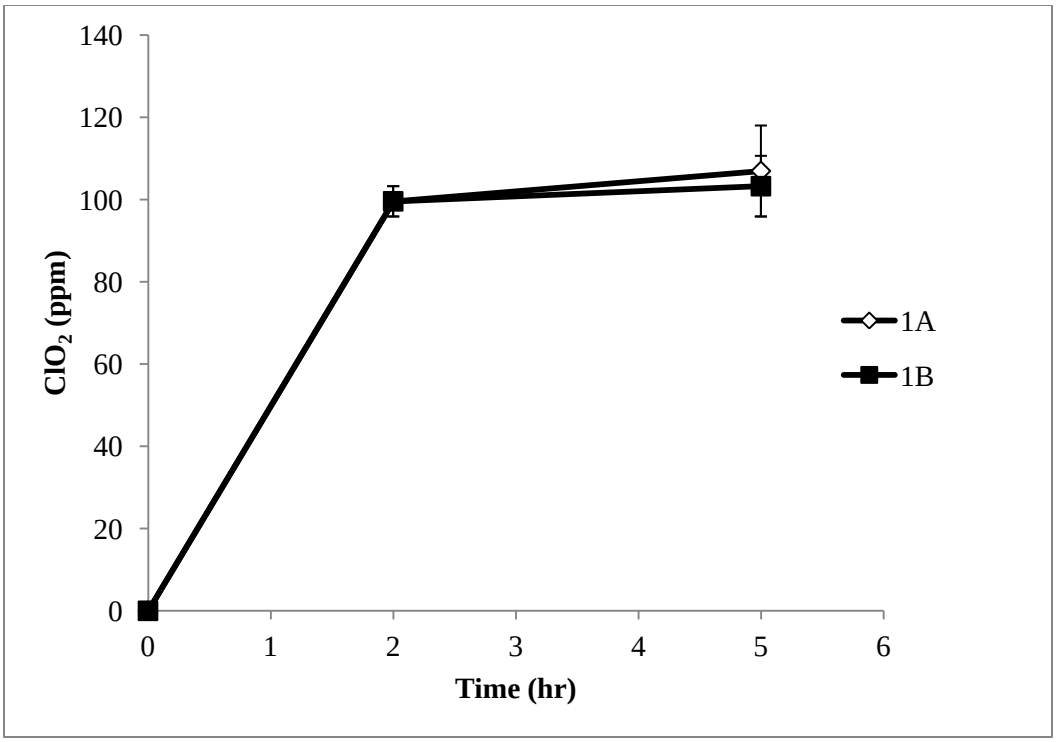


Figure 17. Effect of salt-acid on release of chlorine dioxide from film made with 100 mg PLA (n=3)

3.2.2 Effect of reactant concentration in the film

Reactant percentage is defined as the percentage of total reactants (acid and salt) in PLA. As expected, ClO_2 released from the films increased with an increase of reactants content in the film (Figure 18). ClO_2 released from the film with highest reactant (30%) was 670 ppm at 4 h, while films with 20% and 5% reactants generated 420 ppm and 30 ppm ClO_2 at 5 h, respectively (Figure 18). Similar results were observed with 100 mg PLA films, film with 60% reactants reached 600 ppm at 5 h while that with 20% reactants only had 100 ppm (Figure 19). These data indicate that reactant percentage is critical parameter for controlling the release of ClO_2 from films.

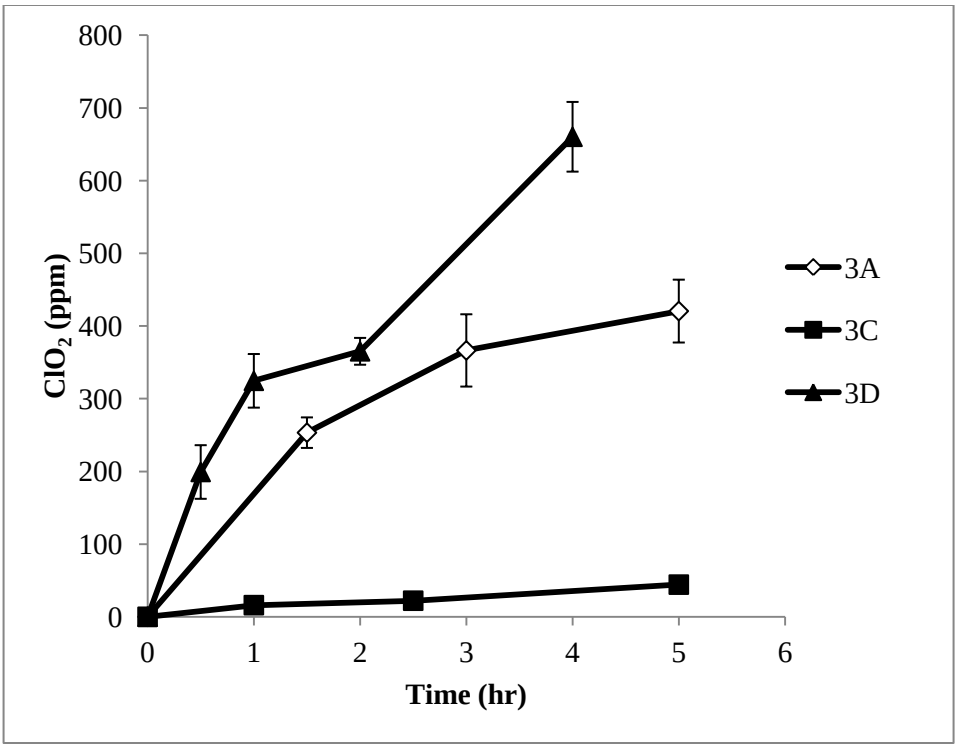


Figure 18. Effect of reactant percentage on release of chlorine dioxide from film made with 300mg PLA

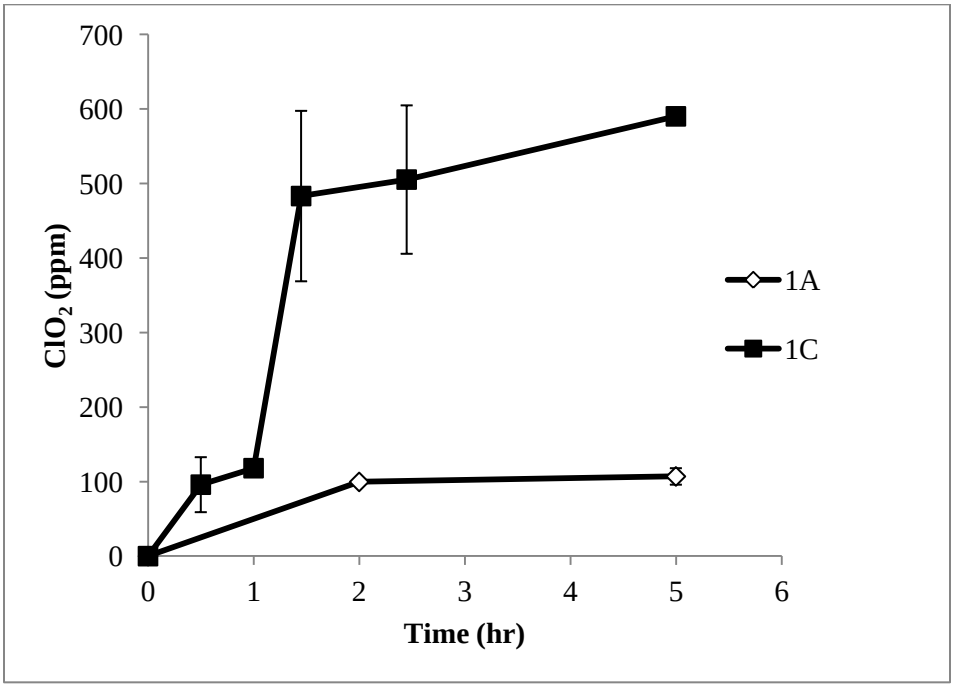


Figure 19. Effect of reactant percentage on release of chlorine dioxide from film made with 100mg PLA

3.2.3 Effect of film thickness

The thickness of PLA film containing 100 mg PLA was 0.11 mm and that of 300 mg PLA was 0.13 mm. ClO_2 released from film made with 300 mg PLA film (420 ppm) was greater than that released from film made with100 mg PLA (110 ppm) at 5 h, both films containing 20% reactant and salt-acid ratio 2:1 (Figure 20). Similar results were observed in the films at 1:2 acid-salt ratios (Figure 21). Because reactant percentage was related to PLA content and increase in PLA content (from 100 mg to 300 mg) also increased the concentration of reactants in the films, the effect of thickness was actually positively associated with the ClO_2 released from the film. Therefore, another experiment was conducted to test real effect of film thickness on ClO_2 release. Both films (300 mg PLA and 100 mg PLA) containing same amount of reactants (60 mg) but not same reactant percentage (1C vs. 3A) were used in this test. The increase of PLA in film resulted in an increase of film thickness and consequently reduced the release of ClO_2 (Figure 22). These results suggested that film thickness can be a controlling factor for release of chlorine dioxide as showed in figure 22, where release of chlorine dioxide reduces with increase in film thickness, keeping the amount of reactant same in both the films. However, chlorine dioxide release increases when both film thickness and reactant concentration in the film increases (Figures 20 & 21). As production of ClO_2 is a relative humidity dependent reaction and PLA is a hydrophobic polymer [63-65], sufficient moisture could not reach inside the polymer matrix [66]. Therefore, ClO_2 was produced only from sodium chlorite and citric acid present on the film surface. For relative humidity dependent reactions, hydrophilic polymers would be a better choice to achieve controlled release as moisture cannot penetrate inside hydrophobic polymers;

reactants present only on the surface of the hydrophobic films would affect the release and molecules entrapped within the polymer matrix would not participate in the reaction or diffuse towards the surface. Another approach could be modification of hydrophobic polymers like PLA with various hydrophilic materials to have a composite with increased moisture absorption capacity [66, 67]. .

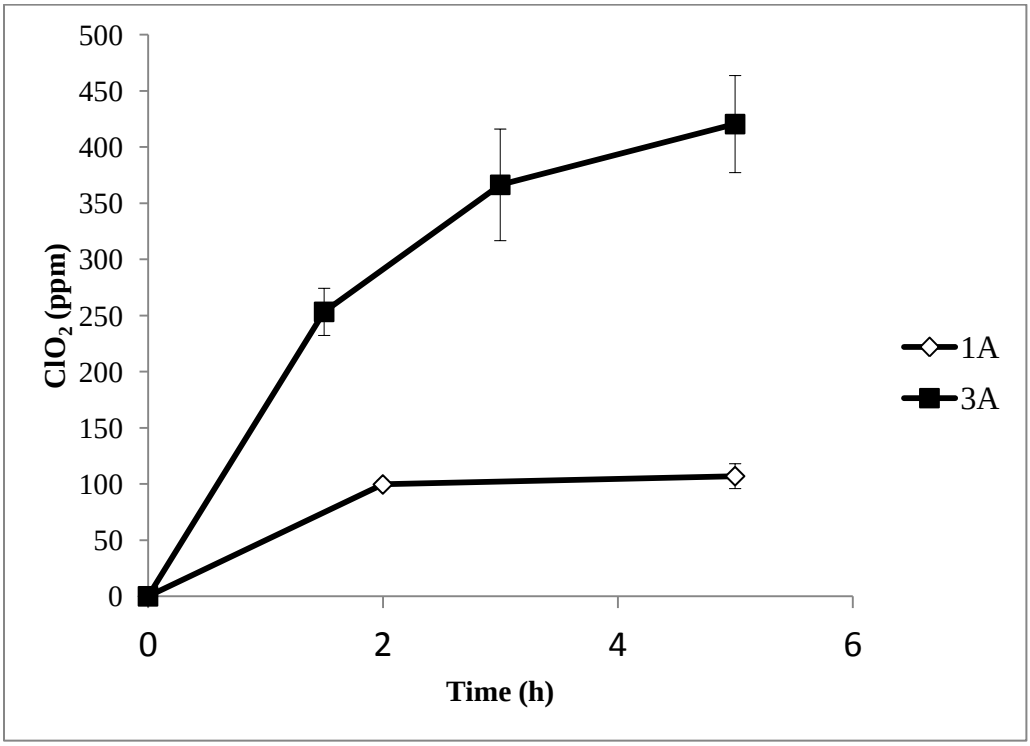


Figure 20. – Effect of film thickness on release of chlorine dioxide from films with salt-acid ratio of 2:1

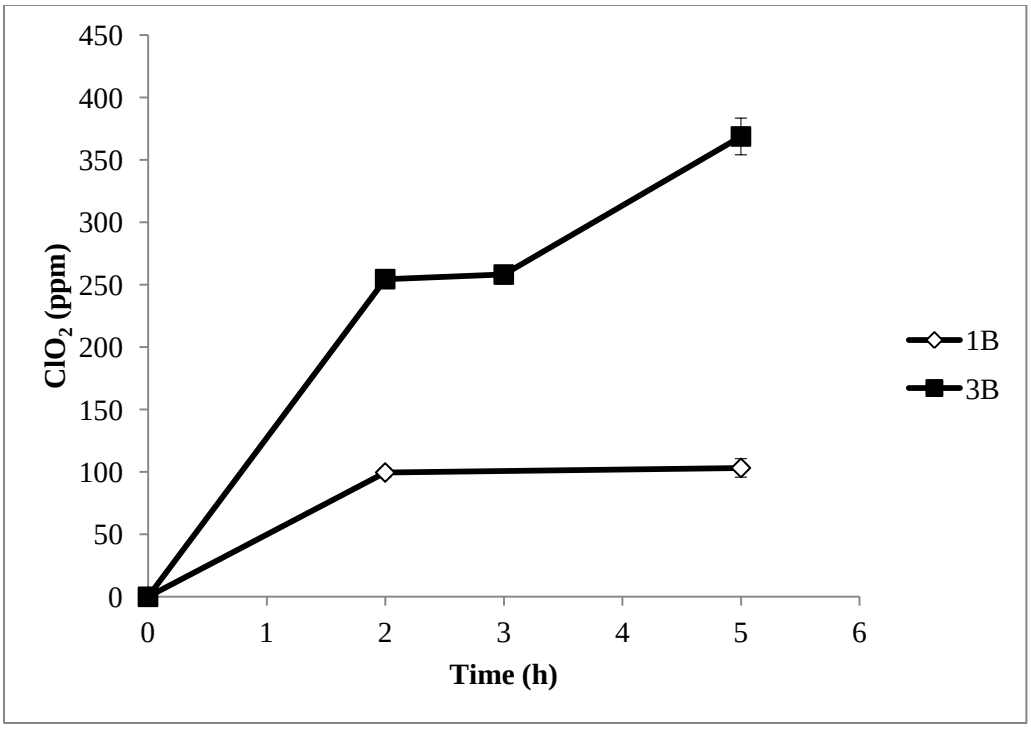


Figure 21. – Effect of film thickness on release of chlorine dioxide from films with salt-acid ratio of 1:2

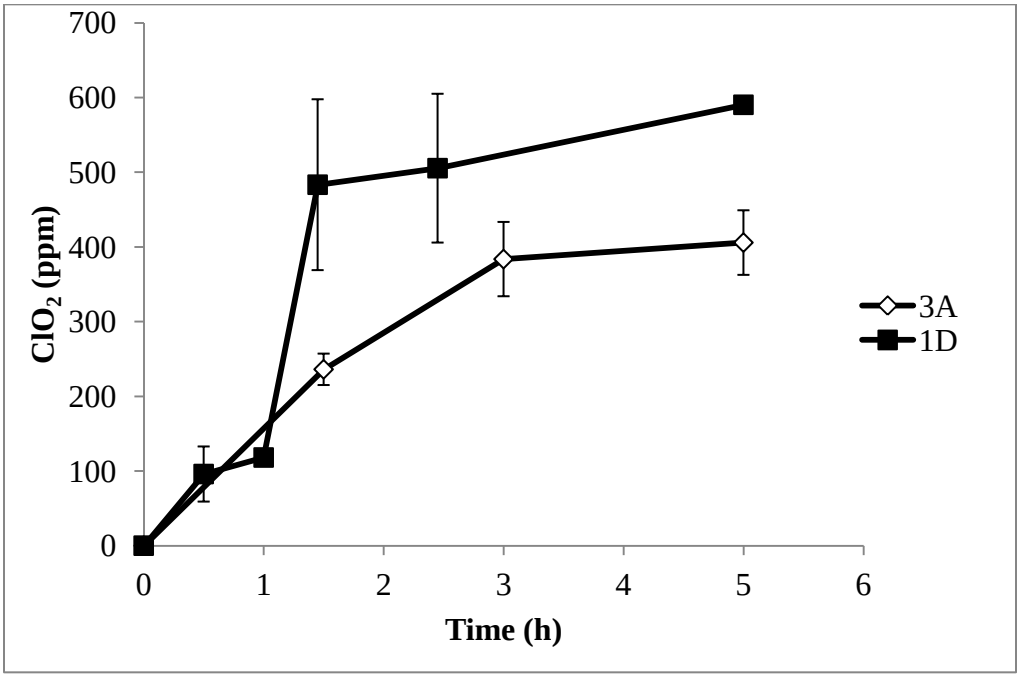


Figure 22. – Effect of film thickness on release of chlorine dioxide from films of different thickness having same amount of reactant with salt-acid ratio of 2:1

3.2.4 Effect of temperature

One film with highest release rate (3E) was selected for a comparison test at 22°C and 10°C. Figure 23 shows that the film at both temperatures had similar release trend. However, the film at room temperature released slightly more ClO_2 than that at 10°C during 1 to 2.5 h while the release rate of film at 10°C was slightly higher than that at room temperature during 2.5 to 5 h. This suggests that activating temperature in general did not affect the release dramatically and therefore these films are suitable for use at 22°C or 10°C.

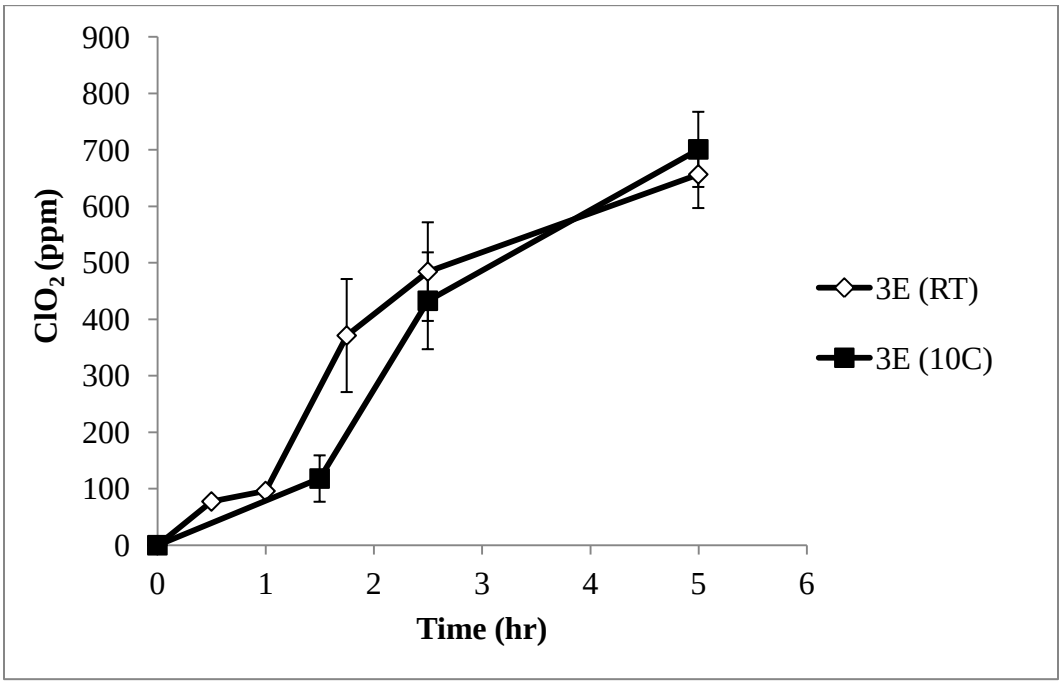


Figure 23. Effect of temperature on release of chlorine dioxide from film. 3E: 3% PLA, 30% reactants, 1:2 salt:acid.

3.3 Antimicrobial activity of chlorine dioxide films

3.3.1 Preliminary microbial assay for film screening

A preliminary microbial study was done with 9 different films using cocktail strain of pathogenic *Salmonella spp*. Based on the result five films were selected in such a way that they cover all the variables under consideration. Selection was done on the basis of results from release study and microbial experiment (Fig.24). Films that provided at least 1 log reduction (films above the red line in Fig.24) were chosen for further antimicrobial study and anything less than that wasn't considered for future experiments. Films selected were 1C – 1% PLA with 60% reactant, 3C – 3% PLA with 5% reactant, 3F – 3% PLA with 25% reactant, 3D & 3E – 3% PLA with 30% reactant and different salt-acid ratio.

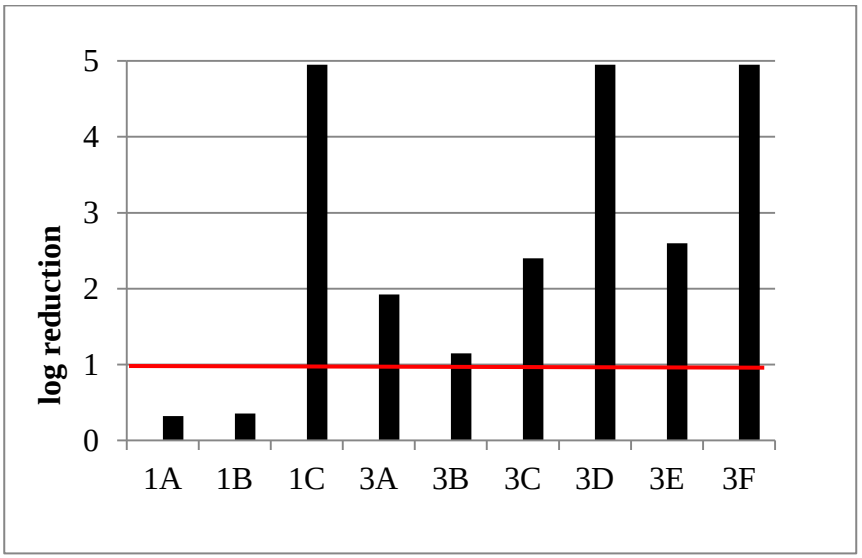


Figure 24. Anti-microbial effectiveness of different films evaluated against *Salmonella spp*.

3.3.2 Antimicrobial efficacy of chlorine dioxide film

Preliminary screening work was done to determine antimicrobial activities of nine types of films initially developed (Fig 24), and five films of those nine films, which showed promising antimicrobial activity against the pathogens in microbial media, were selected for further study. Table 10 shows the survival of *Salmonella spp.* and *E. coli* O157:H7 after 24 h treatments with ClO_2 releasing films. Control samples at 22^oC had 4.26 and 3.61 log CFU/tomato of *Salmonella* and *E. coli* O157:H7, respectively. No *Salmonella* or *E. coli* cells were detected (<5 CFU/tomato) in any treatment except for the film made with 300 mg PLA containing 5% reactant (3C) for *Salmonella*. Additional test for *Salmonella* at 10^oC showed that all film treatments reduced the pathogen to undetectable levels (Table 10). The least antimicrobial activity of 3C film was expected since the film released least ClO_2 gas during the release study (Figure 18). In the present study, the ClO_2 releasing films achieved minimum 3 log reduction of pathogens on tomatoes. More microbial reduction could be achieved if tomato surface contained higher initial bacterial cells, which needs to be further investigated. Antimicrobial effectiveness of the films in this study is comparable with many ClO_2 gas treatments that have been studied so far. Mahmoud and others [12], Richards and Beuchat [68] and Sy and others [25] reported 4.7 log reduction/strawberry and 4.3 log reduction /tomato when treated with 5 mg/L chlorine dioxide for 10 min and 4.1 mg/L for 25 min respectively. Another study showed that approximately 4.87 log reduction/cm^2 of tomato was achieved with 10 mg/L chlorine dioxide for 180s [69].

Table 10. Survival of *Salmonella* and *E.coli* O157:H7 on grape tomato

Microorganism	Temperature	Treatment	Bacterial population (log CFU/tomato)
Salmonella spp.	22^{o}C	Control	4.26±0.23
		3C	1.08±0.26
		3D,3E,3F,1C	ND*
	10^{o}C	Control	5.17±0.35
		3C,3D,3E,3F,1C	ND*
E. coli O157:H7	22 ^{o}C	Control	3.61±0.01
		3C,3D,3E,3F,1C	ND*

*ND – Not detected

3.4 Quality analysis of grape tomato treated with chlorine dioxide film

3.4.1 Effect on color

Changes in tomato color were monitored during storage at 10^{o}C for 21days. L*, Chroma and Hue values are reported in Table 11. During storage, L*, Chroma and Hue values decreased slightly for all samples including controls, however, statistical analysis shows that there was no significant difference ($p>0.05$) in color change among all the samples, indicating that none of the films had a deleterious effect on tomato color during 21 days of storage at 10^{o}C. Other studies have shown that treatment with chlorine dioxide gas and solution did not significantly affect the color of roma tomatoes [69] and strawberry

[70] which agree with our findings. Other researchers also reported that treatment with chlorine dioxide gas did not affect the external color and visual appearance of strawberry and whole cantaloupe [12, 71]. Therefore, the films in these ranges of reactant percentage (5% - 30%) used in our study are suitable for grape tomatoes.

3.4.2 Effect on texture

Changes in texture of tomatoes during storage at 4°C were observed for all the samples including controls (Figure 25). However, statistical analysis showed that no significant difference ($p>0.05$) in texture changes among the film treatments. Apart from firmness, researchers have studied if chlorine dioxide treatment causes any shrinkage or wrinkling of the fruit skin. Trinetti and others (2010) reported that treatment with 10 mg/L ClO_2 gas for 180 s resulted in 4.87 log reduction of *Salmonella* but caused wrinkle on the tomato skin [69]. However, in our study films with highest reactant concentration (3D and 3E) had almost equal (at 22^oC) or even better (at 10^oC) antimicrobial effect but did not cause wrinkle or any other visually perceivable changes in the sample. This may indicate that a long time exposure at lower concentration has better effect than high concentration – short time exposure. Possible reason could be as film releases ClO_2 slowly over a long period of time, samples get enough time to adapt to slowly increasing chlorine dioxide environment instead of exposing them to a very high concentration at once.

Table 11. Changes in color of tomato skin during 21 days storage at 10^{o}C

Parameter	Treatment	Storage time (d)				
		0	2	7	14	21
L*	Control	35.58±1.89	33.14±1.24^{a}	33.72±1.27^{ab}	33.76±1.34^{a}	33.20±1.25^{a}
	3C	35.58±1.89	33.57±1.56^{a}	33.59±1.40^{ab}	33.32±1.06^{a}	32.86±1.20^{ab}
	3D	35.58±1.89	33.57±1.73^{a}	33.04±1.08^{b}	33.42±1.08^{a}	32.37±1.19^{b}
	3E	35.58±1.89	33.22±1.35^{a}	33.68±1.32^{ab}	33.84±1.41^{a}	33.19±1.57^{a}
	3F	35.58±1.89	33.74±1.70^{a}	33.81±1.74^{a}	33.42±1.31^{a}	33.02±1.47^{ab}
	1C	35.58±1.89	33.16±1.32^{a}	33.65±1.33^{ab}	33.26±1.36^{a}	33.12±1.10^{ab}
Chroma value	Control	31.21±2.9	29.09±2.84^{ab}	29.30±3.35^{a}	29.64±3.11^{a}	29.61±3.37^{a}
	3C	31.21±2.9	29.15±2.86^{ab}	28.88±2.54^{a}	28.46±2.71^{a}	29.31±3.47^{a}
	3D	31.21±2.9	28.14±3.48^{b}	28.64±2.26^{a}	29.39±2.34^{a}	27.54±2.34^{a}
	3E	31.21±2.9	29.43±2.86^{ab}	29.44±2.64^{a}	29.82±2.89^{a}	28.72±3.09^{a}
	3F	31.21±2.9	30.47±2.9^{a}	29.37±2.46^{a}	28.70±2.99^{a}	28.78±3.64^{a}
	1C	31.21±2.9	28.71±2.75^{b}	29.59±2.81^{a}	29.35±2.08^{a}	29.25±2.82^{a}
Hue value	Control	41.39±2.84	39.63±2.61^{a}	39.71±2.69^{a}	39.77±2.30^{a}	39.09±2.11^{ab}
	3C	41.39±2.84	40.07±2.54^{a}	39.51±2.06^{a}	39.20±2.68^{a}	38.89±2.30^{b}
	3D	41.39±2.84	41.24±4.55^{a}	38.80±2.34^{a}	39.21±2.37^{a}	39.74±2^{ab}
	3E	41.39±2.84	40.09±2.69^{a}	39.88±2.39^{a}	40.27±2.57^{a}	40.37±3.18^{a}
	3F	41.39±2.84	40.60±2.94^{a}	39.62±2.76^{a}	39.71±2.18^{a}	40.05±2.64^{ab}
	1C	41.39±2.84	40.68±2.51^{a}	38.8±2.34^{a}	39.56±3.04^{a}	40.05±2.92^{ab}

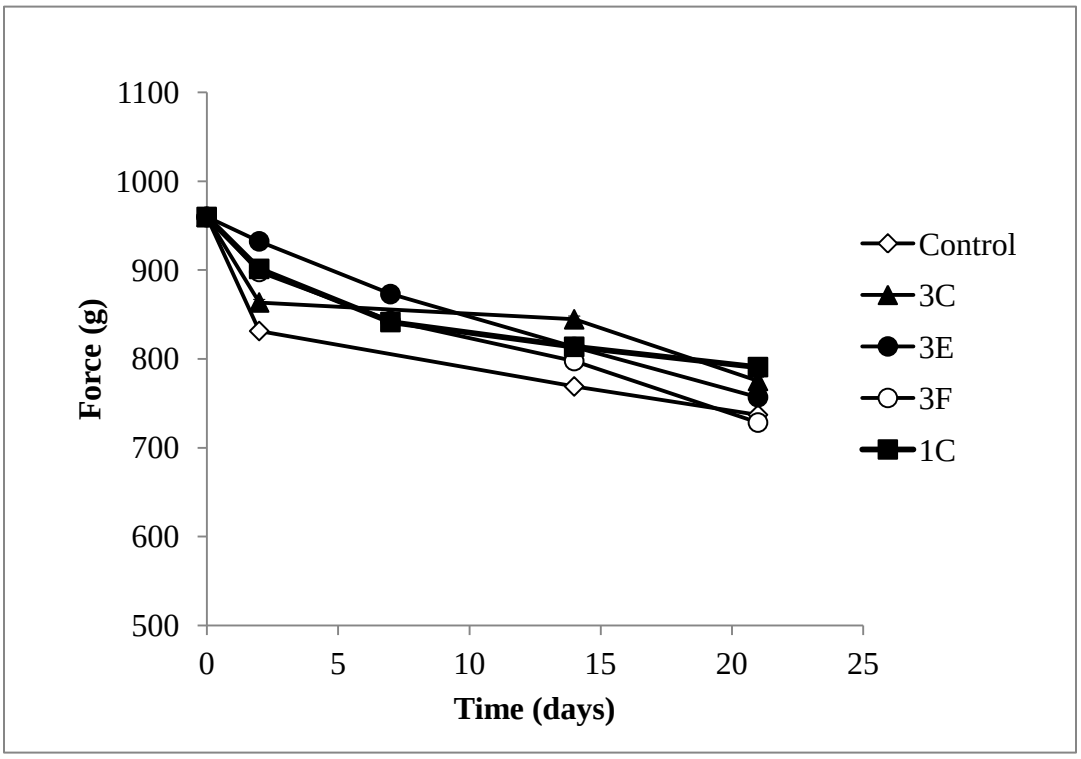


Figure 25. Effect of chlorine dioxide film treatment on texture of grape tomatoes (n=3)

3.4.3 Chlorine dioxide concentration inside package

Concentrations of ClO_2 gas inside each package containing tomatoes were measured at 2, 7, 14, and 21 days using ClO_2 gas detecting tubes. Similar to film tests in glass jars, packages with films containing higher reactant percentage had higher concentration of ClO_2 compared to others when measured at same time. However, ClO_2 depleted in all the packages over time and reached below detection limit after 7 days of storage. This result is expected and could be combination of several factors, such as, absorption of the gas by fruit, loss of gas through seals and leaks of the package and decomposition of chlorine dioxide gas molecules over time as it can participate into series of reaction and

can degrade to chlorite and chlorate which cannot be detected by ClO_2 detecting tubes used in this research [72].

Table 12. Chlorine dioxide gas concentration inside package over 21 days at 10^{o}C

Parameter	Film type	PLA %	Reactant %	Salt-acid ratio	Storage time in Days			
Chlorine dioxide concentration (ppm)					2	7	14	21
	3C	3	5	2:1	0.3	0.2	0.1	BDL*
	3D	3	30	2:1	8	1	0.5	BDL*
	3E	3	30	1:2	9	1.8	BDL*	BDL*
	3F	3	25	2:1	5	4	1.5	BDL*
	1C	1	60	2:1	3	1.5	0.5	0.5

***** BDL – Below detectable limit

3.4.4 Oxygen/carbon dioxide concentration inside package

O_2/CO_2 concentration was monitored primarily to study if presence of chlorine dioxide inside the package for long time affects respiration of the produce. CO_2 concentration in the package increased gradually for all samples, both treated and untreated. However untreated samples reached maximum CO_2 level on Day 7 whereas treated samples reached maximum CO_2 level on Day 14 except for treatment with film containing 5% reactant (3C) which showed an increasing trend till day 21. An increased level of CO_2 suggests that chlorine dioxide treatment suppressed the respiration. Research conducted by Du et al. showed that chlorine dioxide treatment delayed the respiration rate of green bell pepper when stored at 10^{o}C for 40days which corresponds to our findings in current research [73]. Other studies have also shown that chlorine dioxide treatment delayed the respiration peak of plums [74] and respiration rate of apricots [75]. Due to respiration O_2

level in package decreased from 18% to 11% or even lower over time for both treated and untreated samples till Day 14. After that it increased again when measured on Day 21 except for untreated samples which decreased even further. Similar observation was reported for combined application of active packaging and chlorine dioxide treatment to extend shelf life of strawberries [76].

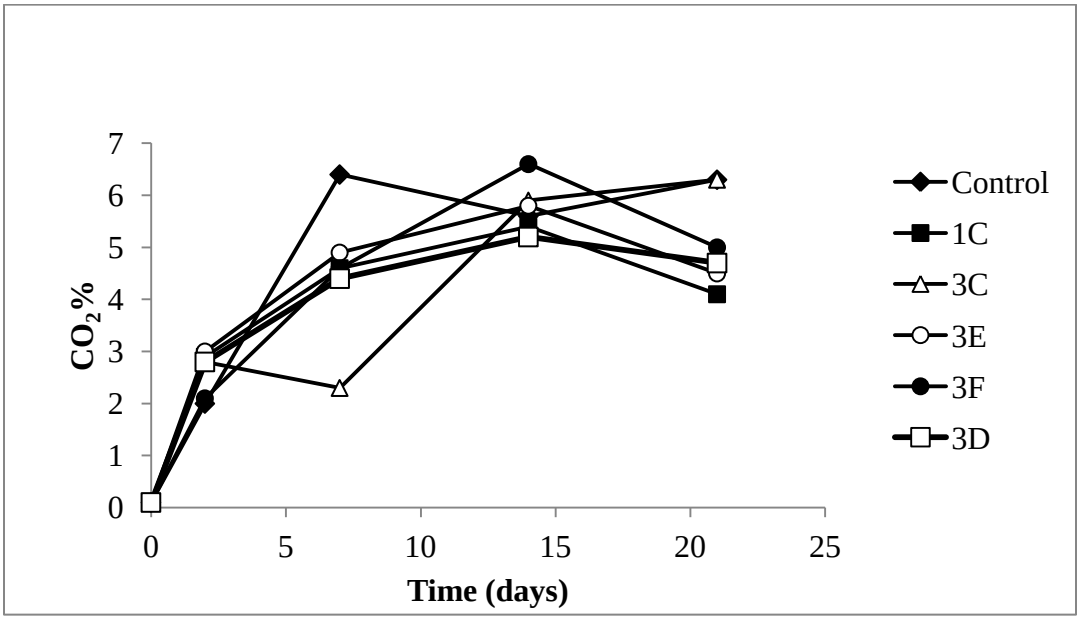


Figure 26. Carbon dioxide concentration inside package over 21days storage at 10^{o}C

4 Conclusion

Results have shown that it is technically feasible to develop chlorine dioxide releasing film by incorporating sodium chlorite and citric acid in poly-lactic acid polymer. The amount of moisture generated by respiration of grape tomato was sufficient to activate the chemical reaction and release of ClO_2 gas. ClO_2 released from films inside the package effectively inactivated *E. coli* O157:H7 and *Salmonella* spp. inoculated on grape tomatoes, achieving more than 3 log reduction of both pathogens and yet not affecting texture, color and visual appearance of the fruit during 21 days storage at 10^oC.

This research has three major components, 1) release study of film, 2) antimicrobial study and 3) quality analysis of the fruit. Results of each component are discussed below in detail.

1) Release study of chlorine dioxide releasing film

a. Reactant ratio – the ratios used in this study did not have much effect on the release profile because of additional barrier as well as uneven distribution of the reactant molecules in the polymer matrix.
b. Reactant concentration – results obtained from the experiment indicated that increase in reactant content from 5-30% or 20-60% increased the release of chlorine dioxide from 100-600 ppm indicating reactant concentration as a key variable affecting the release profile.
c. Film thickness – this is a key factor controlling the release profile of chlorine dioxide mainly due to distribution the reactant molecules along the thickness of the film.

d. Temperature – temperature range (10^{o}C-22^{o}C) used in this study did not affect the release profile dramatically suggesting that films are suitable for use at any temperature within that range. However, a further study outside this temperature range is needed to confirm this.

2) Antimicrobial study – it is evident from the results that concentration of chlorine dioxide released from the film was able to provide a minimum of 3 log reductions of both *Salmonella* and *E.coli* O157:H7. At low temperature, films with higher concentrations were able to achieve over 5 log reductions of *Salmonella*. Therefore depending on the situation, these films can be used to achieve 3-5log reductions of pathogens.

3) Quality analysis – Chlorine dioxide concentrations that provided 3-5 log reduction did not affect the texture, color and visual appearance of grape tomatoes when treated for 21days at 10^{o}C. Moreover, all treatments suppressed the respiration of grape tomatoes to some extent which is an important factor for shelf life of fresh produce.

From the above findings it can be concluded that to develop a single layer chlorine dioxide releasing films, concentration of precursor compounds in the film and film thickness plays an important role controlling the release profile to reduce microorganisms to a safe level without affecting quality of the produce. This study demonstrated the technical feasibility to develop a gaseous chlorine dioxide releasing packaging system and its potential application for decontaminating grape tomato, and perhaps it can be extended to other fresh produce.

5 Limitation

Like any other research, this study also has its own limitation. The chlorine dioxide releasing packaging system proposed in this study is applicable only under specific circumstances and further study is required to make it more generic. Following are the key limitations identified for single layer chlorine dioxide releasing films:

- Films are sensitive to moisture. Since reaction of precursor compounds for the production of chlorine dioxide is moisture controlled, so the film having both the compounds in contact to each other will start generating chlorine dioxide if not stored under proper conditions. Recommended storage condition will be either a room with very low humidity or sealed package with inert gas.
- Since production of chlorine dioxide is moisture dependent, hydrophilic polymer would be a better option as they can absorb moisture. However preparation of films by solution casting method is not a feasible option as most of the hydrophilic polymer dissolves in water and not in organic solvents. Addition of precursor compounds in water will trigger the reaction immediately, so water cannot be used as a solvent in this case to dissolve the polymer.

6 Future work

- In this research, films were tested on grape tomatoes only. Further testing is needed on other categories of produce especially leafy greens as they have uneven surface and are very sensitive to any treatment. Also it would be a good idea to test the films on meat, seafood and dairy product to investigate other potential area of application.
- Since production of chlorine dioxide is moisture dependent, hydrophilic polymer would be a better option for this system. Poly-lactic acid is a hydrophobic polymer, so enough moisture could not get inside the matrix and only the reactants on surface took part in the reaction. Further work may be done to identify hydrophilic polymer or poly-lactic acid polymer in combination with moisture absorbing materials and evaluate their performance for release of chlorine dioxide.
- This research was focused on feasibility study for development of single layer film. Future study could be done to develop multi-layer films or gel for coating application.
- Microscopic imaging could be another area to find the localization of precursor compounds within the polymer matrix. This information will be helpful for selecting other polymer for this system.
- In this research, films were made using solution casting method. To scale it up for commercial application, other design variables need to be evaluated, the most important being film preparation by extrusion. Thermal stability of the precursor compounds and their distribution in the polymer matrix, maintaining low moisture production environment and drying of film are definitely some of the key factors worth researching.

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