FEASIBILITY STUDY OF

NOVEL GASEOUS CHLORINE DIOXIDE GENERATING METHOD

FOR PATHOGEN INHIBITION ON FRESH PRODUCE

by

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And approved by

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ABSTRACT OF THE DISSERTATION

Feasibility Study of Novel Gaseous Chlorine Dioxide Generating Method for Pathogen Inhibition on Fresh Produce

By SIYUAN ZHOU

Dissertation Director:
Professor Kit L. Yam

Based on the understanding of its generating mechanisms, this research hypothesized a novel method of $ClO_{2(g)}$ generation that utilizes two of the major respiration products (i.e. CO_2 and moisture) naturally released from fresh produce to provide H_2CO_3, react with $NaClO_2$ and generate $ClO_{2(g)}$. Through four consecutive experiments, this hypothesis was primarily demonstrated.

Then, the basic chemistry and microbiology mechanisms related to this hypothesis were further investigated. This research demonstrated that only when the three components (i.e. CO_2, moisture and $NaClO_2$) existed in the system, $ClO_{2(g)}$ could be generated and exhibit complete inhibition effects against *Salmonella* spp. The impact of different factors including $NaClO_2$ content, CO_2 content, RH content, temperature

and light conditions in the release profile of $ClO_{2(g)}$ and the inhibition effects against *Salmonella* spp. were systematically investigated. The basic chemistry mechanisms including the relationship between pH and ClO_2 generation profile as well as the microbiology mechanisms including "D value" and "Z value" were obtained.

To help practically apply this demonstrated hypothesis, two easy-to-use delivery systems for $NaClO_2$ as Tyvek sachet and gum arabic paste were developed. The successful generation of $ClO_{2(g)}$ from both systems were confirmed through analytical methods directly and microbial inhibition experiments indirectly. The complete inhibition effects from Tyvek sachet and gum arabic paste against *Salmonella* spp. using real fresh tomato as the source of CO_2 and moisture were observed. For Tyvek sachet, the optimal amount of $NaClO_2$ needed in the delivery system was determined. Besides the two easy-to-use delivery systems, a promising delivery system for $NaClO_2$ as electrospun fiber was also developed. The physical properties of different polyethylene oxide (PEO) and $NaClO_2$ water solutions as well as the morphology and diameter distribution of fibers electrospun from these solutions were obtained and analyzed. The successful loading of $NaClO_2$ onto the electrospun fiber was observed by scanning electron microcopy (SEM) images and the loading efficiency was calculated to be 83.9% (±3.3%). The microbial inhibition effects from electrospun fiber against *Salmonella* spp. was observed both under simulated conditions as well as on the surface of real fresh tomato.

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First of all, I would like to give my deepest gratitude to my supervisor- Dr. Kit L. Yam for his great and constant guidance, help and support during the past 6 years that I stayed in the lab. Without him, I would not have a chance to finish this work in a professional way within such a short time period. He did not only guide me on how to do research, but also taught me in how to develop myself in every way. In this sense, he is more like a mentor or a father to me.

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texture analyzer and colorimeter; Xiaoyuan Wang and Zhenyu Lin, who allowed me to use the electrospinning machine.

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1 INTRODUCTION

"'Fresh produce' is a generalized term for a group of farm-produced crops and goods, including (but not limited to) fruits and vegetables" [1]. It is an important part of healthy diet for human beings, containing different types of necessary nutrients that are essential to good health. In general, its major health-benefits include "helping prevent cardiovascular disease, cancer, diabetes, lower blood pressure as well as maintain gastrointestinal health and good vision" [2-16]. In recent years, its yearly sales value and consumption amount have been increasing sharply and exceeded 100 billion U.S. dollars (USD) and 300 pounds per capita respectively [17-20]. However, the frequent pathogen outbreaks on fresh produce have been placing an obstacle to the revenue of this growing industry and a major safety concern to the consumers [21-23]. Within all the fresh produce related outbreaks, *Salmonella* was one of the most popularly involved pathogens, taking an overall percentage as high as "18%" between the year of 1995 and 2005 [21].

As a common type of fresh produce, tomato is "an edible, often red fruit/berry of the nightshade *Solanum lycopersicum"* [30]. It is considered to be the second largest produced vegetables in the U.S. [31]. The respiration rate of tomato is relatively low within the range of "10 - 20" mg/kg×hr at 5 ^{o}C [28]. Tomato has been involved in a great number of pathogen outbreaks (especially with "*Salmonella*" combination), resulting in more than 1500 related illnesses between the year of 1998 and 2005 [21].

Chlorine dioxide, with the formula as ClO_2, is a commonly used chemical for a variety of industry applications. Its generation could be achieved from different kinds of reactants used. If starting with $NaClO_2$, the first step is usually to generate chlorous acid ($HClO_2$), which is the precusor for chlorine dioxide formation. After chlorous acid is formed, the generation of chlorine dioxide could proceed via the disproportionateness reaction [35,36]. A good number of patens regarding this generating method have been filed [37-41]. Among them, Patent 6,761,872 B2 filed on June 26, 2002 by Roensch Fred et al. has provided an innovative way utilizing carbon dioxide gas to react with sodium chlorite solution for chlorine dioxide generation [37].

Chlorine dioxide has been reported to have a great antimicrobial effectiveness against a board spectrum of bacteria since the first publication in 1949 [42]. Its working mechanism has not been clarified so far but could possibly be owned to the oxidative attack on cell membrane proteins and enzymes [43,44]. Gaseous phase chlorine dioxide usually has a greater antimicrobial effectiveness than its aqueous solution, due to the better penetrating ability [45-47]. Numerous research have observed and reported the great antimicrobial effectiveness of gaseous chlorine dioxide against pathogen growth on fresh produce [48-59].

"Control release" is a novel technique that releases active compounds (such as antimicrobials) in a timely and controlled manner to the food matrix, optimally

maintaining its quality and safety [62,63]. Compared to commonly used "instant addition", "control release" has many advantages [64,65] and its greater antimicrobial effectiveness has been reported by many researchers [66-68]. By successfully applying the "control release" technique, "Ray" developed a packaging system for gaseous chlorine dioxide, generated from a PLA film blended with $NaClO_2$ and citric acid, triggered by the moisture released from fresh produce [48,49]. The system exhibited a great pathogen inhibition effectiveness against both *Salmonella* spp. and *E. coli* O157:H7 inoculated onto the surface of fresh grape tomato [48,49].

Besides PLA film, many other packaging materials could also be developed into delivery systems for gaseous chlorine dioxide generation and release. Tyvek® is a product introduced by DuPont® (Wilmington, DE, U.S.), used mainly for packaging and labeling. It is made from "continuous and extremely fine fibers of randomly distributed and non-directional high-density polyethylene" [69]. Tyvek® has an excellent liquid barrier property though water vapor and gas could pass through it easily [69].

Gum arabic, also widely known as gum acacia, is "a dried gummy exudation obtained from the stems and branches of Acacia senegal (L) Willdenow or of related species of Acacia (*Fam. Leguminosae*)" [70]. Gum arabic is “Generally Recognized as Safe (GRAS)” by Food and Drug Administration (FDA). It's mainly a complex mixture of

glycoproteins and polysaccharide, which provides it with a good solubility in even cold water solution (the solubility could reach 50% by weight) [72,73].

Electrospun fiber, is a novel material produced from "electrospining" technique which was first introduced in the year of 1934. Complying with the current fever of nanotechnology, this technique is gaining more and more scientific attentions, especially in the field of chemistry, materials science, bio-engineering, medical science, food science and etc [74,75]. The electrospun fibers produced through electrospinning technique have many unique properties such as "large surface area (from 10 m^2/g to 1000 m^2/g), highly porous structure, good mechanical strength and great active compounds retention rate" [74,75]. A great number of synthetic polymers could be considered for this technique. Among them, polyethylene oxide (PEO) has been widely utilized by numerous researchers due to its great characteristics (e.g. "good water solubility, suitable viscosity, non-ionic property and nearly neutral pH") [76,77].

2 LITERATURE REVIEW

2.1 Fresh Produce

2.1.1 Definition

By definition from "Wikipedia", "'produce' is a generalized term for a group of farm-produced crops and goods, including (but not limited to) fruits and vegetables" [1].

2.1.2 Health benefits

Fresh produce is an important part of healthy diet for human beings. It contains different types of necessary nutrients such as water, carbohydrate, protein, vitamins, minerals and etc., which are essential to good health.

In general, its health-benefits have been categorized by "School of Public Health, Harvard University" as below [2]: “

1) cardiovascular disease: compelling evidence has shown that fresh produce consumption could help reduce the risk of having heart disease and stroke [3-5].

2) blood pressure: a few research have shown that fresh produce consumption could help lower the blood pressure [6-7].

3) cancer: sufficient early studies have revealed the strong link between fresh produce consumption and the protection against cancer [8-10].

4) diabetes: a few preliminary research results have shown that fresh produce consumption could help lower the risk of having type 2 diabetes [11-13].

5) gastrointestinal health: research have shown that the indigestible fibers fresh produce contains could help improve the gastrointestinal health [14-15].

6) vision: research have shown that the lutein and zeaxanthin fresh produce contains could help maintain good vision [16]. ”

2.1.3 Sales and consumption

The yearly sales value and consumption amount of fresh produce have been increasing continuously. From [17], it could be seen clearly that the yearly sales value of fresh cut produce has increased from 3.3 billion USD in the year of 1994 to 6, 8.9, 11.8 and 15.5 billion USD in the year of 1999, 2003, 2005 and 2007 respectively. More recent data have shown that the yearly sales value of fresh fruits and vegetables in the year of 2013 had reached 34.6 and 68.2 billion USD respectively and is expected to reach 40.4 and 76.4 billion USD respectively in the year of 2018 [18].

Not only the sales value, the per capita consumption amount of fresh produce has also increased sharply. In the year of 1987, the per capita consumption amount of fresh fruits and vegetables was only 121 and 162 pounds respectively. However, in the year of 1995, it had increased 3.3 - 9.2 percent to reach 125 and 177 pounds respectively. Then in the year of 2000, it had further increased 4 - 10.7 percent to reach 130 and 196 pounds respectively [19]. More recent data have also confirmed this trend [20].

2.1.4 Outbreaks and pathogens

Pathogen outbreaks on fresh produce have become one of the biggest food safety issues nowadays. According to the report from "Center for Science in the Public Interest" titled as "Outbreaks by the numbers: fruits and vegetables 1990-2005": between 1990 and 2005, produce outbreaks caused an average of 48 illnesses per outbreak, more than poultry (34 illnesses), beef (27 illnesses) and seafood (10 illnesses) [21].

The situation of fresh produce outbreak is getting more and more serious. From the year of 1990 to the year of 2005, fresh produce related annual outbreaks had increased from around 20 cases to over 60 cases and the resulted annual illnesses had increased from around 1700 cases to over 3000 cases [21]. More recent reports have also confirmed this trend [22,23].

Among all the fresh produce related outbreaks, *Salmonella* was one of the most popularly involved pathogens [22,23]. It took the percentage as high as "28%", "13%" and "21%" in "fruit outbreaks", "produce dish outbreaks" and "vegetable outbreaks" respectively between the year of 1990 and 2005, resulting in an average of "18%" in all "fresh produce outbreaks" [21].

Salmonella are "gram-negative, non-spore forming, facultatively anaerobic and mobile rod (usually 0.7 - 1.5 × 2 - 5 um in dimensions), belonging to the family of

Enterobacteriaceae with more than 2500 serovars" [24]. They are responsible for Salmonellosis and are considered as pathogens in both animals and human [24].

Currently there is no internationally universal agreement on the "acceptable levels" of *Salmonella* on fresh produce. But some related policies established by individual countries, areas and governmental organizations are still available. Generally speaking, for fresh produce to be served as "ready to eat" food product, *Salmonell*a species should not be detected (so called "zero tolerance policy") [25-27].

2.1.5 Post-harvest respiration

Aerobic respiration is an important physiological process for post-harvest fresh produce. "The process of respiration involves combing O_2 in the air with organic molecules in the tissue (usually a sugar) to form various intermediate compounds and eventually CO_2 and water" [28]. The process related reaction is shown as below [29]:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

Different fresh produce have different respiration rates. "Nuts and dates", "apple, citrus, grape, kiwifruit, onion and potato", "apricot, banana, cherry, peach, nectarine, pear, plum, fig, cabbage, carrot, lettuce, pepper and tomato", "strawberry, blackberry, raspberry, cauliflower, lima bean and avocado", "artichoke, snap bean, Brussels sprouts and cut flowers" as well as "asparagus, broccoli, mushroom, pea, spinach and sweet corn" belong to "very low", "low", "moderate", "high", "very high" and

"extremely high" category, with the respiration rate range of CO_2 as "< 5", "5 - 10", "10 - 20", "20 - 40", "40 - 60" and ">60" mg/kg×hr at 5 ºC, respectively [28].

Besides the commodity category (internal factor), there are many external factors that could also impact the respiration rate. They may include "temperature, atmospheric composition (such as CO_2/O_2 content, RH content) and light" [28].

2.1.6 Tomato

By definition of "Wikipedia", "tomato is the edible, often red fruit/berry of the nightshade *Solanum lycopersicum*, commonly known as a tomato plant [30]." Tomato is considered to be the second most produced vegetables in the U.S. [31].

The respiration rate of tomato is relatively low within a typical range of "10 - 20" mg/kg×hr at 5 ºC [28]. This respiration rate is suitable for this research following the "worst case scenario".

"Tomato" and "*Salmonella*" combination resulted in a great number of outbreaks. From [21], it could be seen that "tomato" and "*Salmonella"* combination had a total of 11 outbreaks during the year of 1998-2005 (ranked # 8) causing more than 1500 illnesses (ranked # 2).

2.2 Chlorine Dioxide

2.2.1 Basic information

Chlorine dioxide has the chemical formula as ClO_2 and $NaClO_2$ is its related salt form. Some basic information for chlorine dioxide and sodium chlorite have been summarized by "Wikipedia" in Table 1 and 2 below respectively [32,33].

Table 1 Basic information for chlorine dioxide (copied from [32])

Category	Information
CAS number	10049-04-4
PubChem	24870
ChemSpider	23251
UNII	8061 YMS-4RM
EC number	233-162-8
MeSH	Chlorine+dioxide
ChEBI	CHEBI:29415
RTECS number	FO3000000

Table 2 Basic information for sodium chlorite (copied from [33])

Category	Information
CAS number	7758-19-2
PubChem	23668197
ChemSpider	22860
UNII	G538EBV4VF
EC number	231-836-6
ChEBI	CHEBI:78667
RTECS number	VZ4800000

2.2.2 Physical and chemical properties

The basic physical and chemical properties of chlorine dioxide and sodium chlorite have been summarized by "Wikipedia" and "Center for Disease Control and Prevention" in Table 3 and 4 respectively below [32-34].

Table 3 Properties of chlorine dioxide (copied from [32,34])

Category	Information
Molecular weight	67.5 g/mole
Boiling point	11 ºC
Solubility in water	3.01 g/L at 25 ºC and 34.5 Hg mm
Melting point	-59 ºC
Physical state	Gas
Density	1.640 g/mL
Odor	Pungent
Color	Yellow to reddish yellow
Oxidative ability	Strong
Working pH	2-10
Safety	No chlorine compound formation

Table 4 Properties of sodium chlorite (copied from [33,34])

Category	Information
Molecular weight	90.442 g/mol (anhydrous)
Appearance	White solid
Solubility in water	75.8 g/100 mL (25 °C)
Melting point	Anhydrous decomposes at 180–200 °C
Odor	Odorless
Density	2.468 g/cm^3
Acidity	10-11
Solubility	slightly soluble in methanol and ethanol

2.2.3 Generating methods

There are many methods for chlorine dioxide generation depending on the reactants utilized (mainly are $NaClO_3$ or $NaClO_2$). For $NaClO_3$, the successful generation of chlorine dioxide could be achieved by "reacting it with a strong acid and a reducing agent (such as HCl)". This is the most commonly utilized method for chlorine dioxide generation worldwide, shown as below [35,36]:

$$NaClO_3 + HCl \leftrightarrows HClO_3$$

$$HClO_3 + HCl \leftrightarrows HClO_2$$

$$HClO_3 + HClO_2 \leftrightarrows ClO_2$$

Besides $NaClO_3$, chlorine dioxide could also be generated from $NaClO_2$. When $NaClO_2$ is utilized to generate chlorine dioxide, the first step is usually "to react it with acids to form chlorous acid, which is the precusor for chlorine dioxide formation [35,36]". The reaction is shown as below [35,36]:

$$NaClO_2 + H^+ \leftrightarrows HClO_2 + Na^+$$

For above reaction, "the acids are required to be added in a very high concentration in order to encourage the reaction to complete. However, no matter how, it is impossible for the reaction to be entirely shifted to the right side especially in dilute solutions or when weak acids are utilized". This inability of reaction completion could result in

insufficient amount of chlorous acids formed and therefore limit the later formation of chlorine dioxide [35,36].

After chlorous acid is formed, the generation of chlorine dioxide could proceed via two ways: "1) via the disproportionateness reaction of chlorous acid to chlorine dioxide; 2) via the oxidation reaction of chlorous acid to chlorine dioxide" [35,36]. The reactions for both ways are shown as below [35,36]:

$$HClO_2 \leftrightarrows ClO_2 + HCl$$

$$HClO_2 + e^- \leftrightarrows ClO_2 + H^+$$

For the disproportionateness reaction, usually “the reaction rate is slow (typically the conversion percentage of chlorous acid to chlorine dioxide is less than 65%). However, through this way, the formation of unwanted chlorinated byproducts could be minimized [35,36]”.

For the oxidation reaction, usually “the reaction rate is quite high (the conversion percentage of chlorous acid to chlorine dioxide could be as high as 95% if there are high concentration of chlorous acids being formed from previous step as well as high concentrations of oxidants and additional strong acids being added in current step). However, through this way, some unwanted chlorinated byproducts could possibly be formed [35,36]”.

2.2.4 Patents

A good number of patents regarding the practical methods of utilizing chlorite for chlorine dioxide generation have been filed so far. Some selected patents of impact have been briefly summarized in Table 5 below [37-41]. Among them, Patent 6,761,872 filed on June 26, 2002 by Roensch Fred et al. [37] was of special interest, which provided an innovative way utilizing carbon dioxide gas to react with sodium chlorite solution for chlorine dioxide generation.

Table 5 Patents of impact regarding chlorine dioxide generating method from chlorite [37-41]

Patent No.	Inventors	Filed Date	Brief Descriptions
6,761,872	Roensch Fred Tribble Richard	June 26 2002	Providing a new method for the in situ generation of chlorine dioxide from a solution of sodium chlorite and carbon dioxide [37]
7,407,642	Mussari Frederick Francis David	March 10 2006	Using sodium chlorite solution and diluted sulfuric acid to generate chlorine dioxide with a significantly higher conversion rate [38]
8,563,019	Coughlin Michael	March 19 2009	Providing a method of making a non-aqueous chlorine dioxide solution by combining a chlorite salt and a non-aqueous carboxylic acid [39]
4,292,292	Hicks Bruce Hicks Jerry	May 30 1980	Utilizing concentrated hydrochloric acid solution to react with concentrated sodium chlorite solution resulting in a consistent high efficiency of yield [40]
4,104,190	Hartshorn Stephen	March 21 1977	Chlorine dioxide is generated from aqueous liquids container alkali metal or alkaline earth metal chlorites [41]

2.2.5 Antimicrobial effectiveness

Chlorine dioxide has been reported to have a great antimicrobial effectiveness against a board spectrum of bacteria since the first publication in 1949 [42]. Its working mechanism has not been clarified so far but could possibly be owned to “the oxidative attack on cell membrane proteins and enzymes” [43,44].

Gaseous phase chlorine dioxide usually has a greater antimicrobial effectiveness than its aqueous solution, due to the better penetrating ability, which has been observed and reported by many researchers [45-47]. Prodduk has completed a research regarding the comparison of microbial inhibition effectiveness between aqueous and gaseous phase chlorine dioxide against *Salmonella* spp. inoculated on the surface of mung beans sprout. The results showed that gaseous chlorine dioxide treatment was able to achieve approximately 2 more log reduction of *Salmonella* spp. compared to the aqueous chlorine dioxide treatment [45].

The great antimicrobial effectiveness of gaseous chlorine dioxide on fresh produce have been reported by numerous researchers [50-59] and summarized by "Ray" in her work [48,49].

2.2.6 Safety risks and exposure limits

Chlorine dioxide and sodium chlorite are both toxic, placing certain safety risks to the health of handlers and consumers. Therefore setting the exposure limits is critical to ensure their safe use. The assessments of safety risks (including acute dietary, chronic dietary and carcinogenicity) as well as exposure limits for chlorine dioxide and sodium chlorite have been issued by "Environmental Protection Agency (EPA)", "Agency for toxic substances & disease registry (ATSDR)" and "United States Department of Labor", summarized in Table 6 and 7 below respectively [35,60,61]:

Table 6 Summary of safety risks for chlorine dioxide/ sodium chlorite (copied from [35])

Exposure scenario	Dose used in risk assessment (mg/kg/day)	UF/MOE for risk Assessment	Study and toxicological effects
Acute dietary	LD_{50} = 292 mg/kg (males) and LD_{50} = 340 mg/kg (females)		
Chronic dietary	NOAEL = 3 mg/kg/day	Chronic PAD = 0.03 mg/kg/day	At LOAEL of 6 mg/kg/day
Carcinogenicity	No cancer data is available for chlorine dioxide		

Table 7 Summary of exposure limits for chlorine dioxide/ sodium chlorite (copied from [60,61])

Exposure scenario	Exposure limits
In drinking water	A maximum level of 0.8 mg/L
In working environment	8 hrs permissible exposure limit of 0.1 ppm in air (0.3 milligrams per cubic meter)

2.2.7 Ray's control release system of chlorine dioxide

"Control release" is a novel technique that releases active compounds (such as antimicrobials) in a timely and controlled manner to the food matrix, optimally maintaining its quality and safety [62,63].

Compared with commonly used "instant addition", "control release" has many advantages [64,65] and its greater antimicrobial effectiveness has been reported by many researchers [66-68]. Balasubramanian has completed a research regarding the comparison of microbial inhibiting effectiveness between "control release" and "instant addition" of nisin (as an antimicrobial agent) against *Micrococcus luteus* in TSB broth [68]. The in-detail experimental design was as follows: "10^7 CFU/mL *Micrococcus luteus* were added into 3 TSB broths. One broth was kept as control. One broth was treated with 10 ug/mL nisin instantly. One broth was treated with 10 ug/mL nisin in a "control release" manner (diffusivity was 9.58×10^{-10})." After 48 hours under 30 ºC, the results showed that "control release" had a much greater antimicrobial effectiveness than "instant addition" [68].

By successfully applying the "control release" technique, "Ray" developed a packaging system for chlorine dioxide, generated from a PLA film blended with $NaClO_2$ and citric acid, triggered by the moisture released from fresh produce. The scheme of the system is shown in Figure 1 below [48,49].

This system has demonstrated great pathogen inhibition effectiveness (more than 3 log reduction in general) against both *Salmonella* spp. and *E. coli* O157:H7 inoculated onto the surface of fresh grape tomato [48,49]. However the system developed also had two major drawbacks: the significant loss of chlorine dioxide during film preparation and storage caused by ambient RH as well as the relatively high cost [48,49].

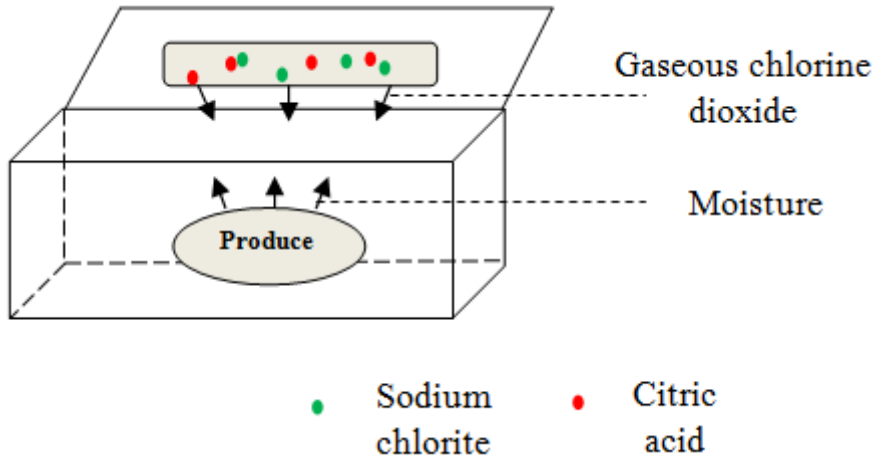


Figure 1. Ray's control release system of chlorine dioxide for fresh produce (copied from [48,49])

2.3 Delivery Systems for $NaClO_2$

2.3.1 Tyvek sachet

Tyvek® is a product introduced by DuPont® (Wilmington, DE, U.S.), used mainly for packaging and labeling. It is made from "continuous and extremely fine fibers of randomly distributed and non-directional high-density polyethylene" [69]. Tyvek® has “an excellent liquid barrier property though water vapor and gas could pass through it easily” [69]. Besides, it has other good properties such as "outstanding chemical resistance, good dimensional stability, remarkable flexibility and etc." [69]. It also meets "the requirements of Title 21 of the United States Code of Federal Regulations (21 CFR 177.1520) for direct food contact applications " [69]. All these properties make it suitable to serve as the delivery system for $NaClO_2$.

2.3.2 Gum arabic paste

Gum arabic is also widely known as gum acacia. According to the description of "United States Food Chemical Code", gum arabic is "a dried gummy exudation obtained from the stems and branches of Acacia senegal (L) Willdenow or of related species of Acacia (*Fam. Leguminosae*)" [70].

Gum arabic is considered to be GRAS by FDA. It's mainly a complex mixture of glycoproteins and polysaccharide [71], which provides it with a good solubility in even cold water solution (the solubility could reach 50% by weight) [72,73]. All these properties make it suitable to serve as the delivery system for $NaClO_2$.

2.3.3 Electrospun fiber

Electrospun fiber, is a novel material produced from "electrospining technique" which was first introduced in the year of 1934. Complying with the current fever of nanotechnology, this technique is gaining more and more scientific attentions, especially in the field of chemistry, materials science, bio-engineering, medical science, food science and etc [74,75] .

The working mechanism for electrospinning technique is shown in Figure 2 and described as below [74,75]: “a syringe pump pushes the polymer solution (in a typical flow rate range of 0.1 - 2 mL/h) to come out of a needle forming a droplet. Since a sufficiently high voltage (in a typical range of 5 - 50 KV) is applied to the droplet, the molecules in the droplet become charged (carrying the same charge "+" or "-"). The electrostatic repulsive force between molecules counteracts the cohesive force so the droplet is stretched. Stretched to a critical point (Taylor Zone), when the force of the electrostatic repulsion is bigger than the cohesive force, a polymer jet could erupt from the surface of the droplet. The jet flies straight in the air for a certain distance, then it is elongated and thinned by a so-called "whipping process". Solvent gets vaporized during the flight and finally the dried fibers are deposited on the grounded collector”.

The polymer fibers deposited on the grounded collector through electrospinning technique have a diameter range from a few nanometers to a few micrometers (a

typical morphology and diameter distribution of the electrospun fiber are shown in Figure 3 below). In general, the electrospun nanofibers produced have "large surface area (from 10 m^2/g to 1000 m^2/g), highly porous structure, good mechanical strength and great active compounds retention rate (non-thermal process)" [74,75].

A great number of synthetic polymers could be considered for electrospinning technique including nylon, polyacrylic acid, polyamide, polycarbonate, polyurethane, polyvinyl alcohol and etc. Among them, polyethylene oxide (PEO) has been widely utilized by numerous researchers due to its great characteristics (i.e. "good water solubility, suitable viscosity, non-ionic property and nearly neutral pH") [76-78].

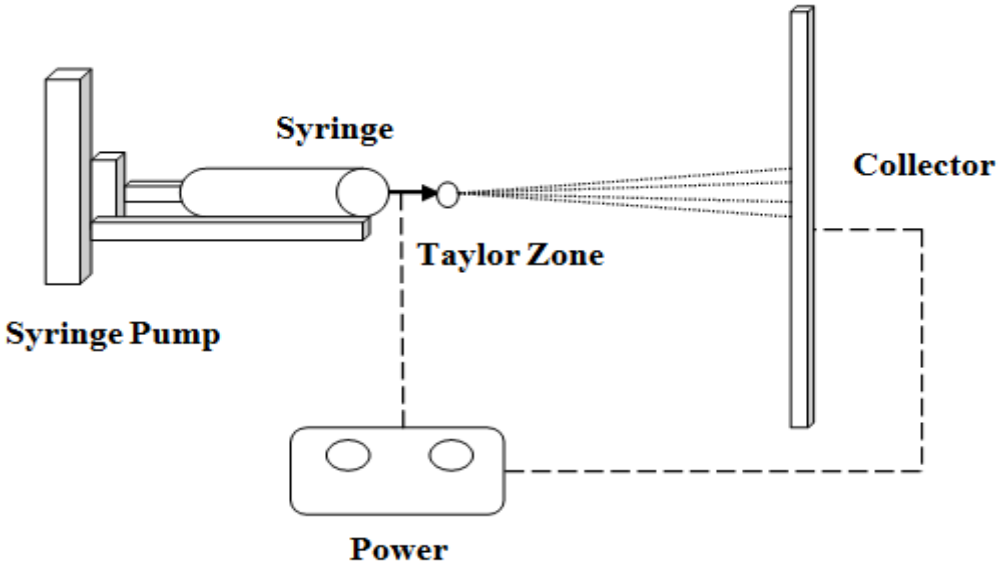
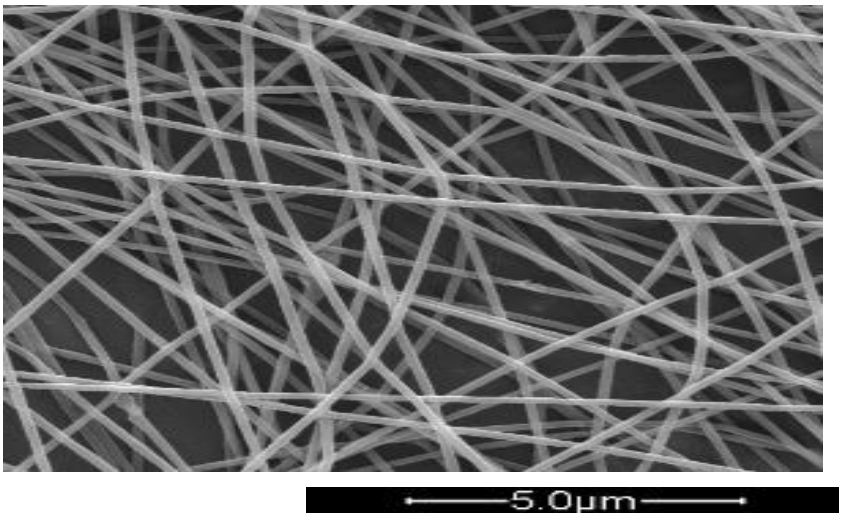


Figure 2. Electrospinning process



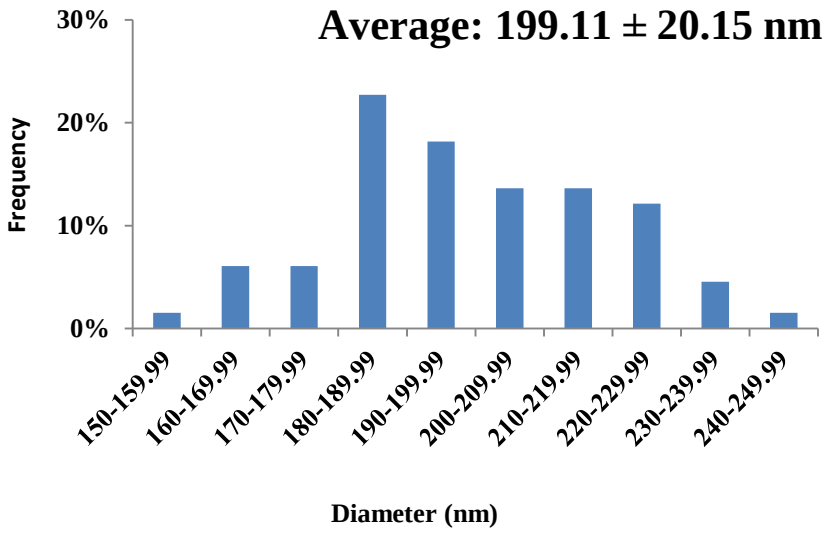


Figure 3. Electrospun fiber morphology (top) and diameter distribution (bottom)

3 HYPOTHESIS, OBJECTIVES AND TASKS

3.1 Formation of Hypothesis

3.1.1 Key points from literature review

Point 1: Fresh produce industry has been growing rapidly.

Point 2: Pathogen outbreak is placing a major safety challenge to fresh produce industry.

Point 3: Gaseous chlorine dioxide has exhibited a great pathogen inhibition effect on fresh produce.

3.1.2 Conclusion

There's an opportunity of developing gaseous chlorine dioxide releasing systems to ensure pathogen safety on fresh produce.

3.1.3 How to develop such a system (system description)

$ClO_{2(g)}$ can't be directly pre-incorporated into any delivery system and then released, due to its unstable and explosive properties. In other words, $ClO_{2(g)}$ must be generated on-site in the delivery system right before release. So the question is how to develop such a delivery system.

$$NaClO_2 + H^+ + H_2O \leftrightarrows ClO_{2(g)} \quad \text{..... (1)}$$

$$C_6H_{12}O_6 + O_2 \rightarrow CO_2 + H_2O \text{ ..... (2)}$$

$$CO_2 + H_2O \leftrightarrows H_2CO_3 \text{ ..... (3)}$$

Reaction (1) describes a common method that $ClO_{2(g)}$ could be generated, basically to react $NaClO_2$ with acids in the presence of water. Reaction (2) describes the aerobic respiration process of fresh produce during post-harvest stage. O_2 from the environment is utilized by the plant to react with its carbohydrate to generate and release relatively large amounts of CO_2 and moisture. Reaction (3) describes a common method to generate an acid (i.e. H_2CO_3), basically to react CO_2 with water. From above three reactions, it is reasonable to speculate that the respiration products (i.e. CO_2 and moisture) from fresh produce during post-harvest stage may form H_2CO_3, react with $NaClO_2$ and generate $ClO_{2(g)}$. Also, since H_2CO_3 is a very weak acid, the generation and release profile of $ClO_{2(g)}$ could be gentle that only effectively inhibiting the pathogen growth on the surface but not significantly affecting the sensory profile of fresh produce in the system.

As the system description shown in Figure 4 below, from the standpoint of mass transfer, the CO_2 and moisture released from fresh produce into the headspace will need to form H_2CO_3, penetrate into the delivery systems to react with $NaClO_2$ incorporated. Then the $ClO_{2(g)}$ generated will need to diffuse out into the boundary, be released into the headspace and reach the surface of fresh produce to fulfill its pathogen inhibition functionality. In order to achieve the optimum system design, extensive knowledge of produce postharvest physiology, chemistry, materials science, mathematics, microbiology and food science will need to be well integrated.

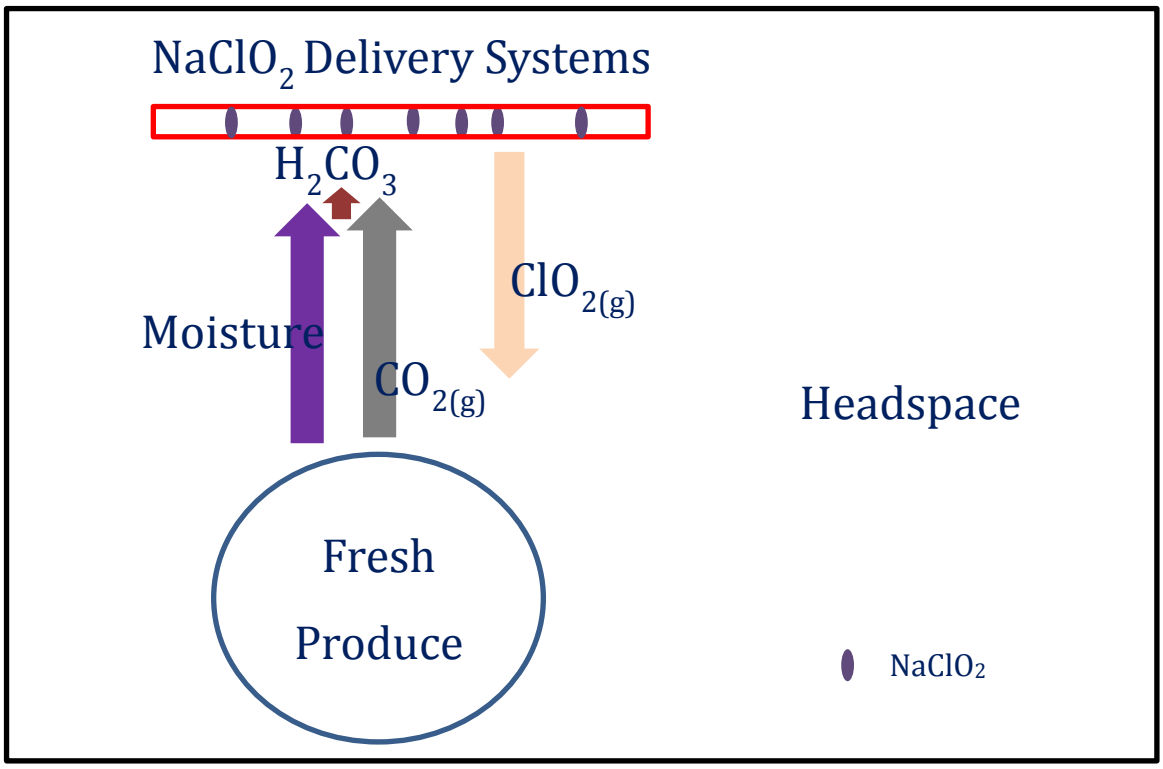


Figure 4. System description

3.2 Hypothesis

CO_2 and moisture released from fresh produce during post-harvest stage is able to react with $NaClO_2$ to generate $ClO_{2(g)}$ inhibiting pathogen growth effectively without damaging the sensory profile.

3.3 Objectives

The objectives of the study are:

1) To test the hypothesis primarily

2) To investigate the basic chemistry mechanisms related to the hypothesis

3) To investigate the basic microbiology mechanisms related to the hypothesis

4) To develop delivery systems of $NaClO_2$ for practical applications of the hypothesis

3.4 Tasks

The specific tasks for the objectives are listed in the flow chart below:

Stage 1 Hypothesis primary testing

- To test whether ClO_2 is able to be generated under ideal conditions ($CO_2 + H_2O + NaClO_2 \leftrightarrows ClO_2$)
- To test whether $ClO_{2(g)}$ is able to be generated under practical conditions of fresh produce storage (CO_2 + moisture + $NaClO_2 \leftrightarrows ClO_{2(g)}$)
- To test whether $ClO_{2(g)}$ generated under practical conditions of fresh produce storage is able to inhibit *Salmonella* spp. growth effectively

- To test whether $ClO_{2(g)}$ is able to be generated from respiration products of fresh produce (i.e. CO_2 and moisture) inhibiting pathogen growth effectively without damaging sensory profiles of tomato

Stage 2 Investigation of basic chemistry mechanisms related to the hypothesis

- To demonstrate the necessity of each component (i.e. $NaClO_2$, H_2O, CO_2) in the generation of $ClO_{2(g)}$
- To understand the impact of each component (i.e. $NaClO_2$, H_2O, CO_2), temperature and light in the release profile of $ClO_{2(g)}$
- To understand the relationship between pH and generation profile of ClO_2 as well as propose possible chemical reactions

Stage 3 Investigation of basic microbiology mechanisms related to the hypothesis

- To demonstrate the necessity of each component (i.e. $NaClO_2$, H_2O, CO_2) in *Salmonella* spp. inhibition effects
- To understand the impact of each component (i.e. $NaClO_2$, H_2O, CO_2), temperature and light in *Salmonella* spp. inhibition effects
- To find out "D value" and "Z value"

Stage 4 $NaClO_2$ delivery system developments for practical application of hypothesis

Tyvek sachet

- To confirm the successful generation of gaseous chlorine dioxide from developed system through chemistry methods directly and microbial inhibition experiments indirectly under simulated conditions at different temperatures
- To confirm the pathogen inhibition effects of developed system against *Salmonella* spp. using fresh tomato as the source of CO_2 and moisture at different temperatures
- To optimize the developed system through both microbial inhibition and sensory tests under long-term storage

Gum arabic paste

- To confirm the successful generation of gaseous chlorine dioxide from developed system through chemistry methods directly and microbial inhibition experiments indirectly under simulated conditions at different temperatures
- To confirm the pathogen inhibition effects of developed system against *Salmonella* spp. using fresh tomato as the source of CO_2 and moisture at different temperatures

Electrospun fiber

- To obtain physical properties of different concentrations of PEO and $NaClO_2$ water solutions
- To obtain morphology and diameter distributions of fibers electrospun from

different concentrations of PEO and $NaClO_2$ water solutions

- $NaClO_2$ loading efficiency measurement
- To confirm the inhibition effects of developed systems against *Salmonella* spp. under simulated conditions
- To confirm the inhibition effects of developed systems against *Salmonella* spp. on fresh tomato

Feasibility Study of Novel Gaseous Chlorine Dioxide Generating Method for Pathogen Inhibition on Fresh Produce

↓

Hypothesis

CO_2 and moisture released from fresh produce is able to react with $NaClO_2$ to generate $ClO_{2(g)}$ inhibiting pathogen growth effectively without damaging the sensory profile

↓

Hypothesis Primary Testing

Testing 1:	**Testing 2:**	**Testing 3:**	**Testing 4:**
Chemical reaction in ideal condition	Chemical reaction in practical condition	Inhibition effects in simulated condition	Inhibition effects using fresh produce

↓

Understanding Basic Science Related

Chemistry Mechanisms	**Microbiology Mechanisms**
1. Necessity study ($NaClO_2$, CO_2, RH - $ClO_{2(g)}$ generation)	1. Necessity study ($NaClO_2$, CO_2, RH - *Salmonella* inhibition)
2. Impact of factor levels on $ClO_{2(g)}$ release profile ($NaClO_2$, CO_2, RH, T, light)	2. Impact of factor levels on microbial inhibition ($NaClO_2$, CO_2, RH, T, light)
3. Impact of pH	3. "D value" and "Z value" obtaining

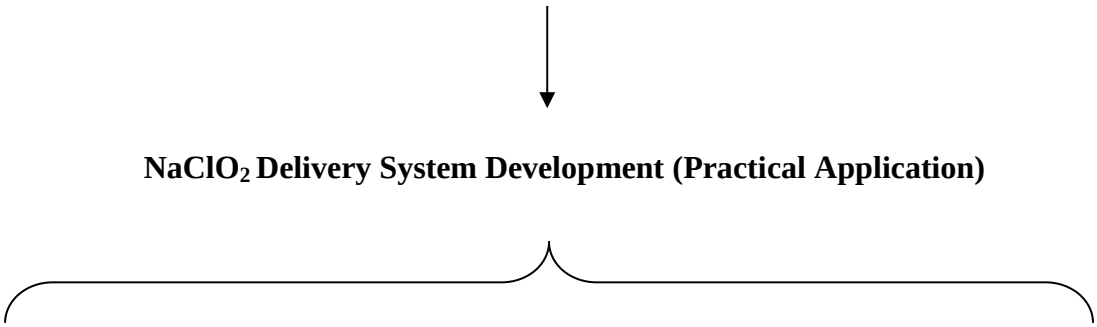


Figure 5. Schematic of research

4 MATERIALS AND METHODS

4.1 Materials

$NaClO_2$ was purchased from ACROS Company (Morris Plains, New Jersey, USA). K_2CO_3 was purchased from Fisher Scientific (Pittsburgh, PA, USA). Gum acacia was purchased from Colloides Naturels Incorporation (Somerville, NJ, USA). Polyethylene oxide powder was purchased from Sigma-Aldrich (St. Louis, MO, USA). CO_2 gas was ordered from Airgas East Inc (Cheshire, CT, USA). 99.5+% ethanol (EtOH) were purchased from ACROS Company (Morris Plains, New Jersey, USA). *Salmonella* spp. were obtained from USDA-ARS-ERRC (Wydmoor, PA, USA). BHI (Brain Heart Infusion) agar powder was purchased from MP Biomedicals (Solon, OH, USA). BHI broth powder was purchased from OXOID LTD (Basinstoke, Hampshire, England). XLT 4 agar powder was purchased from Becton, Dickinson and Company (Sparks, MD, USA). Phosphate buffered saline (PBS) tablets were purchased from Sigma (St. Louis, MO, USA). Fresh tomatoes were purchased from Bravo supermarket (New Brunswick, NJ, USA). Deionized water was prepared by Barnstead E-pure water system (Dubuque, IA, USA).

Parafilms were purchased from Pechiney Plastic Packaging (Menasha, WI, USA). 10 mL and 30 mL sterile plastic tubes as well as petri dish were purchased from Fisher Scientific (Pittsburgh, PA, USA). Polystyrene cuvettes were purchased from VWR (Radnor, PA, USA). Sample bags were purchased from Whirl-pak (Fort Atkinson, WI, USA).

A Model 650 CO_2/O_2 detector was purchased from Mocon (Minneapolis, MN, USA). The incubator was purchased from VWR (Radnor, PA, USA). The fridge was purchased from Haier (Qingdao, Shandong, China). UV spectrometer was purchased from Shimadzu (Kyoto, Japan). ClO_2 detecting strips were purchased from Lamotte (Chestertown, Maryland, USA). ClO_2 detecting tubes were purchased from Gastec (Fukayanaka, Ayase-City, Japan). TA-XT2i Texture analyzer was purchased from Texture Technologies Corp (Scarsdale, NY, USA). CR-200 colorimeter was purchased from Konica-Minolta (Ramsey, NJ, USA). IQ 270G pH meter was purchased from IQ scientific instruments (Loveland, CO, USA). IQ 270G conductivity meter was purchased from IQ scientific instruments (Loveland, CO, USA). TA AR-2000 ETC Viscometer was purchased TA Instruments (New Castle, DE, USA). Fisher Scientific 20 surface tensiometer was purchased from Fish Scientific (Pittsburgh, PA, USA). Guanta 200F scanning electron microscope was purchased from FEI (Hillsboro, OR, USA). Nanofiber electrospinning instrument was purchased from NaBond Technologies Co. (Shenzhen, Guangdong, China).

4.2 Methods

4.2.1 Experimental condition control

Temperatures (10, 22 and 35 ºC) were maintained by fridges or incubators.

CO_2 was injected into the mason jars through a small pipe connected to a CO_2 gas tank. The concentration of CO_2 (7.5% and 15%) inside the jars was monitored by the CO_2/O_2 detector.

RH inside the jars (45% and 90%) were maintained by different concentrations of salt & water solutions. The RH content inside the jars was monitored by the moisture detector.

Environmental condition (dark and light) was maintained by keeping the sun-lamp off and on.

All containers including Mason Jars, glass vials and plastic boxes were first washed with de-ionized water to eliminate any possible chemical residue and then cleaned with 70% ethanol to eliminate any possible background bacteria. All microbial work were conducted inside bio-safety hood with air circulation on to prevent potential pathogen spreads. All chemical work were conducted inside fume hood with air circulation on to prevent any potential chemical spills or gas releases.

4.2.2 Chlorine dioxide concentration detection

The chlorine dioxide concentration was measured through three methods listed below:

1) Chlorine dioxide detecting strips

This method was utilized to roughly detect the generation of chlorine dioxide in water solution. The range of these detecting strips was between 0 – 500 ppm.

2) Chlorine dioxide detecting tubes

This method was utilized to detect the concentration of chlorine dioxide generated in the headspace. The range of these detecting tubes was between 0 – 5 ppm.

3) UV spectrophotometer

This method was utilized to detect the chlorine dioxide concentration both in water solution and in headspace. For in water solution, chlorine dioxide solution was diluted using de-ionized water to a desirable concentration suitable for UV measurement if necessary. For in headspace, a proper volume of chlorine dioxide gas was withdrawn by a syringe and injected into polystyrene cuvettes containing de-ionized water solution sealed with parafilm for UV measurement [79,80]. The proper volume to be withdrawn depended on its original concentration in the headspace.

The relativity of detected results between chlorine dioxide detecting tubes and UV-spectrophotometer were compared through following experiments: two samples of gaseous chlorine dioxide with different concentrations were prepared inside Mason jars respectively. The detection results using both methods were shown in Table 8.

From the table, it could be seen that the detected results using both detecting tubes and UV-spectrophotometer were very close. For measuring such low concentrations (ppm level) of a gas, the difference was negligible. The comparison between the two detecting methods was listed in Table 9.

Table 8 Detected results of gaseous chlorine dioxide concentration using chlorine dioxide detecting tubes and UV-spectrophotometer

	Detecting tube	**UV-spectrophotometer**
Result 1	0.73 mg/L	0.44 mg/L
Result 2	0.19 mg/L	0.0939 mg/L

Table 9 Comparison between measurements of gaseous chlorine dioxide concentration using chlorine dioxide detecting tubes and UV-spectrophotometer

	Detecting tubes	**UV-spectrophotometer**
Advantage	Direct reading; Smaller sampling volume	Reading itself is accurate
Disadvantage	Reading marks on the tube are not very clear	Indirect reading, loss during transaction; Larger sampling volume

4.2.3 Bacteria incubation, inoculation and enumeration

1) In simulated conditions

Salmonella spp. (containing three different stains) were utilized in the research. A loopful of each strain was transferred from a -80 ºC stock culture into a 10 mL Brain Heart Infusion (BHI) broth and incubated at 37 ºC for 6 hrs. An equal amount of cell suspension of each strain was then separately transferred to a fresh 10 mL BHI broth and incubated at 37 ºC for 24 hrs (at this point, the bacteria concentration in the BHI broth reached approximately 10^9 CFU mL^{-1}) [81]. 10^9 CFU mL^{-1} *Salmonella* spp. BHI broth was diluted by 10^4 times to reach a concentration of 10^5 CFU mL^{-1}. Then 0.1 ml of the diluted solution was plated on TSA agar in petri dish (the concentration of *Salmonella* spp. was around 10^4 CFU on TSA agar at this time). If 10^2 CFU *Salmonella* spp. was needed, 10^9 CFU mL^{-1} *Salmonella* spp. BHI broth was diluted by 10^6 times to reach a concentration of 10^3 CFU mL^{-1}. Then 0.1 ml of the diluted solution was plated on TSA agar in petri dish (the concentration of *Salmonella* spp. was around 10^2 CFU on TSA agar at this time). At different time intervals, the growth profile of *Salmonella* spp. was checked and recorded.

2) On fresh tomato

Salmonella spp. (containing three different stains) were utilized in the research. A loopful of each strain was transferred from a -80 ºC stock culture into a 10 mL Brain Heart Infusion (BHI) broth and incubated at 37 ºC for 6 hrs. An equal amount of cell

suspension of each strain was then separately transferred to a fresh 10 mL BHI broth and incubated at 37 ºC for 24 hrs (at this point, the bacteria concentration in the BHI broth reached approximately 10^9 CFU mL^{-1}) [81]. Then the original broth was diluted by 10^2 times with BHI broth and 0.02 ml of the diluted broth was spot inoculated onto the surface of fresh tomatoes, ready for use (the concentration of *Salmonella* spp. was around 10^5 CFU on surface at this time). At different time intervals, the tomatoes inoculated with *Salmonella* spp. were put inside a sterile plastic bag, washed with enough peptone water thoroughly, then 0.1 mL of the peptone water solution was plated to check the *Salmonella* spp. growth profile.

4.2.4 Fresh tomato selections and pretreatment

Fresh tomato bought from a local supermarket was utilized in the research. All the fresh tomatoes utilized were firm in texture and had no soft or black spots on the surface. According to the color classification requirement in tomato published by United Fresh Fruits and Vegetable Association (shown in Figure 6), the tomato used in this research belonged to stage 6: "more than 90% of the surface is not green; in the aggregate, shows red color". All tomato were washed with deionized water and then rinsed with 70% ethanol to completely eliminate any possible background microorganisms before use.

Figure 6. Stage classification of tomato
(copied from "http://awaytogarden.com/what-color-is-your-tomato-how-to-ripen-them/")

4.2.5 Sensory profile measurement of fresh tomato

Firmness of tomatoes was measured using a TA-XT2i texture analyzer and surface color of tomato was measured using a CR-200 colorimeter. Lightness (L), redness (a) and yellowness (b) were obtained then Hue value and Chroma value were calculated following equations below:

$$\text{Hue value} = \text{Atan}(b/a) \times 57.3$$

$$\text{Chroma value} = \sqrt{(a^2 + b^2)}$$

A sensory panel consisted of a few Rutgers Food Science graduate students (65 Dudley Road, New Brunswick, NJ 08901) was utilized for discrimination sensory

tests including surface color, texture and smell between treated and control fresh tomatoes. The decision (whether samples were same or different) was made based on all the feedback collected.

4.2.6 $NaClO_2$ delivery system preparations

Tyvek sachet utilized in the research was a small 1 cm × 2 cm rectangular envelop with the thickness as 1.1×10^{-3} m and the weight as 0.1 g. After $NaClO_2$ was placed inside the sachet, it was sealed tight, ready for use.

Gum arabic paste was made by first adding a right amount of $NaClO_2$ into water solution. After all $NaClO_2$ has been dissolved, a right amount of gum arabic powder was added into the solution and well mixed. The freshly made paste was sticky in texture with a slightly yellowish appearance and a pleasant smell. It's stable in structure during long-term storage under ambient conditions.

Electrospun fibers were produced first by dissolving the right amount of $NaClO_2$ and polyethylene oxide powder into the right amount of water. After overnight stirring, all homogenate (i.e. 2%, 3% and 4% PEO as well as 3% PEO with 0.15% and 0.3% $NaClO_2$) were electrospun to produce fibers. Fibers produced were collected and stored in sealed plastic bags, ready for use.

5 RESULTS AND DISCUSSIONS

5.1 Hypothesis Primary Testing

5.1.1 To test whether ClO_2 is able to be generated under ideal conditions

After 20 hrs, the color of the water solution turned yellowish (see Figure 7). The average ClO_2 concentration in the solution was detected to be 3.096 mg/L, which indicated the successful generation of ClO_2 in ideal conditions.

So hypothesis testing (1) was supported. In other words, ClO_2 is able to be generated under ideal conditions.

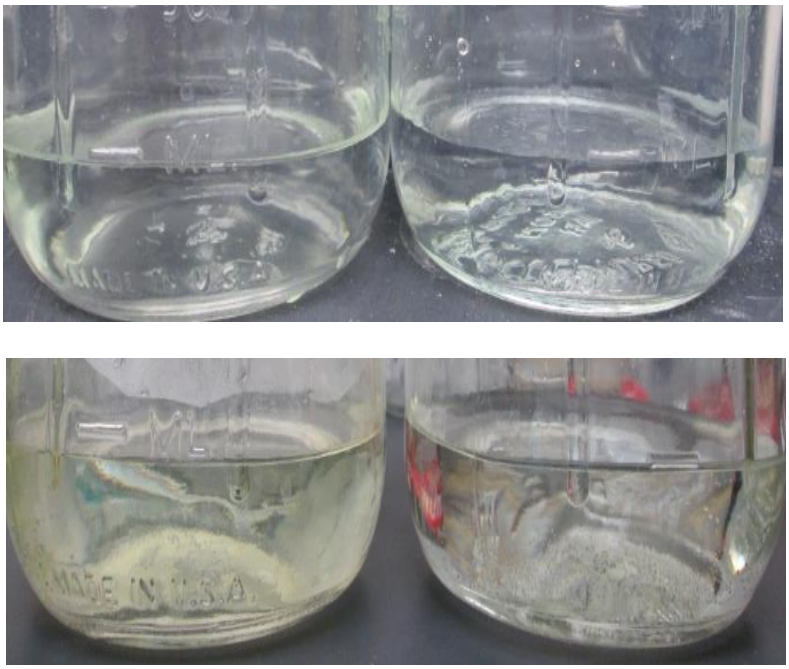


Figure 7. Color of water solutions at 0 hrs (top) and 20 hrs (bottom) for hypothesis testing (1)

5.1.2 To test whether $ClO_{2(g)}$ is able to be generated under practical conditions of fresh produce storage

After 24 hrs, the concentration of gaseous chlorine dioxide in headspace was first detected to be 5 (± 5) uL/L. After 120 hrs, the concentration of gaseous chlorine dioxide detected in headspace reached the peak as 110 (± 26) uL/L. After that, the concentration of gaseous chlorine dioxide detected in headspace slowly decreased (shown in Figure 8). This experiment demonstrated the feasibility of proposed reaction in practical fresh produce storage conditions.

So hypothesis testing (2) was supported. In other words, $ClO_{2(g)}$ is able to be generated under practical conditions of fresh produce storage [82-85].

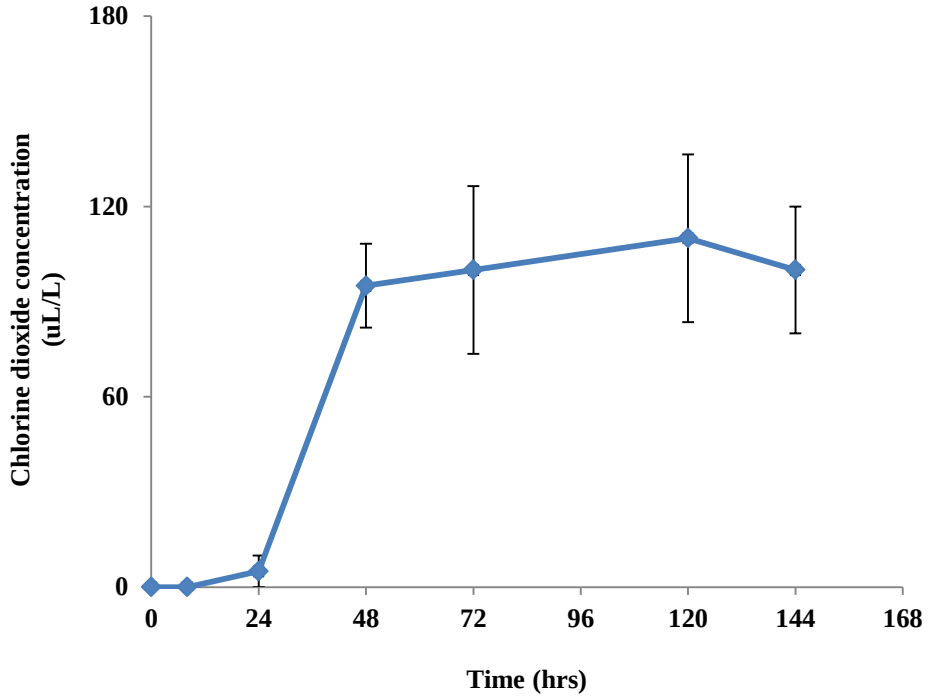


Figure 8. Release profile of $ClO_{2(g)}$ for hypothesis testing (2)

5.1.3 To test whether $ClO_{2(g)}$ generated under practical conditions is able to inhibit pathogen growth effectively

After 48 hrs, 10^4 CFU *Salmonella* spp. on TSA agar were completely inhibited [86,87]. All agars were further incubated at 37 ºC overnight and found no *Salmonella* spp. grew back (shown in Figure 9). In order to demonstrate that the complete inhibition effectiveness was not mainly from the CO_2 content in the headspace, experiments under the mere impact of CO_2 were also performed and the results showed that only slight growth inhibition of *Salmonella* spp. was observed (shown in Figure 9). Similar effect has also been reported by Zhou and Pang [90,91].

So hypothesis testing (3) was supported. In other words, $ClO_{2(g)}$ generated under practical conditions is able to inhibit pathogen growth effectively.

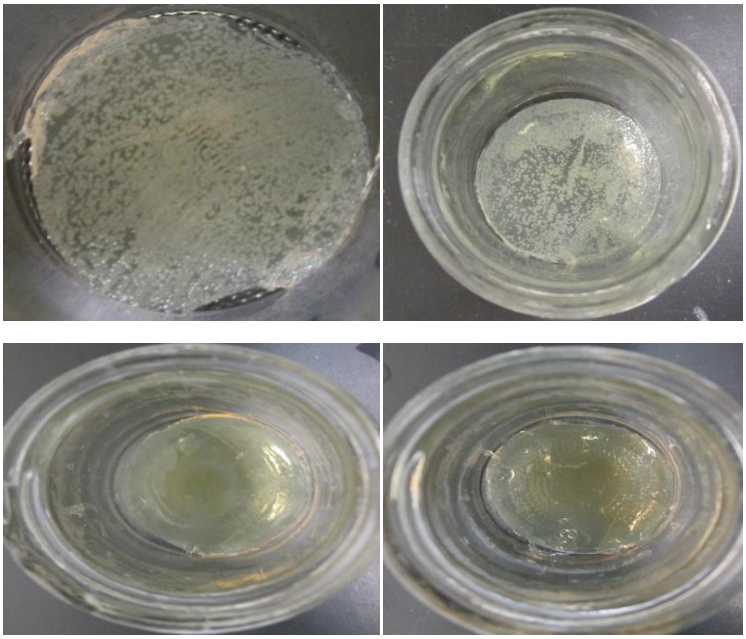


Figure 9. *Salmonella* spp. growth profiles of control (top left), under CO_2 (top right) and under treatment of CO_2 + $NaClO_2$ + moisture (bottom two) for hypothesis testing (3)

5.1.4 To test whether $ClO_{2(g)}$ is able to be generated from respiration products of fresh produce inhibiting pathogen growth effectively without damaging sensory profiles of tomato

At 0 hrs, sensory evaluations including appearance and texture of randomly selected samples from both controls and treatments were conducted. For the appearance, L, Hue and Chroma values obtained through colorimeters for both controls (as 40.79 ± 2.85, 46.08 ± 5.45 and 26.10 ± 3.78 respectively) and treatments (as 45.99 ± 0.79, 43.55 ± 2.56 and 26.88 ± 1.15 respectively) were shown in Figure 10. From the figure, it could be concluded that there was no significant difference ($p>0.05$) between samples randomly selected from controls and treatments in appearance (shown in Table 10). For the texture, the maximum force obtained through texture analyzer for both controls (as 1389 ± 98.80 g) and treatments (as 1336.9 ± 294.48 g) were shown in Figure 11. Even though the significance analysis showed there was significant difference ($p<0.05$) between samples randomly selected from controls and treatments in texture (shown in Table 10), the sensory panel concluded that the practical difference was insignificant. This significant difference could possibly be owned to the sampling error during measurements.

After 48 hrs, on the agar there was completely no growth of any *Salmonella* spp. for treatments (see Figure 14). All agar were further incubated at 37 ºC overnight and found no bacteria grew back. Sensory evaluations including appearance and texture of randomly selected samples from both controls and treatments were conducted. For the appearance, L, Hue and Chroma values obtained through colorimeters for both

controls (as 41.59 ± 1.07, 41.69 ± 4.45 and 26.66 ± 2.56 respectively) and treatments (as 43.12 ± 0.72, 45.83 ± 3.65 and 27.47 ± 1.49 respectively) were shown in Figure 12. For the texture, the maximum force obtained through texture analyzer for both controls (as 1159.58 ± 90.12 g) and treatments (as 1117.2 ± 103.38 g) were shown in Figure 13. From both figures, it could be concluded that there was no significant difference ($p>0.05$) between samples randomly selected from controls and treatments in both appearance and texture (shown in Table 11), which agreed with the visual observations (shown in Figure 15) and the feedback from the sensory panel.

So hypothesis testing (4) was supported. In other words, $ClO_{2(g)}$ is able to be generated from respiration products of fresh produce (i.e. CO_2 and moisture) inhibiting pathogen growth effectively without damaging sensory profiles of tomato.

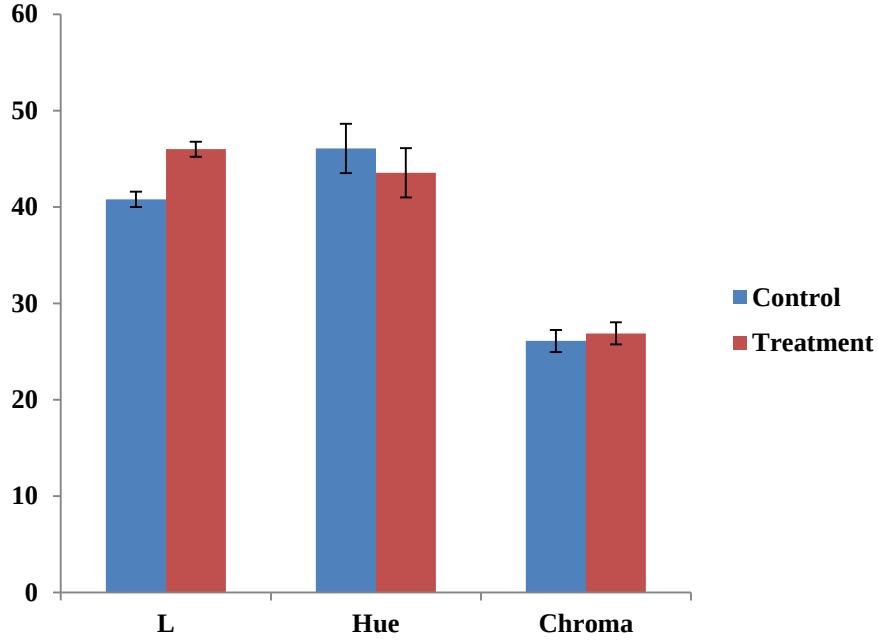


Figure 10. L, Hue and Chroma values of control (left) and treated samples (right) at 0 hrs for hypothesis testing (4)

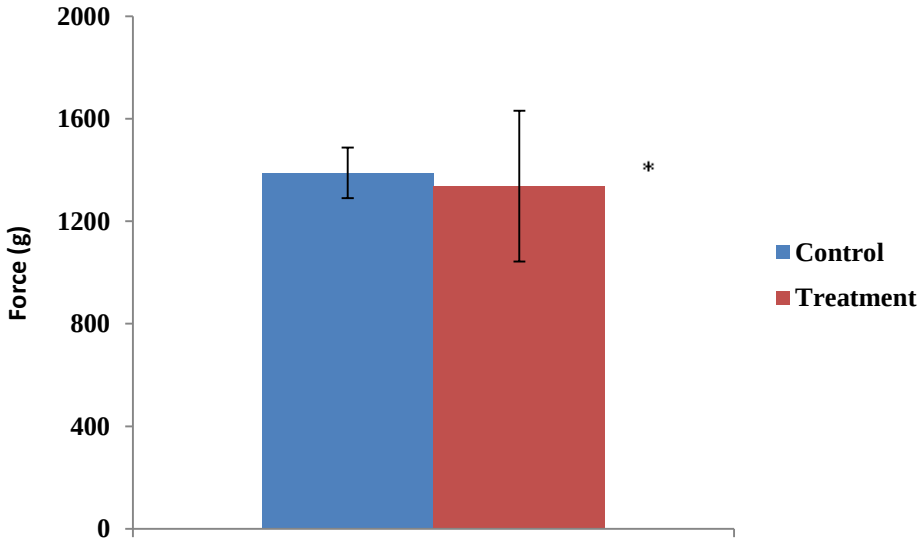


Figure 11. Firmness of control (left) and treated samples (right) at 0 hrs for hypothesis testing (4)

Table 10 Significance analysis between control and treated samples at 0 hrs for hypothesis testing (4) (from top to bottom: L, Hue, Chroma values and texture)

Source of Variation	SS	MS	F	P-value	F crit
Between Groups	12.81828	12.81828	0.707104	0.432635	5.987378
Within Groups	108.7671	18.12785			
Total	121.5854				

Source of Variation	SS	MS	F	P-value	F crit
Between Groups	1.229634	1.229634	0.15776	0.704971	5.987378
Within Groups	46.76591	7.794318			
Total	47.99554				

Source of Variation	SS	MS	F	P-value	F crit
Between Groups	6786.025	6786.025	0.140675	0.717359	5.317655
Within Groups	385912	48239			
Total	392698				

Source of Variation	SS	MS	F	P-value	F crit
Between Groups	54.2882	54.2882	12.39843	0.012501	5.987378
Within Groups	26.2718	4.378633			
Total	80.56				

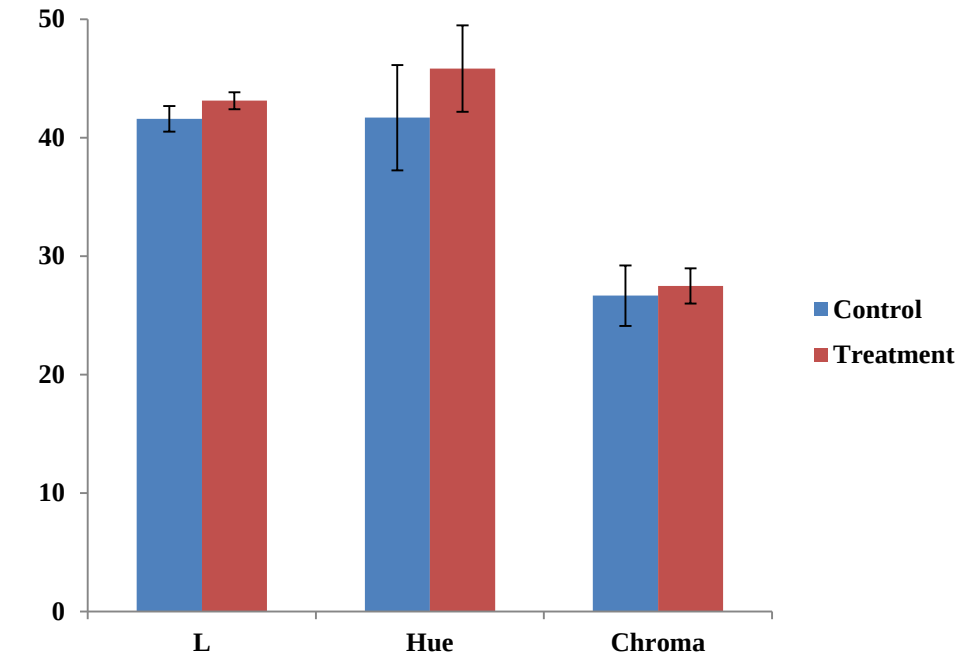


Figure 12. L, Hue and Chroma values of control (left) and treated samples (right) after 48 hrs for hypothesis testing (4)

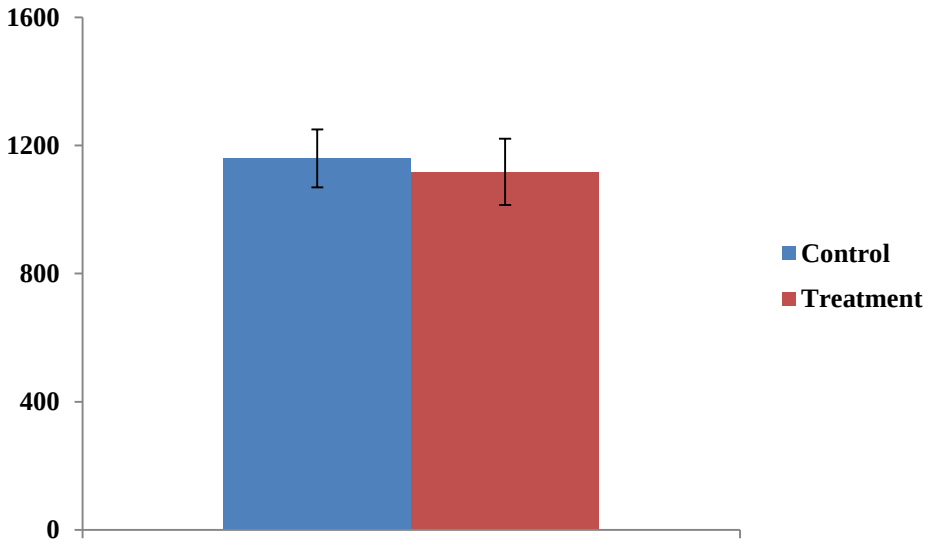


Figure 13. Firmness of control (left) and treated samples (right) after 48 hrs for hypothesis testing (4)

Table 11 Significance analysis between control and treated samples at 48 hrs for hypothesis testing (4) (from top to bottom: L, Hue, Chroma values and texture)

Source of Variation	SS	MS	F	P-value	F crit
Between Groups	4.727813	4.727813	5.667056	0.054726	5.987378
Within Groups	5.005575	0.834262			
Total	9.733387				

Source of Variation	SS	MS	F	P-value	F crit
Between Groups	34.30222	34.30222	2.070397	0.200235	5.987378
Within Groups	99.40767	16.56795			
Total	133.7099				

Source of Variation	SS	MS	F	P-value	F crit
Between Groups	1.318875	1.318875	0.301395	0.602825	5.987378
Within Groups	26.25542	4.375903			
Total	27.57429				

Source of Variation	SS	MS	F	P-value	F crit
Between Groups	4490.161	4490.161	0.477417	0.509143	5.317655
Within Groups	75240.89	9405.111			
Total	79731.05				

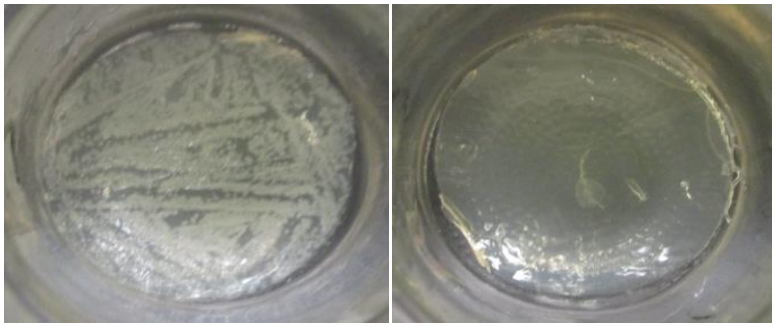


Figure 14. *Salmonella* spp. growth profiles of control (left) and treated samples (right) after 48 hrs for hypothesis testing (4)

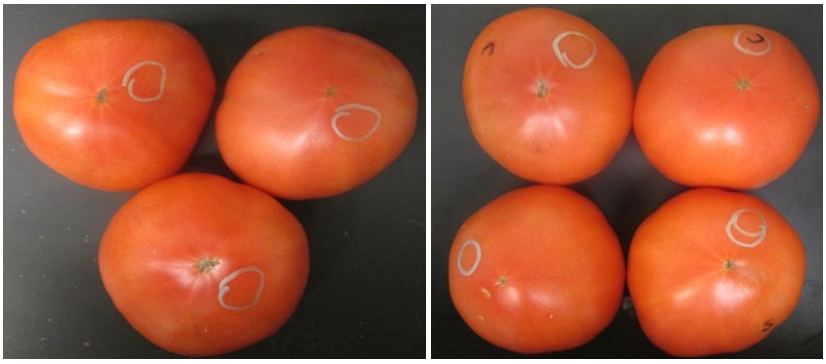


Figure 15. Visual observations of control (top) and treated samples (bottom) at 0 hrs (left picture) and 48 hrs (right picture) for hypothesis testing (4)

5.2 Chemistry Mechanism Investigation

5.2.1 To demonstrate the necessity of each component ($NaClO_2$, H_2O, CO_2) in $ClO_{2(g)}$ generation

The result of detected concentration for gaseous chlorine dioxide in headspace was shown in Figure 16. From the figure, it could be seen that with all three components (i.e. $NaClO_2$, RH and CO_2) being present in the system, the detected concentration of gaseous chlorine dioxide in headspace was 5 (± 5), 95 (± 13), 100 (± 26), 110 (± 26) and 100 (± 20) uL/L at 24, 48, 72, 120 and 144 hrs respectively. However, with only one component or two components being present in the system, gaseous chlorine dioxide was not able to be detected or be detected in a negligible amount in headspace (interfered by CO_2 and moisture in ambient environment).

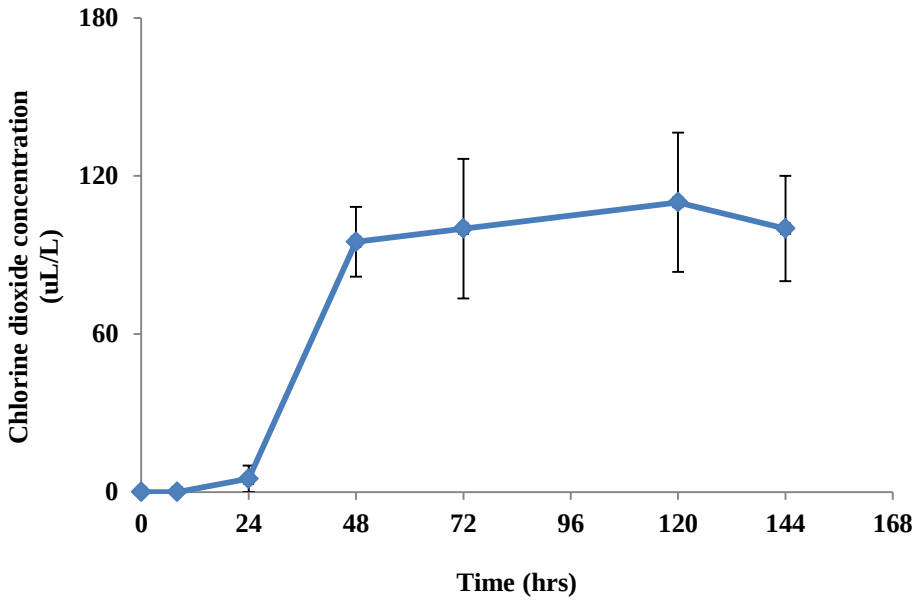


Figure 16. Release profile of $ClO_{2(g)}$ for chemistry necessity study

5.2.2 To understand the impact of each component ($NaClO_2$, H_2O, CO_2), temperature and light in the release profile of $ClO_{2(g)}$

1) $NaClO_2$ content

The impact of different $NaClO_2$ contents (0.2, 0.1 and 0.05 g) in the release profile of $ClO_{2(g)}$ was shown in Figure 17.

From Figure 17, it could be seen that with smaller $NaClO_2$ contents (0.1 and 0.05 g), 5 (± 5) uL/L gaseous chlorine dioxide was able to be detected at as early as 8 hrs in the headspace while with larger $NaClO_2$ content (0.2 g), the detection of similar concentration of gaseous chlorine in headspace was delayed by 16 hrs. It could be due to the original pH difference in the $NaClO_2$ water solution formed after moisture from headspace was absorbed by $NaClO_2$ powder. $NaClO_2$ is an alkaline compound [92,93], so larger $NaClO_2$ content would lead to a more alkaline solution environment while the generation of ClO_2 is favored at acidic pH.

Under 0.05, 0.1 and 0.2 g of $NaClO_2$, the maximum amount of $ClO_{2(g)}$ detected in headspace was 25 (± 5), 65 (± 18) and 110 (± 26) uL/L respectively. It demonstrated that the maximum amount of $ClO_{2(g)}$ detected in headspace increased with the increasing of $NaClO_2$ contents (though not in an exactly linear relationship).

In general, this experiment suggested that changing $NaClO_2$ content (within the range of 0.05 - 0.2 g) significantly affected the $ClO_{2(g)}$ release profile in headspace.

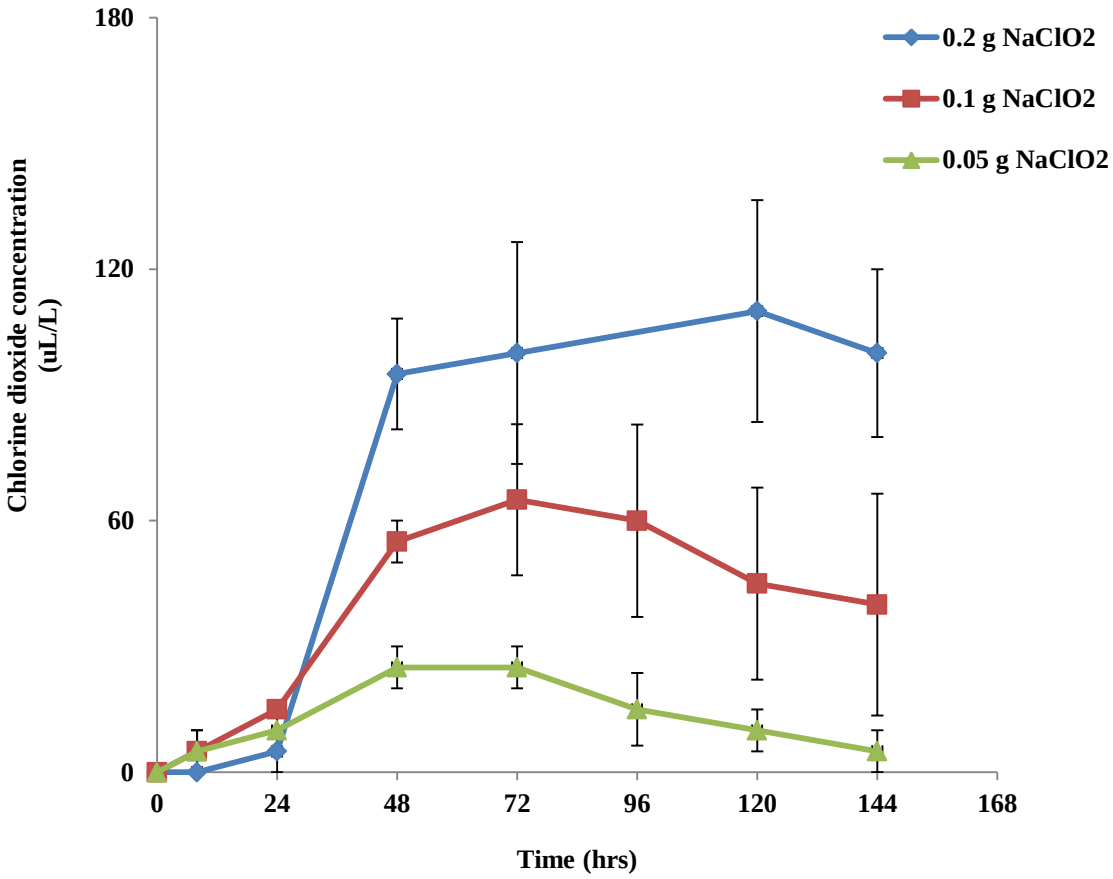


Figure 17. Concentration profiles of $ClO_{2(g)}$ in headspace under impact of different $NaClO_2$ contents

2) CO_2 content

The impact of different CO_2 contents (15% and 7.5%) in the release profile of $ClO_{2(g)}$ was shown in Figure 18.

From Figure 18, it could be seen that with larger CO_2 content (15%), gaseous chlorine dioxide in the headspace was able to be detected at 8 hrs (5 ± 5 uL/L), which was earlier than with lower CO_2 content (7.5%). Other than that, the two curves had no significant difference. At 24 and 120 hrs, the average concentrations of gaseous chlorine dioxide detected in the headspace under 15% and 7.5% CO_2 contents were identical (as 15 and 45 uL/L respectively). At 48 and 72 hrs, the average concentrations of gaseous chlorine dioxide detected in the headspace under 15% CO_2 content was slightly higher than under 7.5% CO_2 content (as 55 uL/L vs 45 uL/L and 65 uL/L vs 55 uL/L respectively) while at 96 hrs, the average concentration of gaseous chlorine dioxide detected in the headspace under 7.5% CO_2 content was slightly higher than under 15% CO_2 content (as 70 uL/L vs 60 uL/L).

The results suggested that 7.5% CO_2 content was already excessive in the system, so further increasing CO_2 content to 15% didn't create any significant difference in the gaseous chlorine dioxide generation and release profiles.

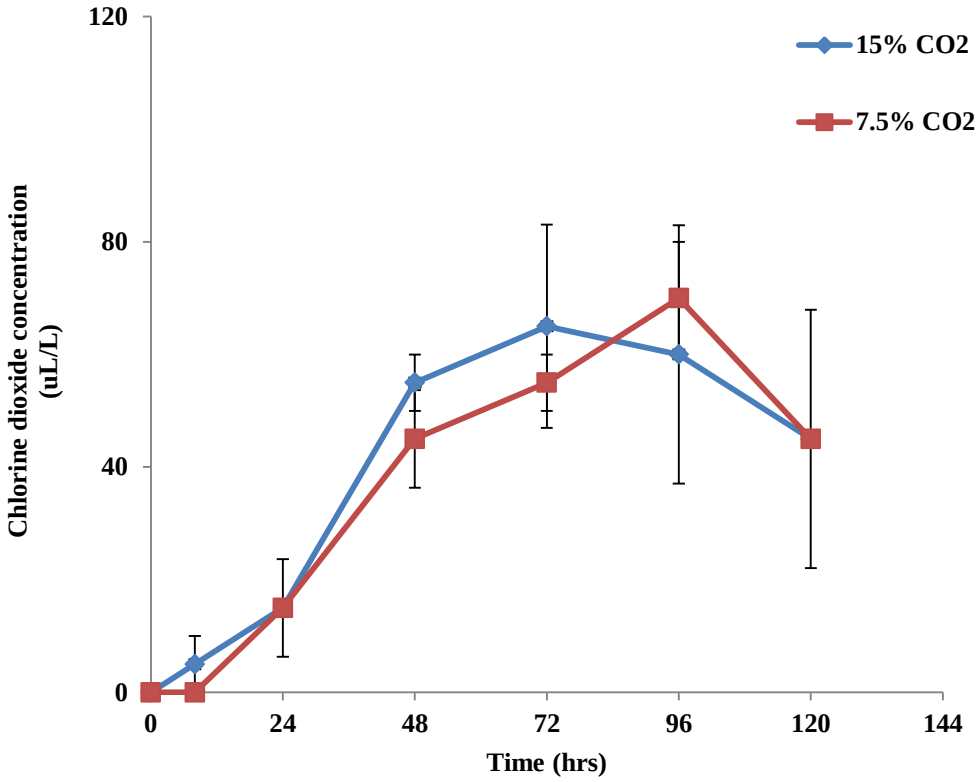


Figure 18. Concentration profiles of $ClO_{2(g)}$ in headspace under impact of different CO_2 contents

3) RH content

The impact of different RH contents (90% and 45%) in the release profile of $ClO_{2(g)}$ was shown in Figure 19.

From Figure 19, it could be seen that the two curves (under 90 and 45% RH) had very similar shapes. The only difference was that with higher RH content (90% RH), the detected concentration profile of gaseous chlorine dioxide in headspace was around 24 hrs earlier than that with lower RH content (45% RH), which suggested that with higher RH content, gaseous chlorine dioxide was generated faster. However higher RH content didn't increase the maximum concentration of gaseous chlorine dioxide detected in headspace (as 65 ± 18 uL/L for under 90% RH and 70 ± 5 uL/L for under 45% RH), which confirmed with the theory that moisture was not a reactant in the reaction of chlorine dioxide generation.

It has to be pointed out that higher RH content may not always accelerate the generation of chlorine dioxide. When it's excessive, it may dilute the reactant concentrations and decelerate the reaction.

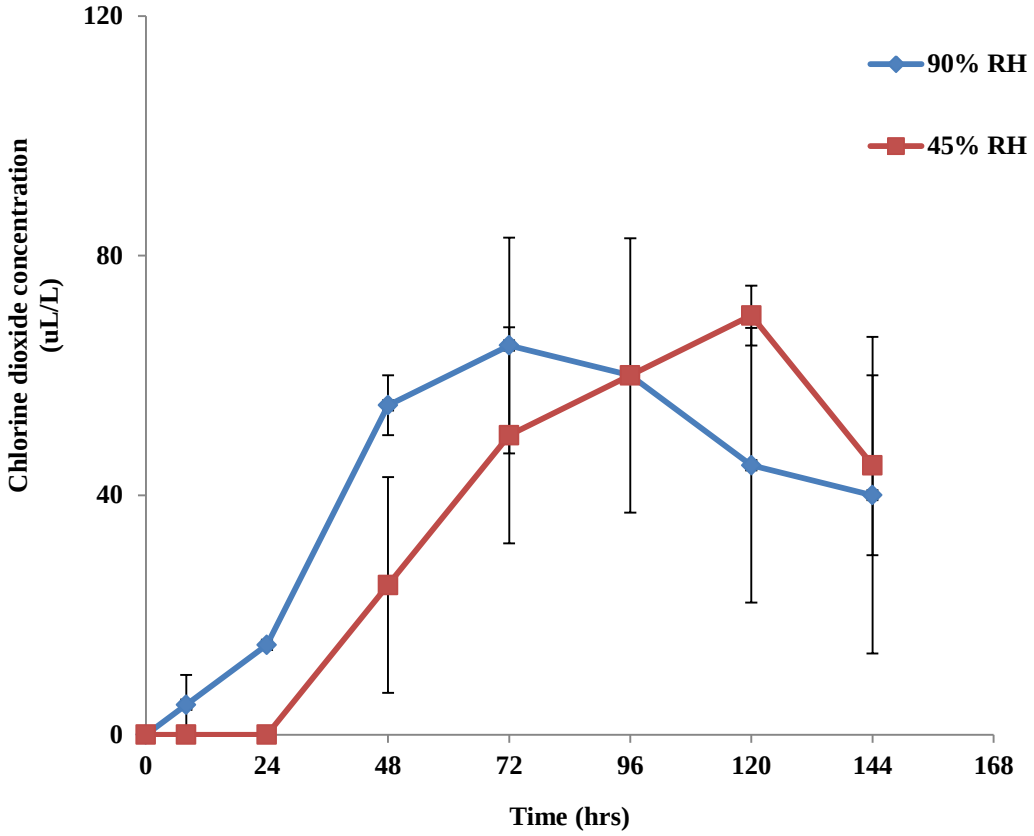


Figure 19. Concentration profiles of $ClO_{2(g)}$ in headspace under impact of different RH contents

4) Temperature

The impact of different temperatures (10, 22 and 35 ºC) in the release profile of $ClO_{2(g)}$ was shown in Figure 20.

From Figure 20, it could be seen that at 35 ºC, the concentration of gaseous chlorine dioxide (60 ± 0 uL/L) in headspace was able to be detected at as early as 8 hrs, and then it reached the maximum concentration of 90 (± 0) uL/L at 24 hrs. After 24 hrs, its detected concentration in headspace started to decrease sharply (as 65 ± 10, 60 ± 8, 40 ± 5 and 25 ± 5 uL/L at 48, 72, 96 and 144 hrs respectively). It's because when the generation of chlorine dioxide slowed down after 24 hrs, the decomposition of chlorine dioxide dominated at such a high temperature (35 ºC). At lower temperatures (22 and 10 ºC), chlorine dioxide generation in headspace was delayed compared to at 35 ºC (especially for under 10 ºC).

The maximum concentration of chlorine dioxide detected in headspace was temperature dependent. When temperature increased within 10 - 35 ºC range, the maximum concentration detected (as 90 ± 0, 65 ± 18 and 40 ± 10 uL/L for under 35, 22 and 10 ºC respectively) also increased (though not in a exactly linear relationship), which indicated that the reaction was high temperature favorable: at higher temperature, the equilibrium of reaction ($CO_2 + H_2O + NaClO_2 \leftrightarrows ClO_2$) moves towards the right side, which means that more chlorine dioxide will be generated.

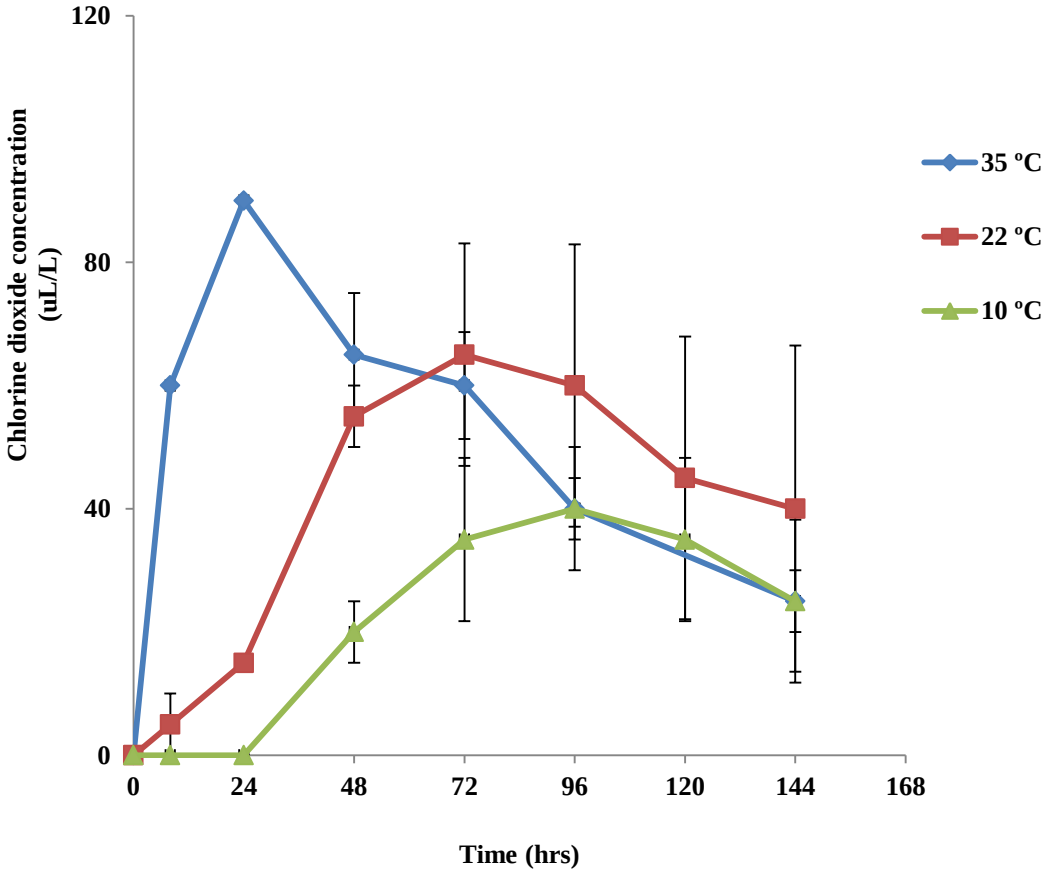


Figure 20. Concentration profiles of $ClO_{2(g)}$ in headspace under impact of different temperatures

5) Light condition

The impact of different light condition (dark and light) in the release profile of $ClO_{2(g)}$ was shown in Figure 22.

From Figure 22, it could be seen that under light, chlorine dioxide generation in headspace was much faster than under dark within 24 hrs. For example, at 8 hrs, 10 ± 5 uL/L gaseous chlorine dioxide was detected under light while only 5 ± 5 uL/L gaseous chlorine dioxide was detected under dark. At 24 hrs, 50 ± 10 uL/L gaseous chlorine dioxide was detected under light while only 15 ± 0 uL/L gaseous chlorine dioxide was detected under dark. The reason could be due to the extra energy provided from the light to the system, which accelerated the initial generation of gaseous chlorine dioxide under light within 24 hrs. However, the light exposure also accelerated the decomposition of ClO_2 in headspace [94,95] (shown in Figure 21), so after 24 hrs, when the generation of chlorine dioxide slowed down, the detectable chlorine dioxide concentration in headspace kept low.

Figure 21. Appearance of decomposition product of gaseous chlorine dioxide after 120 hrs (left: with light; right: in dark)

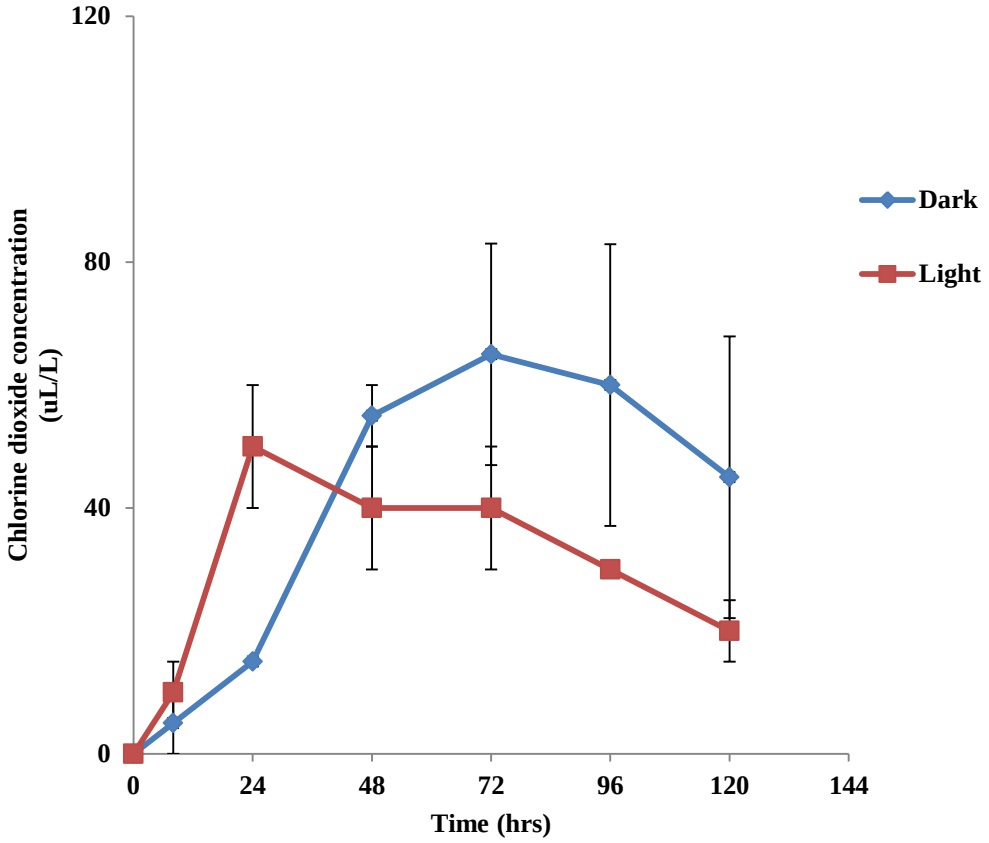


Figure 22. Concentration profiles of $ClO_{2(g)}$ in headspace under impact of different light environments

5.2.3 To understand the chemistry principles and propose possible chemical reactions

The major steps of chlorine dioxide generation within the proposed system are shown in Figure 23 below:

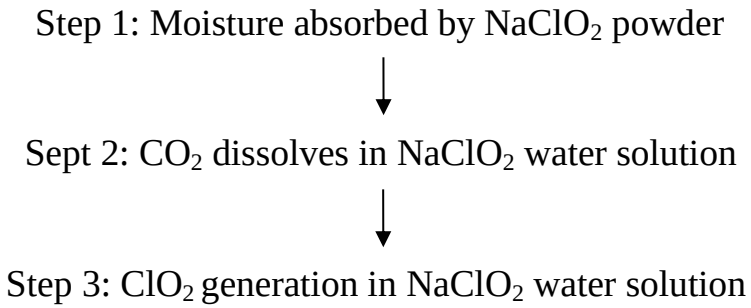


Figure 23. Chlorine dioxide generation steps

Step 2 is extremely important. Dissolving of CO_2 would lower the pH in $NaClO_2$ water solution (originally alkaline) and start the generation of chlorine dioxide. The relationship between pH and chlorine dioxide generation profile in water solution was investigated. The results were shown in Figure 24. From the figure, it could be seen that under alkaline pH (pH = 9.5) and neutral pH (pH = 7), the generation of chlorine dioxide in water solution was negligible. When pH was acidic, chlorine dioxide started to generate. The lower the pH, the more chlorine dioxide was able to be generated, which confirmed with the theory that the concentration of H^+ played a very important role in the reaction.

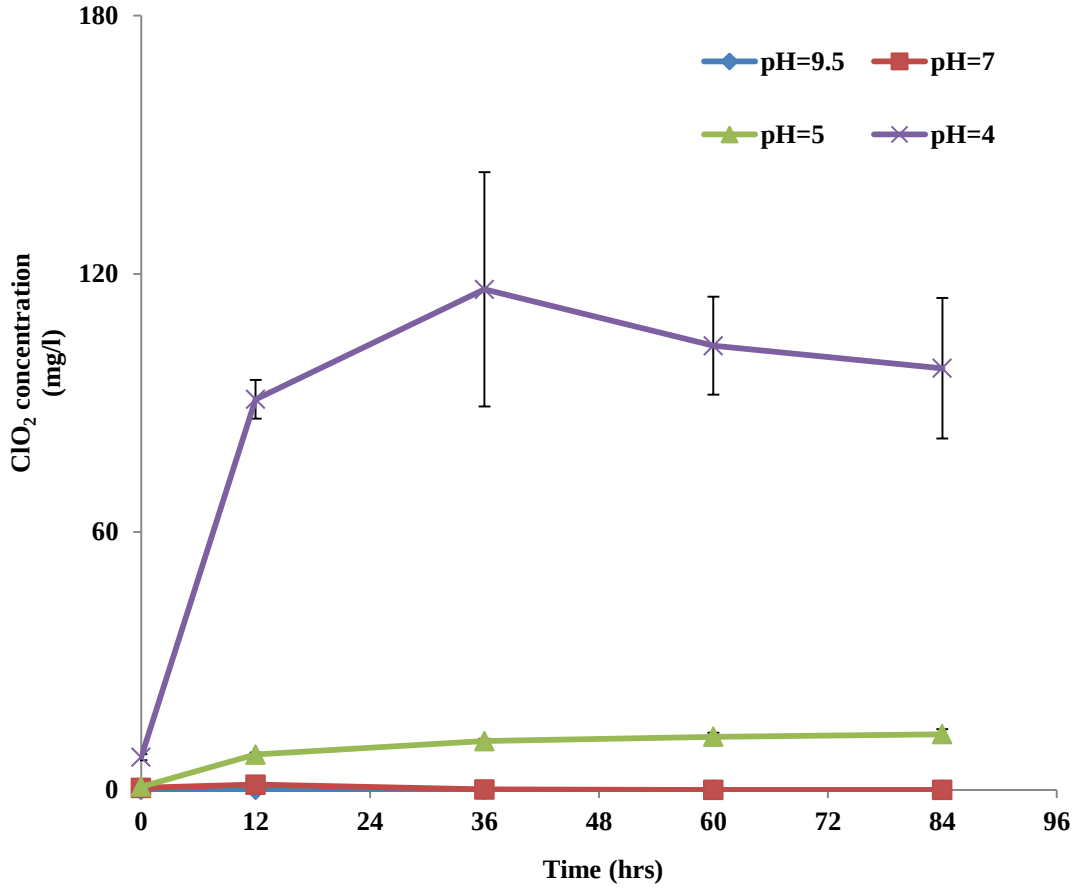


Figure 24. Chlorine dioxide generation profile in water solutions with different pH

There is always a balance between ClO_2 generated in solution, released in headspace and decomposed in headspace:

$$ClO_{2(\text{generated in solution})} \leftrightarrows ClO_{2(\text{released in headspace})} \leftrightarrows ClO_{2(\text{decomposed in headspace})}$$

More details regarding this balance are shown below:

1st stage: as more and more CO_2 dissolves in $NaClO_2$ water solution, pH in water solution (originally above 7 due to the alkalinity of $NaClO_2$ [92,93]) goes down to below 7, ClO_2 starts generating in water solution.

2nd stage: pH in water solution continues going down, more ClO_2 is generated in water solution therefore leads to more ClO_2 released in headspace. The rate of ClO_2 released in headspace is more than the rate of ClO_2 decomposed in headspace. ClO_2 concentration detectable in headspace ($ClO_{2(\text{released})} - ClO_{2(\text{generated})}$) increases.

3rd stage: At a certain pH or pH range in water solution, the rate of ClO_2 released from water solution into headspace is equal to the rate of ClO_2 decomposed in headspace, ClO_2 concentration detectable in headspace stays stable.

4th stage: pH slowly goes up in water solution, less ClO_2 is generated in water solution therefore leads to less ClO_2 released in headspace. The rate of ClO_2 decomposed in the headspace is more than the rate of ClO_2 released into headspace. ClO_2 concentration detectable in headspace decreases.

5th stage: pH continues to go up to be 7 or beyond 7, ClO_2 generation in water solution stops, no ClO_2 is released into headspace anymore. After previously accumulated ClO_2 in the headspace is decomposed, concentration of ClO_2 in headspace becomes undetectable.

The possible chemical equations involved in the reaction are shown below:

1) ClO_2 generation

Carbonic acid formation [96]

$CO_{2(gas)} \leftrightarrows CO_{2(dissolved\ in\ water\ solution)}$

$CO_{2(dissolved\ in\ water\ solution)} + H_2O \leftrightarrows H_2CO_3$

Dissociation of carbonic acid and formation of proton [96]

$H_2CO_3 \leftrightarrows H^+ + HCO_3^-$

$HCO_3^- \leftrightarrows H^+ + CO_3^{2-}$

The fractional relationship between H_2CO_3, HCO_3^-, CO_3^{2-} under different pH is shown as below [96]: when pH is < 6, H_2CO_3 dominates; when pH is between 6 and 10, HCO_3^- dominates; when pH is > 10, CO_3^{2-} dominates.

ClO_2 formation [35,36]

$H^+ + NaClO_2 \leftrightarrows HClO_2 + Na^+$

$5HClO_2 \leftrightarrows 4ClO_2 + HCl + 2H_2O$

In summary

$4CO_{2(\text{dissolved in water solution})} + 4H_2O + 5NaClO_2 \leftrightarrows 4ClO_2 + NaCl + 4NaHCO_3 + 2H_2O$

or

$2CO_{2(\text{dissolved in water solution})} + 2H_2O + 5NaClO_2 \leftrightarrows 4ClO_2 + NaCl + 2Na_2CO_3 + 2H_2O$

2) ClO_2 decomposition

$ClO_2 \leftrightarrows Cl_2 + O_2 +$ other by complex by-products (Cl_yO_z) [32,36]

$ClO_2 + H_2O \leftrightarrows ClO^- + ClO_3^- + H^+ + Cl^- + O_2 + O_3 +$ other complex by-products [94,95]

5.3 Microbiology Mechanism Investigation

5.3.1 To demonstrate the necessity of each component ($NaClO_2$, CO_2 and H_2O) in the inhibition effects against *Salmonella* spp.

The experimental results were shown in Figure 25. From the figure, it could be seen that after 48 hrs, compared to controls (with only moisture), the adding of $NaClO_2$ had no significant impact ($p>0.05$) in *Salmonella* spp. growth inhibition. The adding of CO_2 exhibited significant inhibition effects ($p<0.05$), with both the bacterial colony size and colony formation unit (CFU) being reduced. But only when $NaClO_2$, CO_2 and moisture were combined, complete inhibition effects ($p<0.01$) were observed. Agars were further incubated at 37 ºC overnight and found no bacteria grew back.

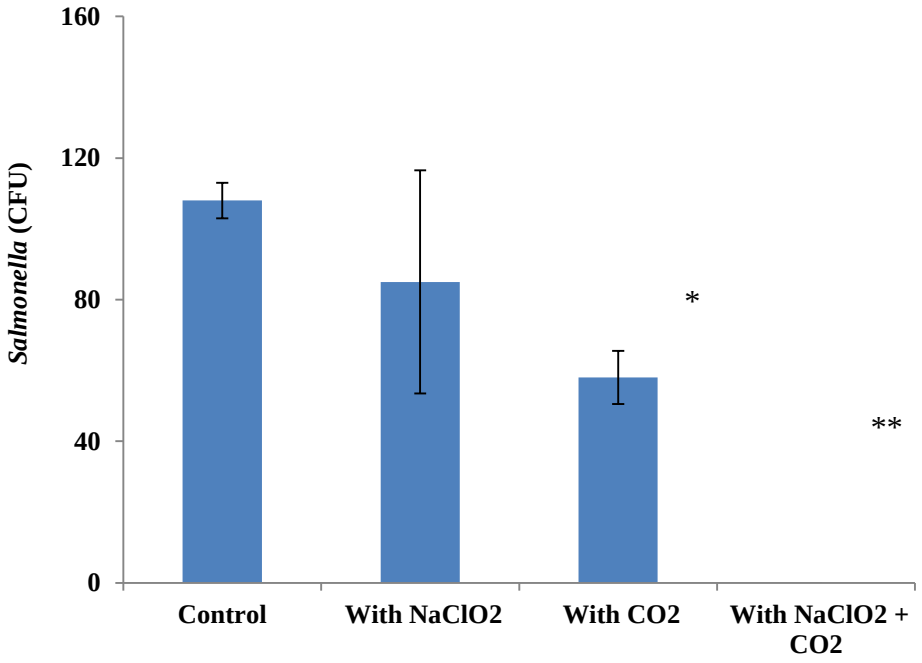


Figure 25. Microbial necessity study inhibition results

Table 12 Significance analysis in Microbial necessity study
(top: between control and with $NaClO_2$; middle: between control and with CO_2; bottom: between control and with $NaClO_2 + CO_2$)

Source of Variation	SS	MS	F	P-value	F crit
Between Groups	506.25	506.25	0.497665	0.553623	18.51282
Within Groups	2034.5	1017.25			
Total	2540.75				

Source of Variation	SS	MS	F	P-value	F crit
Between Groups	2550.25	2550.25	31.38769	0.030414	18.51282
Within Groups	162.5	81.25			
Total	2712.75				

Source of Variation	SS	MS	F	P-value	F crit
Between Groups	11664	11664	466.56	0.002136	18.51282
Within Groups	50	25			
Total	11714				

5.3.2 To understand the impact of each component ($NaClO_2$, H_2O, CO_2), temperature and light in the inhibition effects against *Salmonella* spp.

1) $NaClO_2$ content

The impact of different $NaClO_2$ contents (0.05, 0.1, 0.2 g) in the inhibition effects against *Salmonella* spp. was shown in Figure 26.

The results showed that after 48 hrs, under all three adding amount (0.05, 0.1, 0.2 g) of $NaClO_2$, 10^4 CFU *Salmonella* spp. on TSA agar were completely inhibited within 48 hrs. All agar were further incubated at 37 ºC overnight and found no bacteria grew back. This experiment demonstrated that adding tiny amount (as low as 0.05 g) of $NaClO_2$ could be enough to exhibit complete inhibition effects against 10^4 CFU *Salmonella* spp. on TSA agar within 48 hrs.

2) CO_2 content

The impact of different CO_2 contents (15% and 7.5%) in the inhibition effects against *Salmonella* spp. was shown in Figure 26.

The results showed that after 48 hrs under both 7.5% and 15% of CO_2, 10^4 CFU *Salmonella* spp. on TSA agar were completely inhibited within 48 hrs. All agar were further incubated at 37 ºC overnight and found no bacteria grew back. This experiment demonstrated that very low content (as low as 7.5%) of CO_2 could be enough to exhibit complete inhibition effects against 10^4 CFU *Salmonella* spp. on TSA agar within 48 hrs.

3) RH content

The impact of different RH contents (90% and 45%) in the inhibition effects against *Salmonella* spp. was shown in Figure 26.

The results showed that after 48 hrs under both 45% and 90% of RH, 10^4 CFU *Salmonella* spp. on TSA agar were completely inhibited within 48 hrs. All agar were further incubated at 37 ºC overnight and found no bacteria grew back. This experiment demonstrated that very low content (as low as 45%) of RH could be enough to exhibit complete inhibition effects against 10^4 CFU *Salmonella* spp. on TSA agar within 48 hrs.

4) Temperature

The impact of different temperatures (10, 22 and 35 ºC) in the inhibition effects against *Salmonella* spp. was shown in Figure 26.

The results showed that after 48 hrs under all three temperatures (10, 22 and 35 ºC), 10^4 CFU *Salmonella* spp. on TSA agar was completely inhibited within 48 hrs. All agar were further incubated at 37 ºC overnight and found no bacteria grew back. Temperature affects both ClO_2 generation and bacteria growth in the same direction. When temperature rises, both ClO_2 generation and bacteria growth are promoted. When temperature drops, both ClO_2 generation and bacteria growth are inhibited. This experiment demonstrated that within the 10 - 35 ºC temperature range, ClO_2

generation rate matched with bacteria growth rate, so complete inhibition effects against 10^4 CFU *Salmonella* spp. on TSA were observed at any temperature (between 10 - 35 ºC) within 48 hrs.

5) Light condition

The impact of different light conditions (dark and light) to the inhibition effects against *Salmonella* spp. was shown in Figure 26.

The results showed that after 48 hrs under both dark and light conditions, 10^4 CFU *Salmonella* spp. on TSA agar were completely inhibited within 48 hrs. All agar were further incubated at 37 ºC overnight and found no bacteria grew back. This experiment demonstrated that the light condition didn't affect the complete inhibition effects against 10^4 CFU *Salmonella* spp. in TSA agar within 48hrs.

6) Conclusion

The level variations (within the experimental range) of the factors investigated above (i.e. $NaClO_2$ content, CO_2 content, RH, temperature and light condition) didn't affect the complete inhibition effects against 10^4 CFU *Salmonella* spp. on TSA agar within 48 hrs.

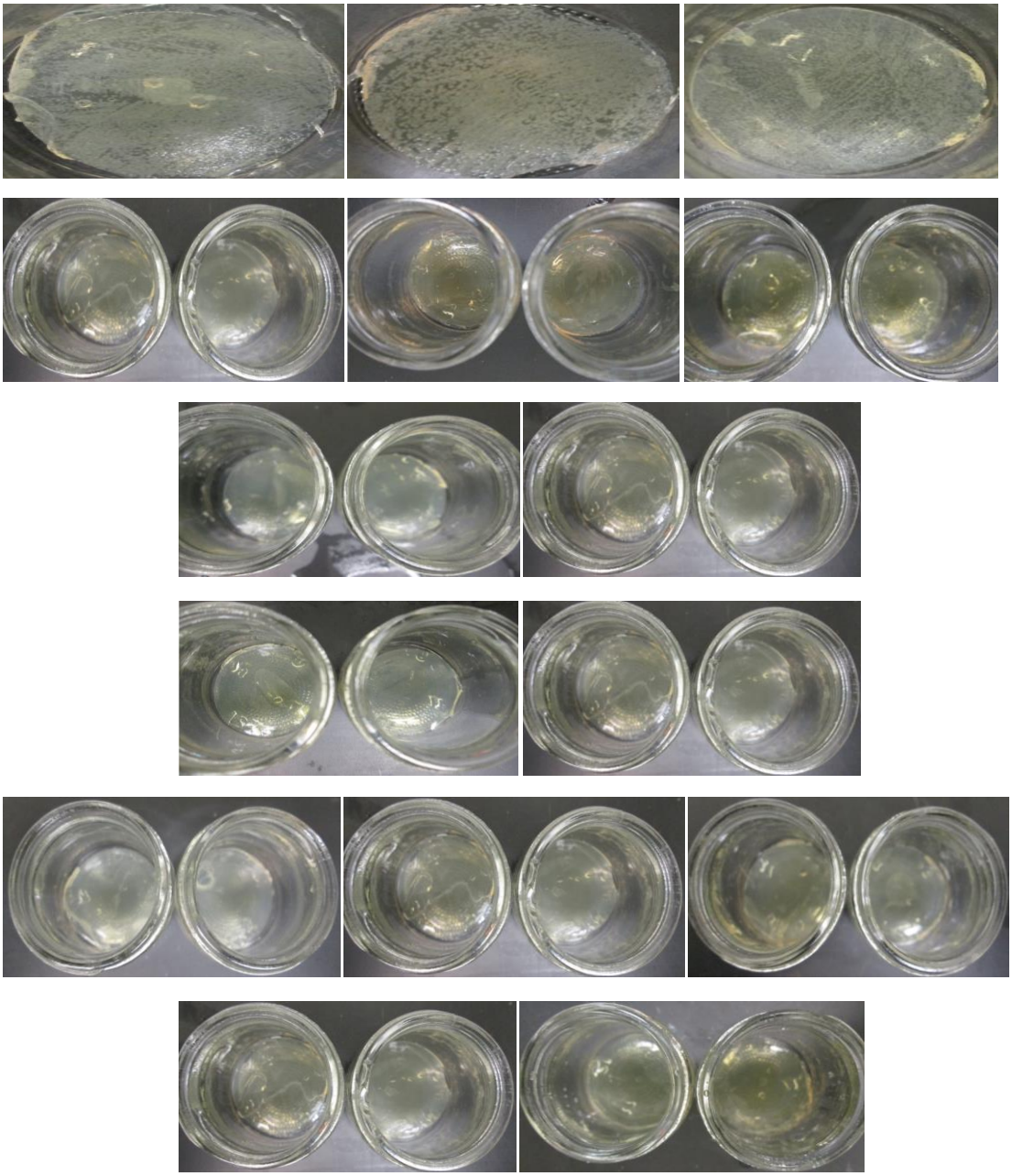


Figure 26. *Salmonella* spp. growth profile under different conditions after 48 hrs (first line: controls; second line: 0.05, 0.1 and 0.2 g $NaClO_2$; third line: 7.5% and 15% CO_2; Fourth line: 45% and 90% RH; fifth line: 10, 22 and 35 ºC; sixth line: dark and light)

5.3.3 "D value" and "Z value" obtaining

"D value" is defined as "decimal reduction time" and is "the time required at a certain temperature to kill 90% of the organisms being studied [97]."

"Z value" is defined as "the temperature required for one log reduction in the D-values [98]."

In order to obtain "D value" and "Z value", the inhibition results for *Salmonella* spp. at different time intervals under different temperatures were investigated and shown in Figure 27. From Figure 27, it could be seen that under 10 ºC, after 12, 24 and 48 hrs, a 1.7, 2.5 and 4 log reduction of *Salmonella* spp. CFU on TSA agar was achieved respectively. Under 22 ºC, after 6, 12 and 24 hrs, a 2, 2.8 and 4 log reduction of *Salmonella* spp. CFU on TSA agar was achieved respectively. Under 35 ºC, after 3, 6 and 12 hrs, a 2.1, 3 and 3.7 log reduction of *Salmonella* spp. CFU on TSA agar was achieved respectively.

"D value" and "Z value" were therefore calculated (shown in Figure 28 and 29) and displayed in Table 13. From the table, it could be seen that as temperature increased from 10 to 35 ºC, "D value" decreased from 12.6 hrs to 3.5 hrs, which meant that it took less time for the concentration of *Salmonella* spp. on TSA agar to exhibit 1 log reduction at higher temperature. From the three D values obtained, Z value was calculated to be 45.5 ºC, which meant that it required an increase of 45.5 ºC to exhibit one log reduction in D-value (between 10 to 35 ºC).

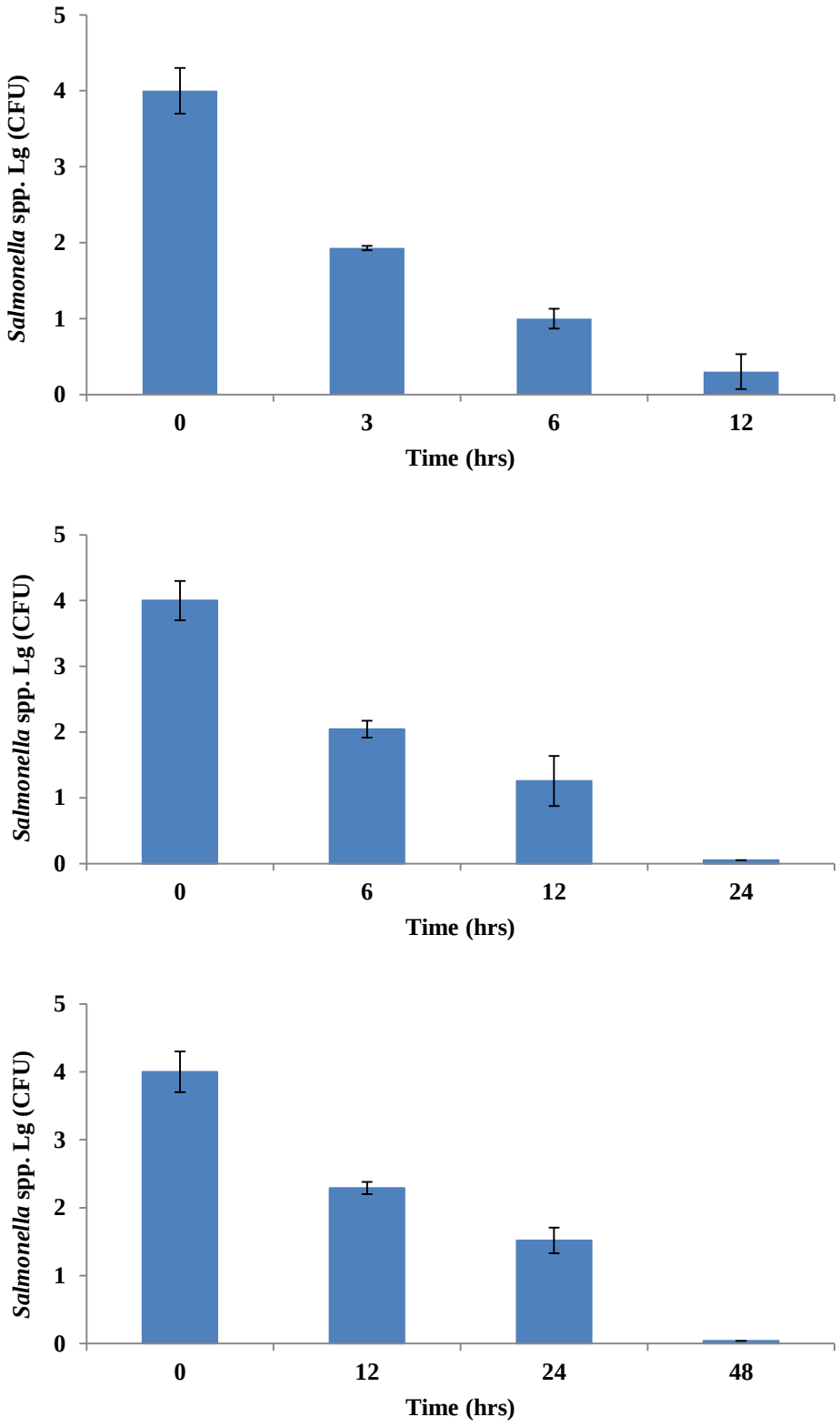


Figure 27. *Salmonella* spp. profiles on TSA agar at different time intervals for D value obtaining (top: 35 ºC; middle 22 ºC; bottom 10 ºC)

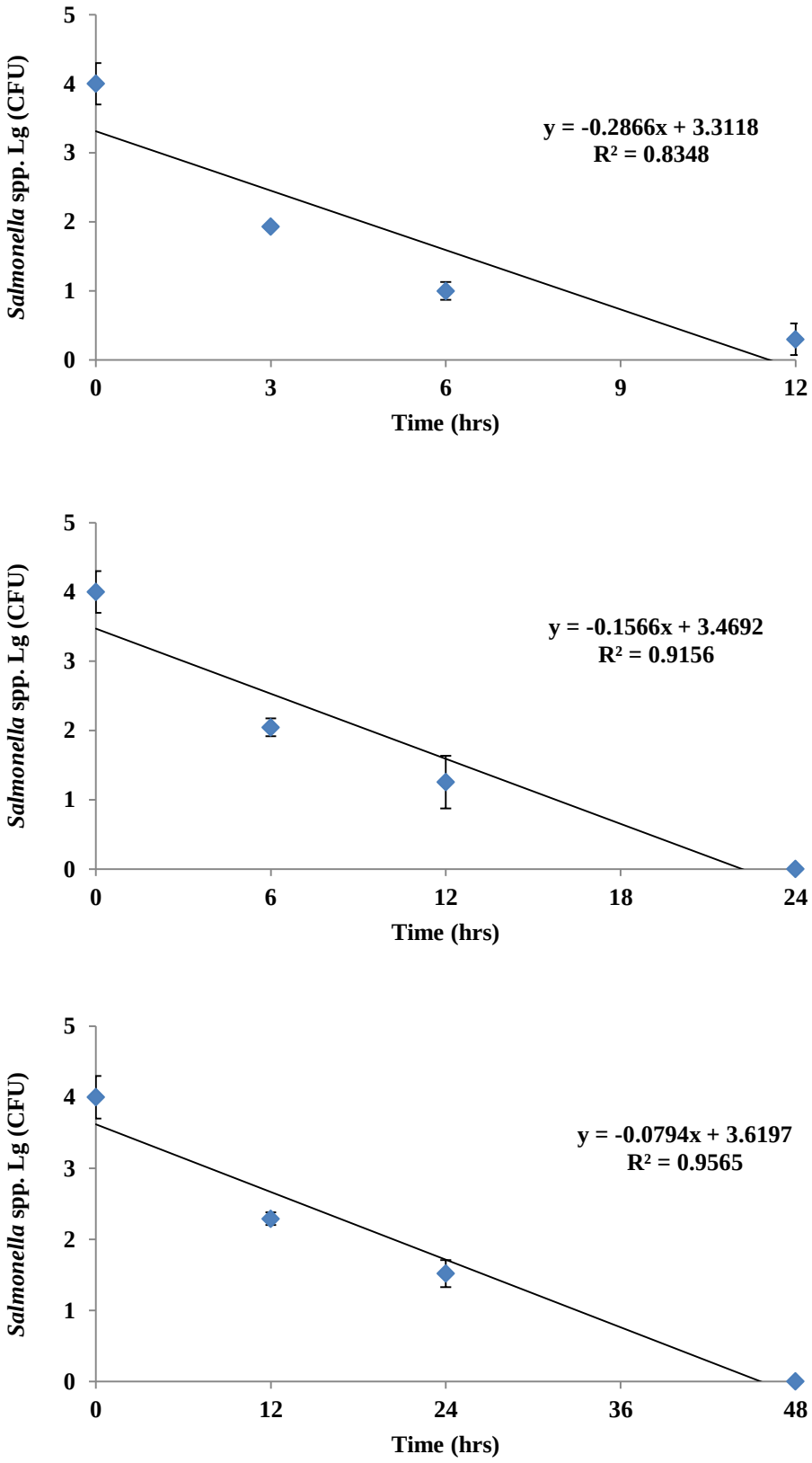


Figure 28. D value obtaining

(top: 35 ºC; middle 22 ºC; bottom 10 ºC)

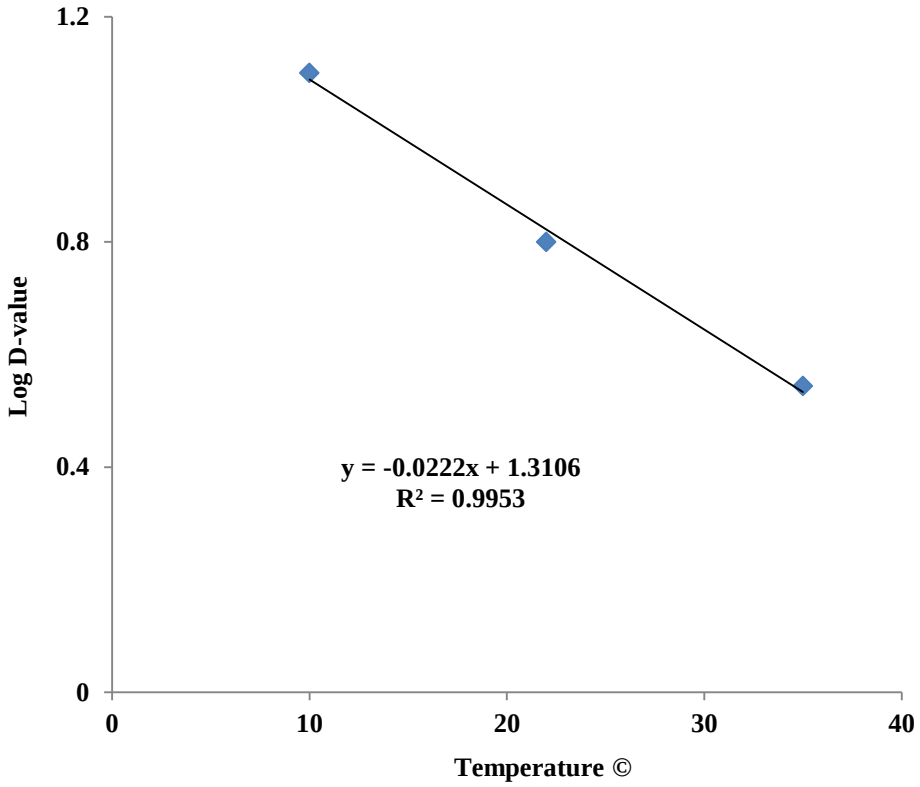


Figure 29. Z value obtaining

(from 10 to 35 ºC)

Table 13 D value and Z value summary

	10 ºC	**22 ºC**	**35 ºC**
D value (hrs)	12.6	6.4	3.5
Z value (ºC)		45.5 (between 10 to 35 ºC)	

5.4 $NaClO_2$ Delivery System Developments

5.4.1 Tyvek sachet

1) To confirm the successful generation of gaseous chlorine dioxide from developed system through analytical methods directly and microbial inhibition experiments indirectly under simulated conditions at different temperatures

The concentration profiles of gaseous ClO_2 detected in headspace using Tyvek sachet as a delivery system at different time intervals under different temperatures were shown in Figure 30 below. From the figure, it directly demonstrated the successful generation and release of gaseous chlorine dioxide using Tyvek sachet as a delivery system.

The microbial inhibition results using Tyvek sachet as a delivery system at different time intervals under different temperatures were shown in Table 14. From the table, it showed that for under 35 ºC, within 12 hrs, 10^4 *Salmonella* spp. on TSA agar was completely inhibited. For under 22 ºC, within 24 hrs, 10^4 *Salmonella* spp. on TSA agar was completely inhibited. For under 10 ºC, within 48 hrs, 10^4 *Salmonella* spp. on TSA agar was completely inhibited. All agar were further incubated at 37 ºC for 8 hrs and found no bacteria grew back. The microbial inhibition results indirectly demonstrated the successful generation and release of gaseous chlorine dioxide using Tyvek sachet as a delivery system.

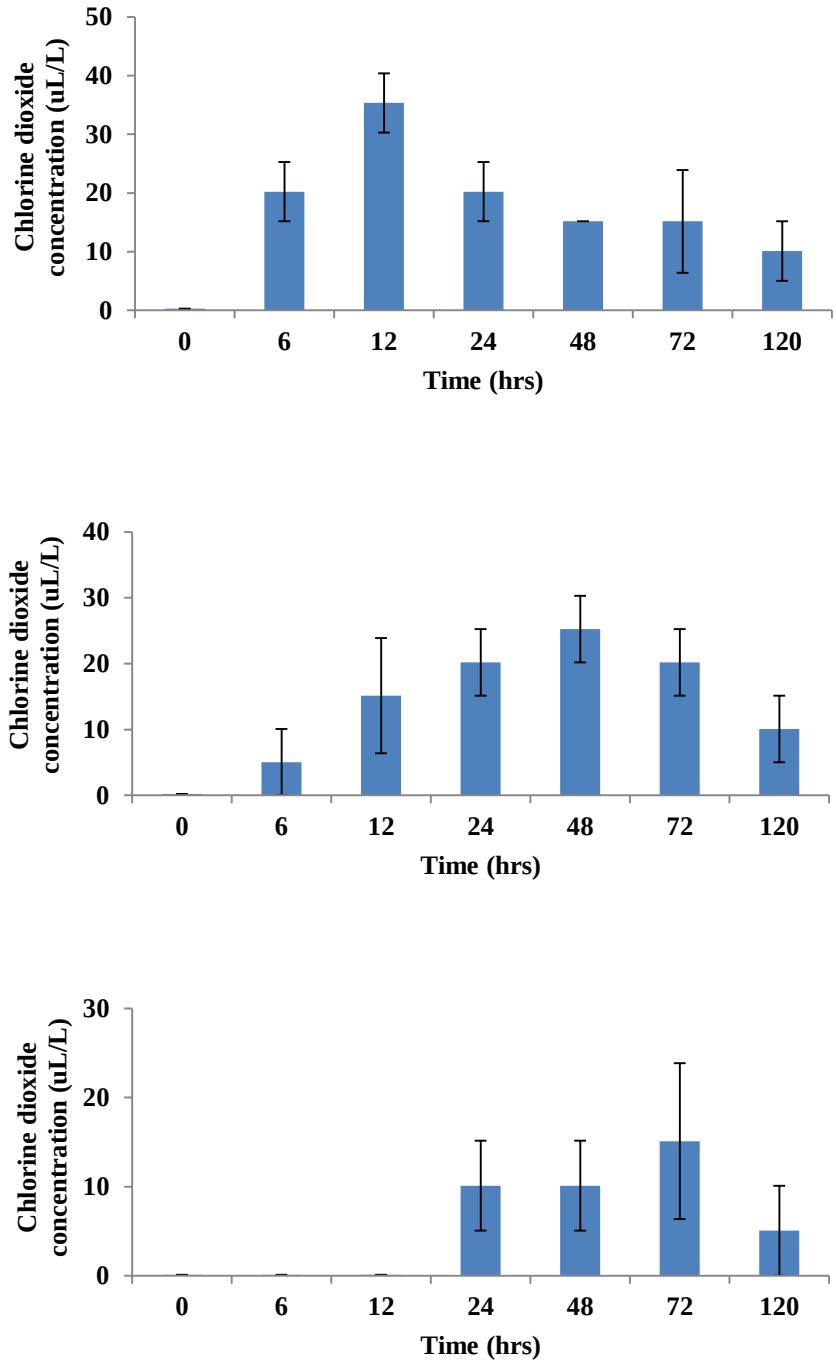


Figure 30. Profiles of $ClO_{2(g)}$ concentration in headspace using Tyvek bag (top: 35 ºC; middle: 22 ºC and bottom: 10 ºC)

Table 14 Microbial inhibition results for Tyvek sachet under simulated conditions

Temperature	Time	Results
35 ºC	12 hrs	Completely inhibited
22 ºC	24 hrs	Completely inhibited
10 ºC	48 hrs	Completely inhibited

Table 15 Microbial inhibition results for Tyvek sachet using fresh tomato

Microbial loads	Results		
10^2 CFU	35 ºC	12 hrs	Completely inhibited
	22 ºC	24 hrs	Completely inhibited
	10 ºC	48 hrs	Completely inhibited
10^4 CFU	35 ºC	12 hrs	Completely inhibited
	22 ºC	24 hrs	Completely inhibited
	10 ºC	48 hrs	Completely inhibited

2) To confirm the pathogen inhibition effects of developed system against *Salmonella* spp. using fresh tomato as the source of CO_2 and moisture at different temperatures

The microbial inhibition results using Tyvek sachet were shown in Table 15 above. From the table, it showed that for under 35 ºC, within 12 hrs, both 10^2 and 10^4 *Salmonella* spp. on TSA agar was completely inhibited. For under 22 ºC, within 24 hrs, both 10^2 and 10^4 *Salmonella* spp. on TSA agar was completely inhibited. For under 10 ºC, within 48 hrs, both 10^2 and 10^4 *Salmonella* spp. on TSA agar was completely inhibited. All agar were further incubated at 37 ºC for 8 hrs and found no bacteria grew back.

The profiles of CO_2 concentration detected in the headspace for both controls and treatments were shown in Figure 31 below. From Figure 31, it's easy to see that within the treatment time, the CO_2 concentration in headspace was below 10% in general and its difference between treated fresh tomato and controls was insignificant ($p>0.05$). Also, preliminary sensory evaluation including appearance and texture showed the sensory profile of both treated fresh tomato and controls were acceptable and had no practically significant difference.

In general, this experiment confirmed the pathogen inhibition effects of developed Tyvek sachet system using fresh tomato as source of CO_2 and RH under 10, 22 and 35 ºC. No significant sensory impact was detected between control and treated fresh tomatoes within 12, 24 and 48 hrs under 10, 22 and 35 ºC respectively.

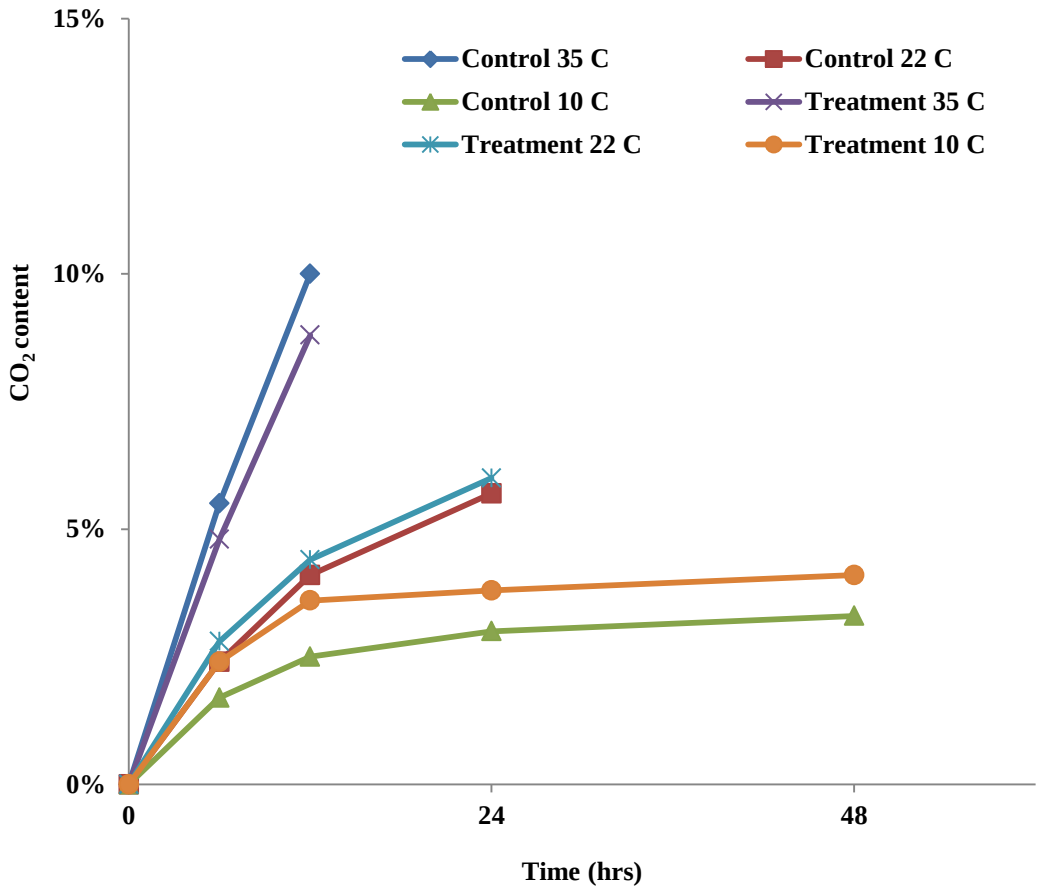


Figure 31. Profiles of CO_2 concentration in headspace for Tyvek sachet using fresh tomato

3) To optimize the developed system through both microbial inhibition and sensory tests under long-term storage

At 0 days, sensory evaluations including appearance and texture of randomly selected samples from both controls and treatments were conducted. For the appearance, L, Hue and Chroma values obtained through colorimeters for both controls (as 41.71 ± 1.11, 41.49 ± 1.49 and 28.95 ± 0.34) and treatments (as 42.01 ± 0.89, 41.20 ± 1.26 and 31.21 ± 2.23) were shown in Figure 32. For the texture, the maximum force obtained through texture analyzer for both controls (as 1118.9 ± 186.44 g) and treatments (as 1130.06 ± 221.42 g) were shown in Figure 33. From both figures, it could be concluded that there was no significant difference ($p>0.05$) between samples randomly selected from controls and treatments in appearance and texture (shown in Table 16), which agreed with the visual observations and the feedback from the sensory panel.

The microbial growth profile of *Salmonella* spp. on the surface of fresh tomato during 21-day storage was shown in Figure 34. For the control, the microbial concentration slightly dropped around half a log (from log 5.3 ± 0.3 CFU to log 5.0 ± 0.05 CFU) within first 24 hrs, it could be due to the low temperature shock. Then after the bacteria adapted to the temperature, it gradually grew back to the original concentration (log 5.3 - 5.5 CFU) and stayed stable. For the treatment, the microbial

concentration dropped from log 5.3 ± 0.3 CFU to log 4.5 ± 0.2 CFU within first 24 hrs. Then it dropped to below undetected level after 3 days and never grew back.

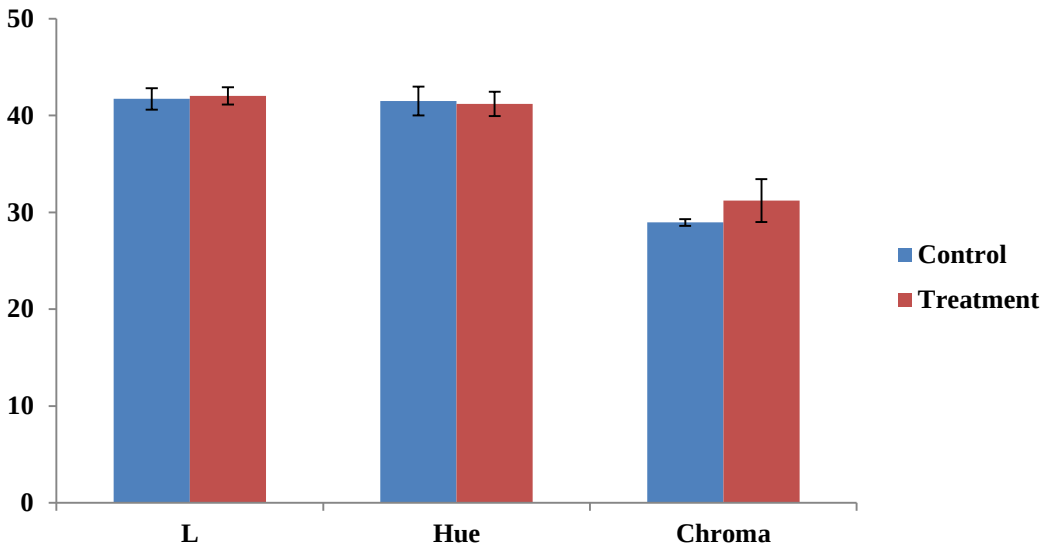


Figure 32. L, Hue and Chroma values of control (left) and treated samples (right) at 0 hrs using Tyvek sachet

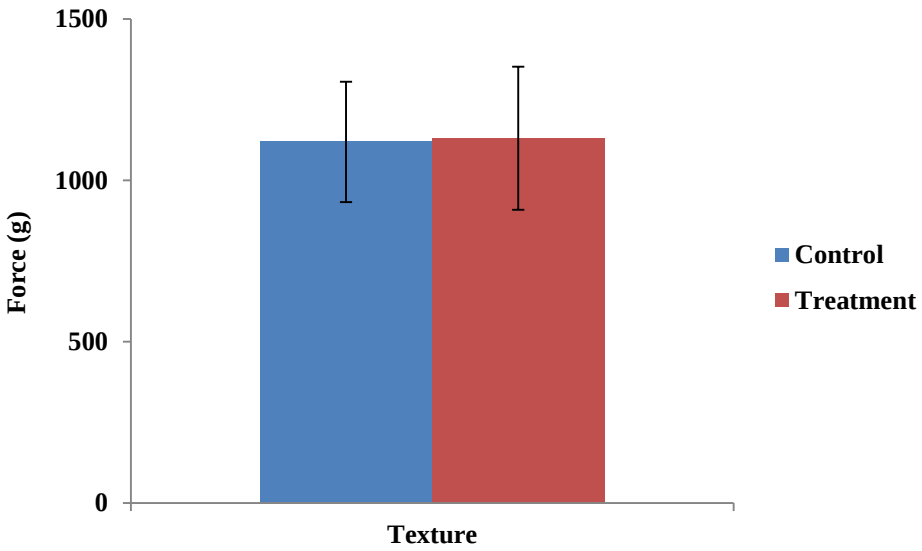


Figure 33. Firmness of control (left) and treated samples (right) at 0 hrs using Tyvek sachet

Table 16 Significance analysis between control and treated samples at 0 hrs using Tyvek sachet (from top to bottom: L, Hue, Chroma values and texture)

Source of Variation	SS	MS	F	P-value	F crit
Between Groups	0.23104	0.23104	0.226046	0.647182	5.317655
Within Groups	8.17676	1.022095			
Total	8.4078				

Source of Variation	SS	MS	F	P-value	F crit
Between Groups	0.209191	0.209191	0.109344	0.749386	5.317655
Within Groups	15.30518	1.913147			
Total	15.51437				

Source of Variation	SS	MS	F	P-value	F crit
Between Groups	12.76953	12.76953	5.01333	0.055508	5.317655
Within Groups	20.37692	2.547115			
Total	33.14645				

Source of Variation	SS	MS	F	P-value	F crit
Between Groups	311.364	311.364	0.007432	0.933418	5.317655
Within Groups	335154.7	41894.33			
Total	335466				

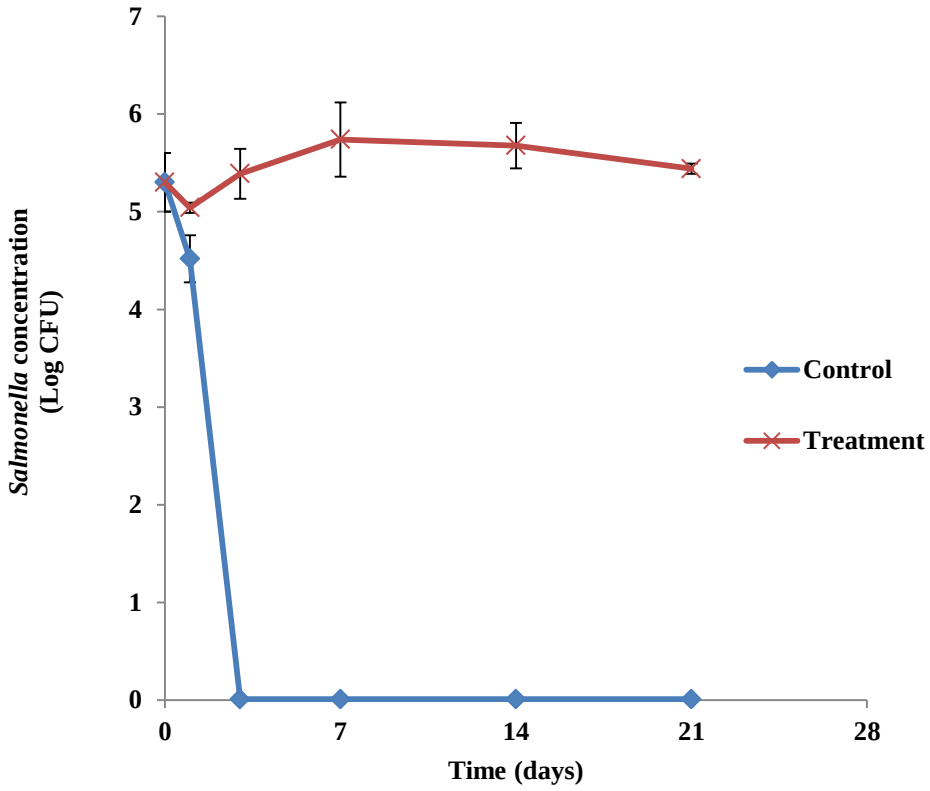


Figure 34. Microbial growth profiles of *Salmonella* spp. on fresh tomato during 21 day storage using Tyvek sachet

At 21 days, sensory evaluations including appearance and texture of randomly selected samples from both controls and treatments were conducted. For the appearance, L, Hue and Chroma values obtained through colorimeters for both controls (as 39.92 ± 0.86, 38.37 ± 1.82 and 32.24 ± 2.32) and treatments (as 38.93 ± 1.39, 38.26 ± 2.15 and 33.85 ± 1.96) were shown in Figure 35. For the texture, the maximum force obtained through texture analyzer for both controls (as 913.55 ± 201.71 g) and treatments (as 921.16 ± 109.44 g) were shown in Figure 36. From both figures, it could be concluded that there was no significant difference ($p>0.05$) between samples randomly selected from controls and treatments in appearance and texture (shown in Table 17), which agreed with the visual observations (shown in Figure 37) and the feedback from the sensory panel. However, the smell of treated tomato lacked the natural typical tomato aroma compared to controls. The profiles of CO_2 concentration detected in the headspace for both controls and treatments during 21-day storage were shown in Figure 38 below. From Figure 38, it's easy to see that within the treatment time, the CO_2 concentration in headspace was below 10% and its difference between treated fresh tomato and controls in the headspace was insignificant ($p>0.05$).

In conclusion, the Tyvek sachet treatment completely inhibited 10^5 CFU *Salmonella* spp. inoculated on the surface of fresh tomato within 3 days and no bacteria grew back within 21-day storage time. Also, the treatment didn't significantly affect the appearance and texture of tomato though the typical tomato smell was scarified.

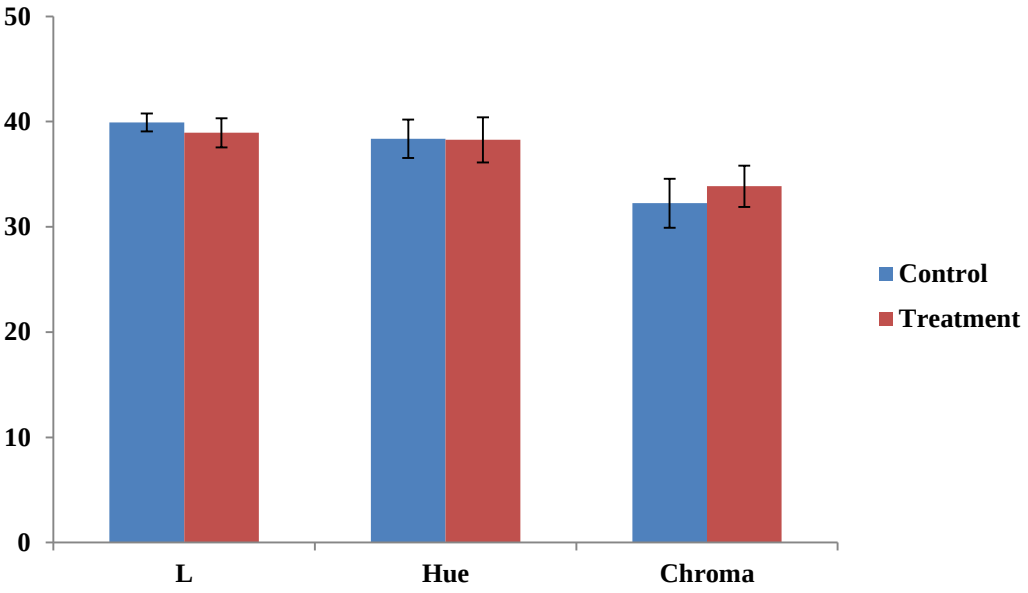


Figure 35. L, Hue and Chroma values of control (left) and treated samples (right) at 21 days using Tyvek sachet

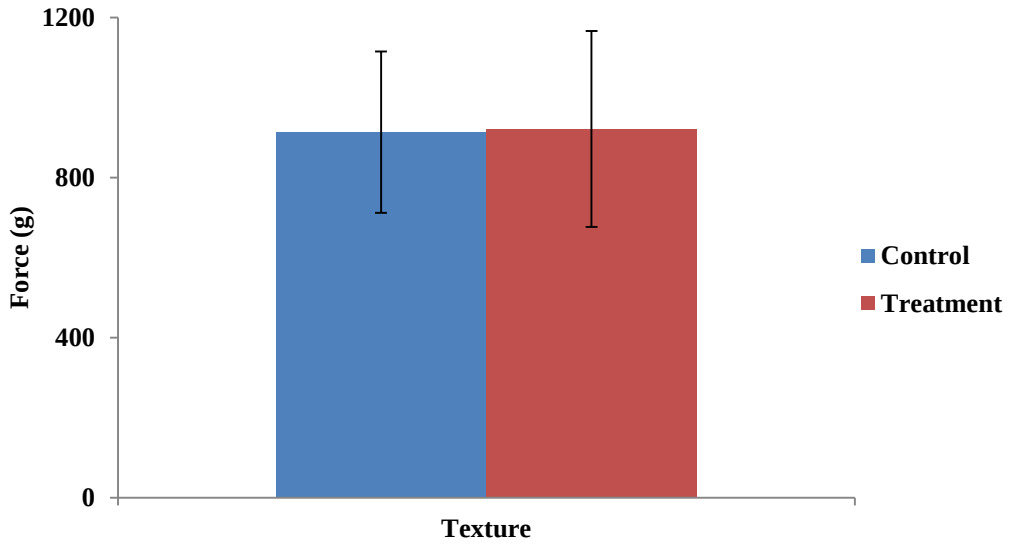


Figure 36. Firmness of control (left) and treated samples (right) at 21 days using Tyvek sachet

Table 17 Significance analysis between control and treated samples at 21 days using Tyvek sachet (from top to bottom: L, Hue, Chroma values and texture)

Source of Variation	SS	MS	F	P-value	F crit
Between Groups	3.900625	3.900625	2.913966	0.109887	4.60011
Within Groups	18.74035	1.338596429			
Total	22.64098				

Source of Variation	SS	MS	F	P-value	F crit
Between Groups	0.044074	0.044073596	0.01109	0.917626	4.60011
Within Groups	55.63981	3.974272213			
Total	55.68388				

Source of Variation	SS	MS	F	P-value	F crit
Between Groups	10.41469	10.4146923	2.24719	0.156066	4.60011
Within Groups	64.88357	4.634540609			
Total	75.29826				

Source of Variation	SS	MS	F	P-value	F crit
Between Groups	231.8006	231.800625	0.004609	0.946831	4.60011
Within Groups	704028.5	50287.75277			
Total	704260.3				

Figure 37. Visual observations of control (top) and treated samples (bottom) at 21 days using Tyvek sachet

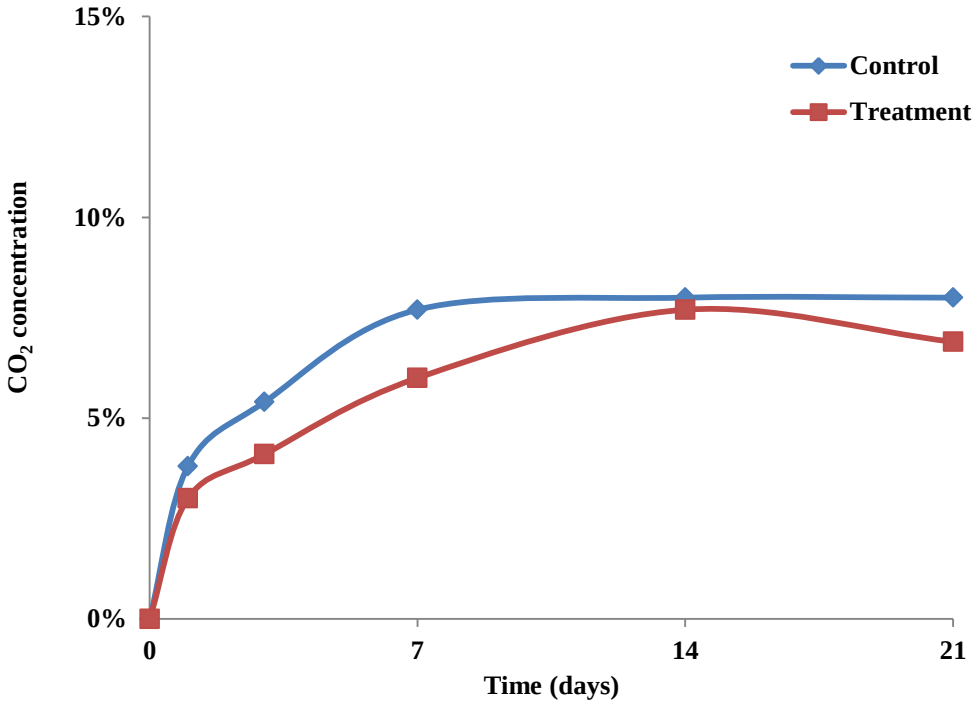


Figure 38. Profiles of CO_2 concentration in headspace during 21 days using Tyvek sachet

5.4.2 Gum arabic paste

1) To confirm the successful generation of gaseous chlorine dioxide from developed system through analytical methods directly and microbial inhibition experiments indirectly under simulated conditions at different temperatures

The concentration profiles of gaseous ClO_2 detected in headspace using gum arabic paste as a delivery system at different time intervals under different temperatures were shown in Figure 39 below. From the figure, it directly demonstrated the successful generation and release of gaseous chlorine dioxide using gum arabic paste as a delivery system.

The microbial inhibition results using gum arabic paste as a delivery system at different time intervals under different temperatures were shown in Table 18. From the table, it could be seen that under 35 ºC, within 12 hrs, 10^4 *Salmonella* spp. on TSA agar was completely inhibited. For under 22 ºC, within 24 hrs, 10^4 *Salmonella* spp. on TSA agar was significantly inhibited. For under 10 ºC, within 48 hrs, 10^4 *Salmonella* spp. on TSA agar was completely inhibited. All agars were further incubated at 37 ºC for 8 hrs and found no bacteria grew back except agars previously treated under 22 ºC (less than 20 colonies grew back). This microbial inhibition result indirectly demonstrated the successful generation and release of gaseous chlorine dioxide using gum arabic paste as a delivery system.

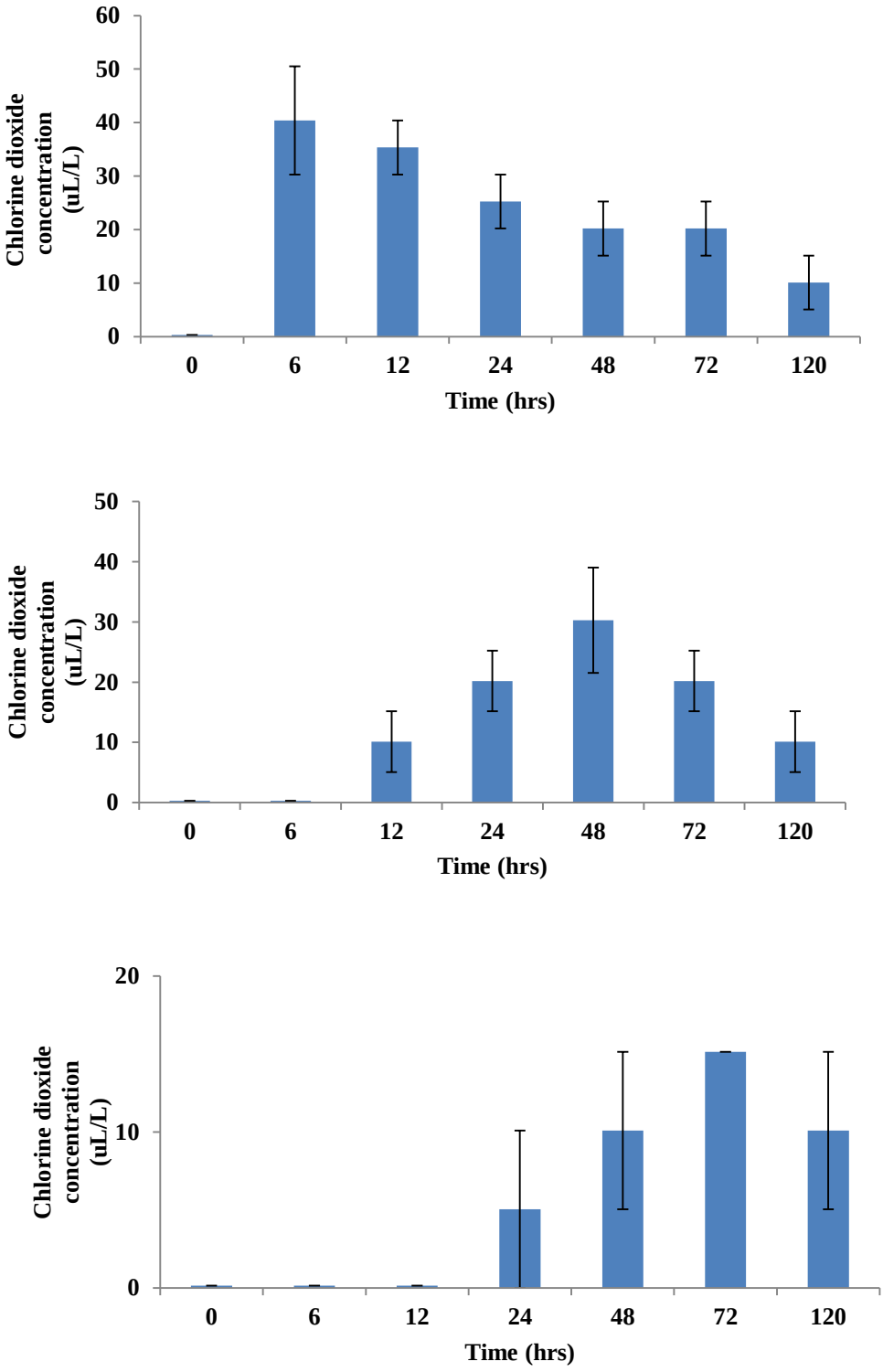


Figure 39. Profiles of $ClO_{2(g)}$ concentration in headspace using gum arabic paste (top: 35 ºC; middle: 22 ºC and bottom: 10 ºC)

Table 18 Microbial inhibition results for gum arabic paste under simulated conditions

Temperature	Time	Results
35 ºC	12 hrs	Completely inhibited
22 ºC	24 hrs	Significantly inhibited
10 ºC	48 hrs	Completely inhibited

Table 19 Microbial inhibition results for gum arabic paste using fresh tomato

Microbial loads	Results		
10^5 CFU	35 ºC	12 hrs	Completely inhibited
	22 ºC	48 hrs	Completely inhibited
	10 ºC	72 hrs	Completely inhibited

2) To confirm the pathogen inhibition effects of developed system against *Salmonella* spp. using fresh tomato as the source of CO_2 and moisture at different temperatures

The microbial inhibition results using gum arabic paste were shown as Table 25 above. From Table 19, it showed that for under 35 ºC, within 12 hrs, 10^4 *Salmonella* spp. on TSA agar was completely inhibited. For under 22 ºC, within 48 hrs, both 10^4 *Salmonella* spp. on TSA agar was completely inhibited. For under 10 ºC, within 72 hrs, 10^4 *Salmonella* spp. on TSA agar was completely inhibited. All agar were further incubated at 37 ºC for 8 hrs and found no bacteria grew back.

The profiles of CO_2 concentration detected in the headspace for controls and treatments were shown in Figure 40 below. From Figure 40, it's easy to see that within the treatment time, the CO_2 concentration in headspace was below 10% in general and its difference between treated fresh tomato and controls in the headspace was insignificant ($p>0.05$). However, preliminary sensory evaluation including appearance and texture showed the sensory profile of treated fresh tomato and controls had practically significant difference. It's mainly due to the dripping of the gum paste onto the surface of fresh tomato after moisture gain.

In general, this experiment confirmed the pathogen inhibition effects of developed gum arabic paste system using fresh tomato as the source of CO_2 and moisture under 10, 22 and 35 ºC. However significant sensory impact has been detected between control and treated fresh tomatoes within 12, 24 and 48 hrs under 10, 22 and 35 ºC

respectively. Therefore, this system was excluded from future long-term (21 days) storage experiments.

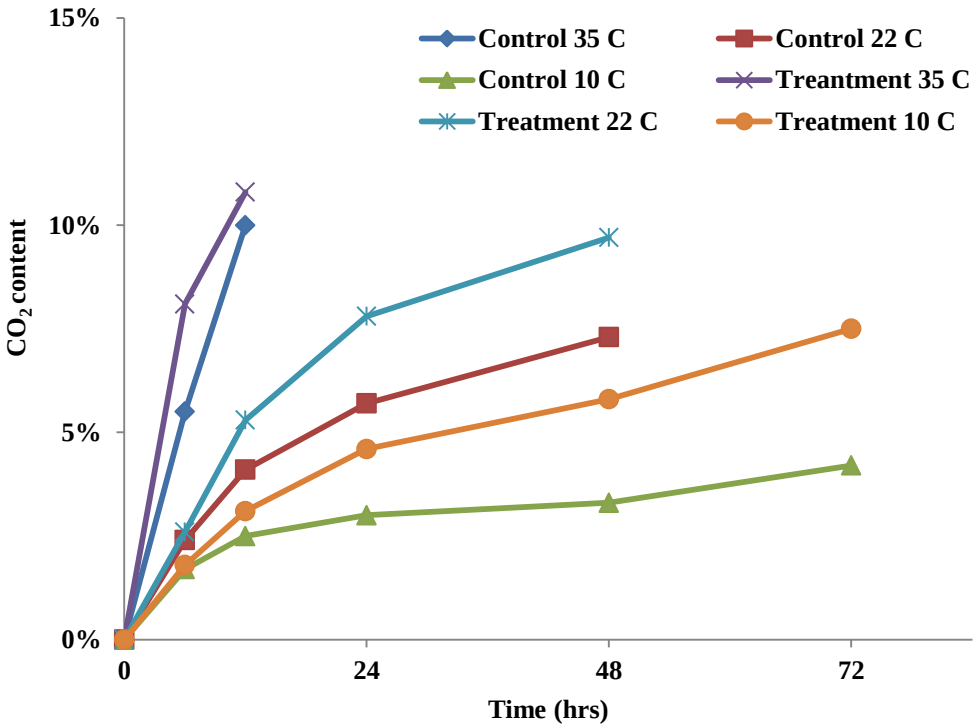


Figure 40. Profiles of CO_2 concentration in headspace for gum arabic paste using fresh tomato

5.4.3 Electrospun fiber

1) To obtain physical properties of different concentrations of PEO an $NaClO_2$ water solutions

The physical properties (including pH, conductivity, surface tension and viscosity) of 2%, 3% and 4% PEO water solutions were shown in Figure 41. From the figure, it could be seen that as the concentration of PEO increased in the water solutions, the conductivity decreased (as 110.8, 104.9 and 91.2 us for 2%, 3% and 4% PEO water solutions respectively) while viscosity increased (as 79.75, 552.15 and 1233 cP for 2%, 3% and 4% PEO water solutions respectively).

The physical properties (including pH, conductivity, surface tension and viscosity) of 3% PEO water solutions containing 0%, 0.15% and 0.3% $NaClO_2$ were shown in Figure 42. From the figure, it could be seen that as the concentration of $NaClO_2$ (as a salt) increased in the polymer solutions, the conductivity increased sharply (as 104.9, 1654 and 3220 us for 0%, 0.15% and 0.3% $NaClO_2$ in 3% PEO water solutions respectively). $NaClO_2$ is an alkaline compound [33], so as its concentration increased in polymer solutions, the pH also increased (as 7.7, 8.4 and 9.2 for 0%, 0.15% and 0.3% $NaClO_2$ in 3% PEO water solutions respectively). Furthermore, the addition of $NaClO_2$ slightly lowered the viscosity of the polymer solutions (as 552.15, 531.4 and 417.15 cP for 0%, 0.15% and 0.3% $NaClO_2$ in 3% PEO water solutions respectively),

it could due to the degradation of PEO polymer caused by $NaClO_2$ since it's a strong oxidant in water solution [33].

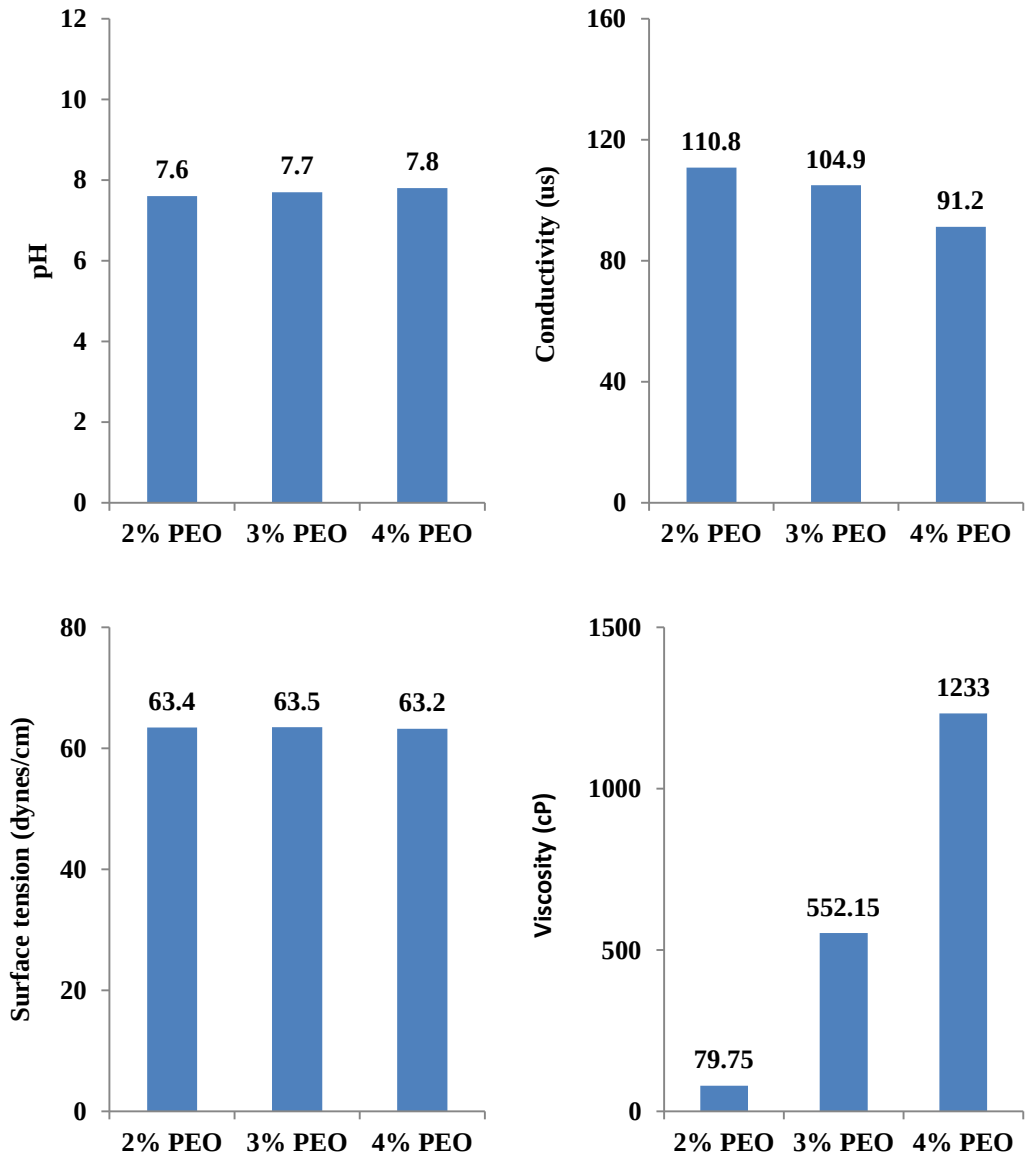


Figure 41. Physical properties of 2%, 3% and 4% PEO water solutions

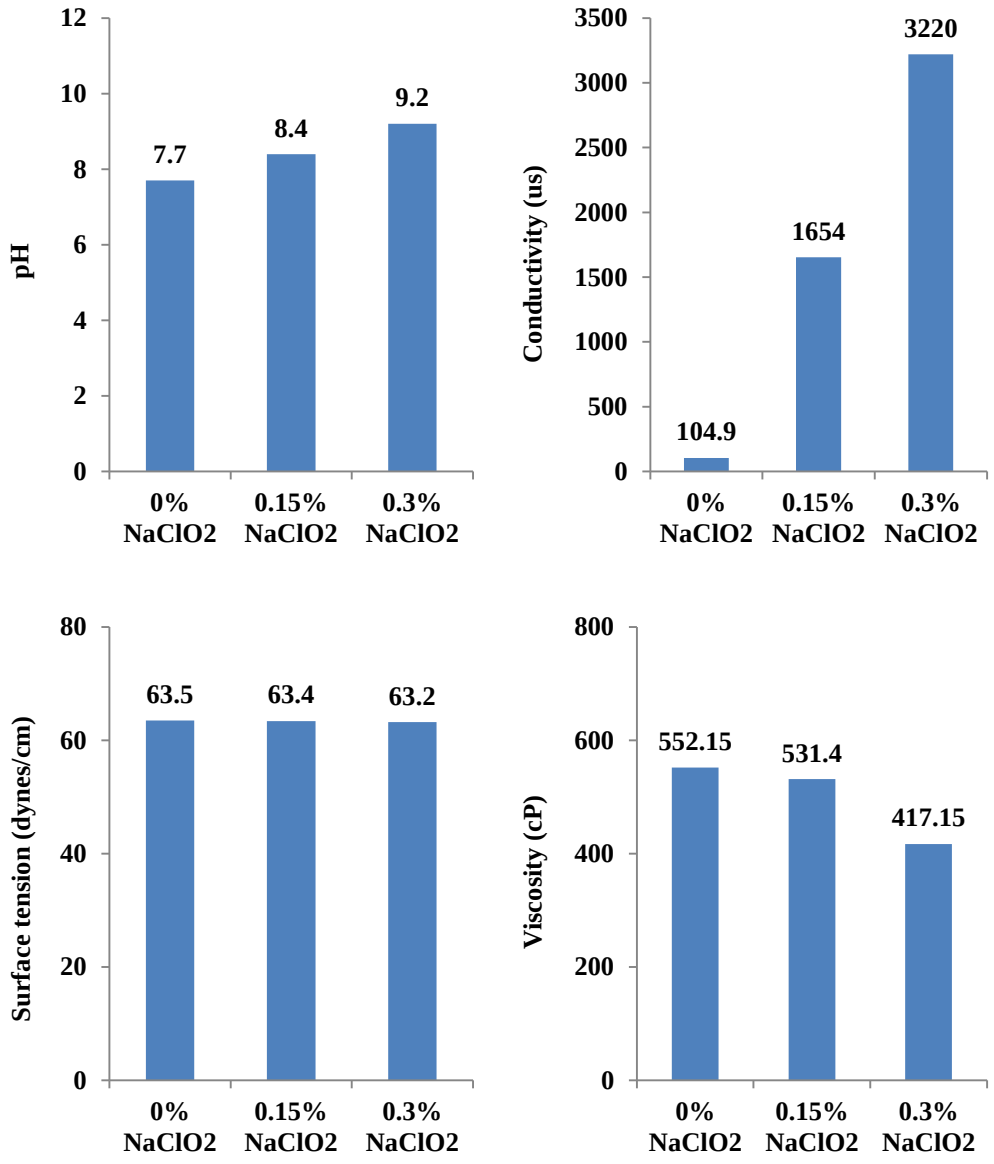


Figure 42. Physical properties of 3% PEO water solutions containing 0%, 0.15% and 0.3% $NaClO_2$

2) To obtain morphology and diameter distributions of fibers electrospun from different concentrations of PEO and $NaClO_2$ water solutions

The morphology and diameter distribution of fibers electrospun from 2%, 3% and 4% PEO were shown in Figure 43. From the figure it could be seen that, as the concentration of PEO polymer increased in water solution, the beads formation was reduced while the average diameter of fibers produced increased (as 160.37 ± 18.09 nm, 199.11 ± 20.15 nm and 243.29 ± 43.41 nm for 2%, 3% and 4% PEO water solutions respectively). The beads formation is related to the instability of the jet of polymer solution (mainly due to the break-up of water molecules during electrospinning process). So higher polymer contents (less water contents) intend to have reduced beads formation [99,100].

The morphology and diameter distribution of fibers electrospun from 3% PEO water solutions containing 0%, 0.15% and 0.3% $NaClO_2$ were shown in Figure 44. From the figure it could be seen that as the concentration of $NaClO_2$ increased in PEO water solution, the average diameter of fibers produced decreased (as 243.29 ± 43.41 nm, 176.36 ± 16.04 nm and 162.18 ± 15.02 nm for 0%, 0.15% and 0.3% $NaClO_2$ in 3% PEO water solutions respectively). As the concentration of $NaClO_2$ increased in PEO water solutions, the solution conductivity also increased sharply, which leads to more electric charges carried by the polymer jet. This results in an increased elongation force exerted on the polymer jet during elongation stage. Therefore, reduced fiber

diameter would be observed [101].

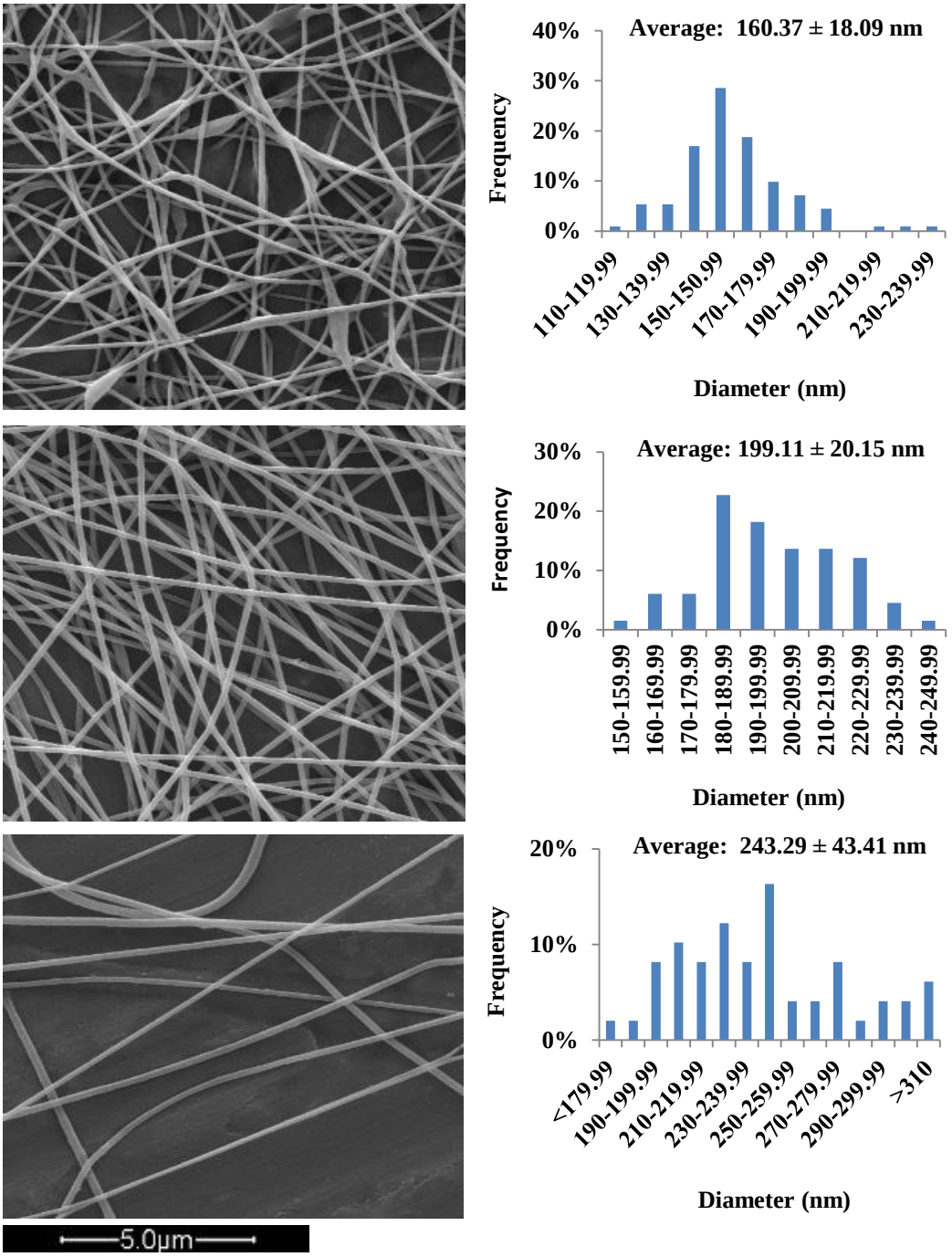


Figure 43. Morphology and diameter distribution of fibers electrospun from 2%, 3% and 4% PEO (from top to bottom)

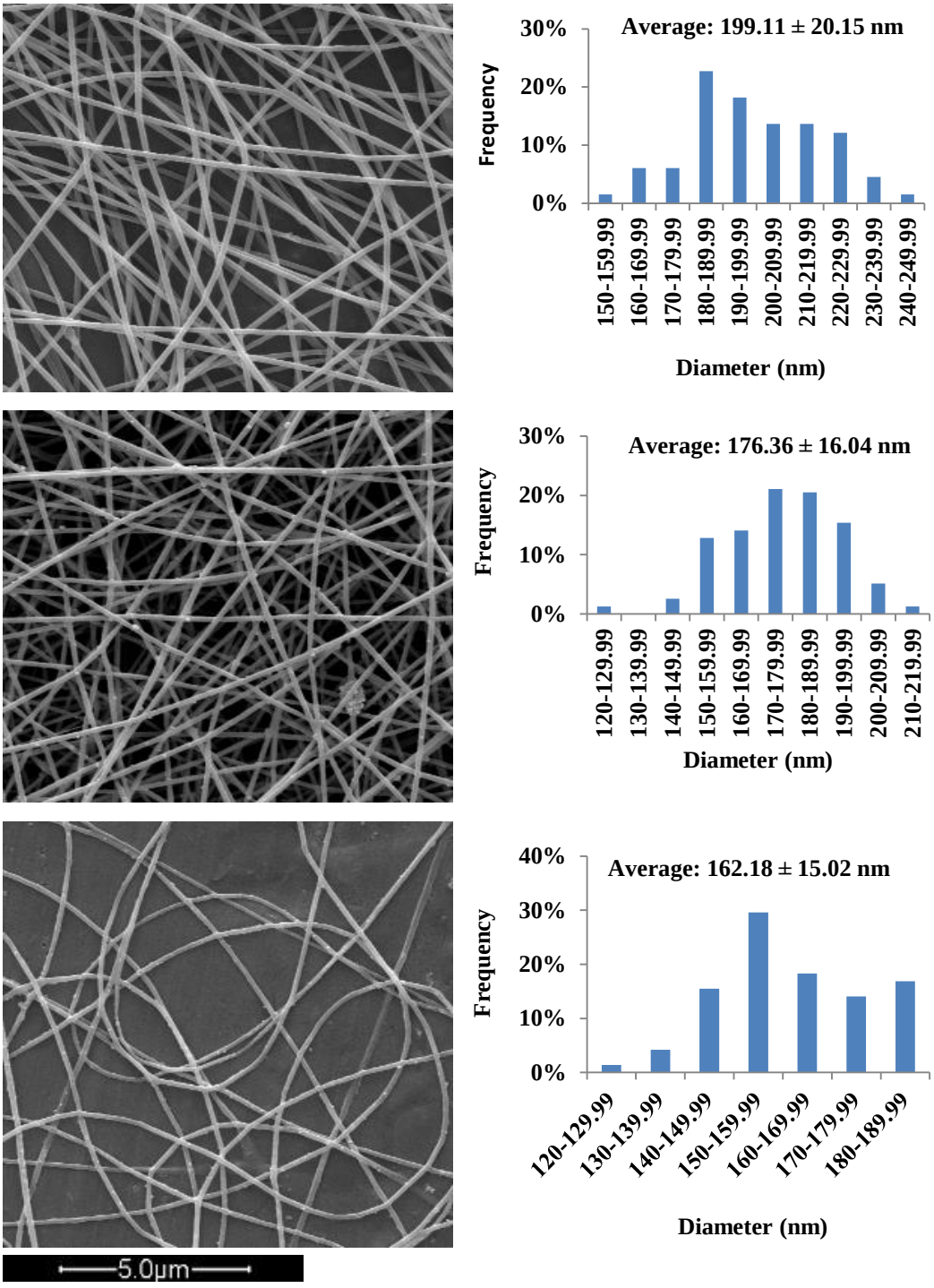


Figure 44. Morphology and diameter distribution of fiber electrospun from 3% PEO water solutions containing 0%, 0.15% and 0.3% $NaClO_2$ (from top to bottom)

3) $NaClO_2$ loading efficiency measurement

From the images obtained (shown in Figure 46) by scanning electric microscopy (SEM), the white small dots located on the surface of the fibers may indicate the successful loading of $NaClO_2$ onto fibers.

The accurate loading efficiency was calculated using standard curve of $NaClO_2$ in water solution (see below Figure 45) through UV-spectrophotometer at 260 nm [88,89]. The calculated loading efficiency was 83.9% (±3.3%), which indicated a good retention of $NaClO_2$ through electrospinning process. Compared with many other processing methods (such as film extrusion), the non-thermal characteristic of electrospinning process would better protect the active compounds from degradation and therefore leads to a higher retention rate.

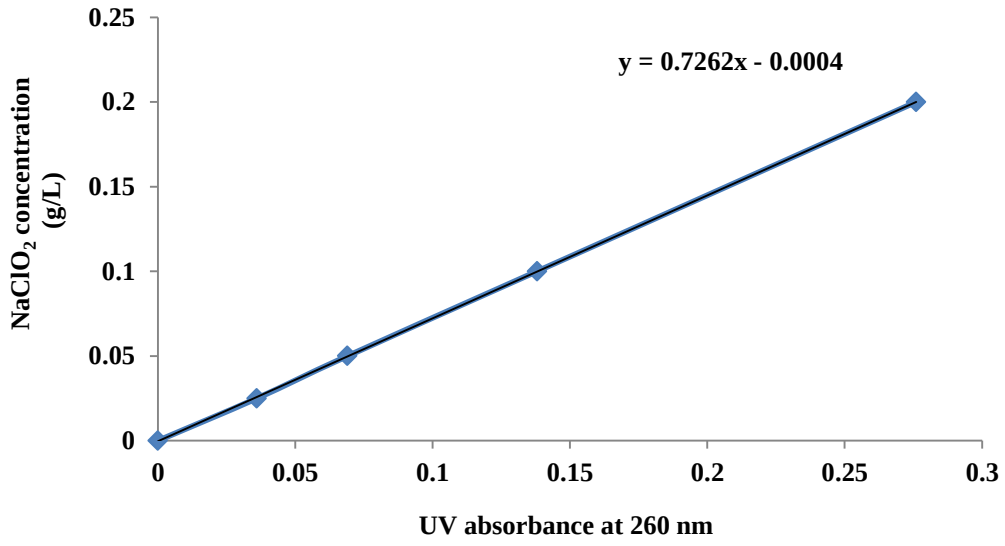


Figure 45. Standard curve of $NaClO_2$ concentration in water solution (g/mL)

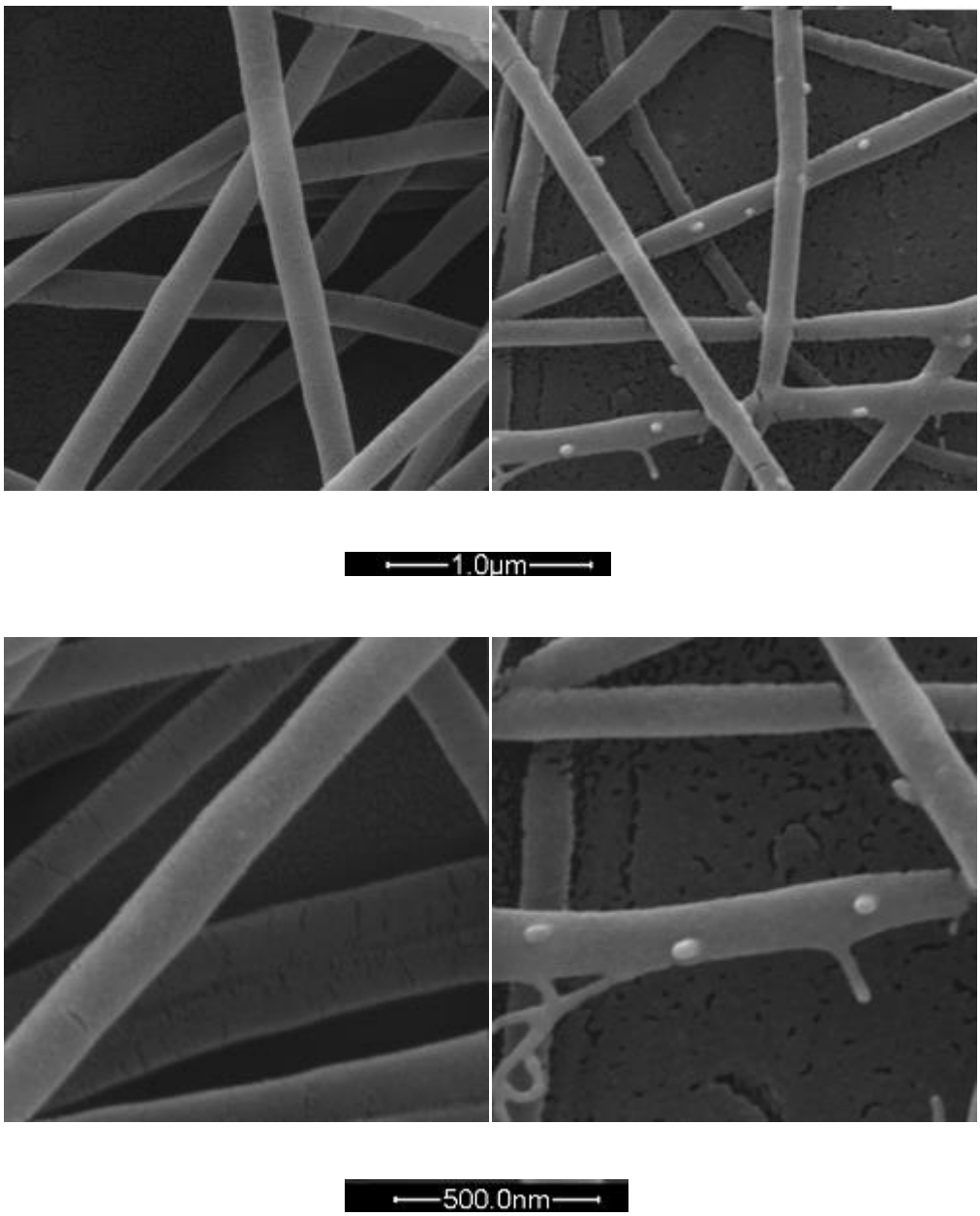


Figure 46. Morphology of fibers electrospun from 3% PEO (left) and 3% PEO with 0.3% $NaClO_2$ (right)

4) To confirm the inhibition effects of developed systems against *Salmonella* spp. under simulated conditions

The microbial inhibition results using electrospun fibers under simulated conditions were shown below in Figure 47. From the figure, it could be seen that after 48 hrs storage, compared to control, more than 10^2 log reduction of *Salmonella* spp. on TSA was observed (as 10^4 CFU *Salmonella* spp. on TSA agar for control and less than 10^2 CFU *Salmonella* spp. on TSA agar for treatments). Though no complete inhibition effect was observed (unlike using Tevyk sachet and gum arabic paste as delivery systems in previous studies), considering the extremely tiny amount of $NaClO_2$ loaded on the fiber, the inhibition effectiveness was still great.

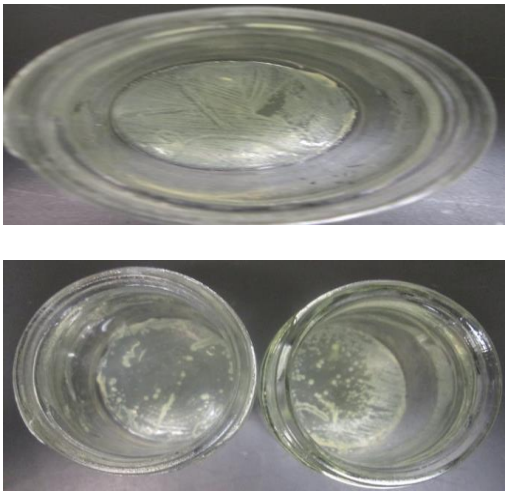


Figure 47. Inhibition results for PEO electrospun fiber under simulated conditions (top: control; bottom: with $NaClO_2$ in PEO fiber)

5) To confirm the inhibition effects of developed systems against *Salmonella* spp. on fresh tomato

The microbial inhibition results using electrospun fibers on fresh tomato were shown below in Figure 48. From the figure, it could be seen that after 48 hrs storage, compared with controls, more than 10^5 CFU *Salmonella* spp. was inhibited on the surface of fresh tomato, which confirmed the great inhibiting effects by using electrospun fiber as the delivery system for $NaClO_2$ against pathogen growth on fresh tomato.

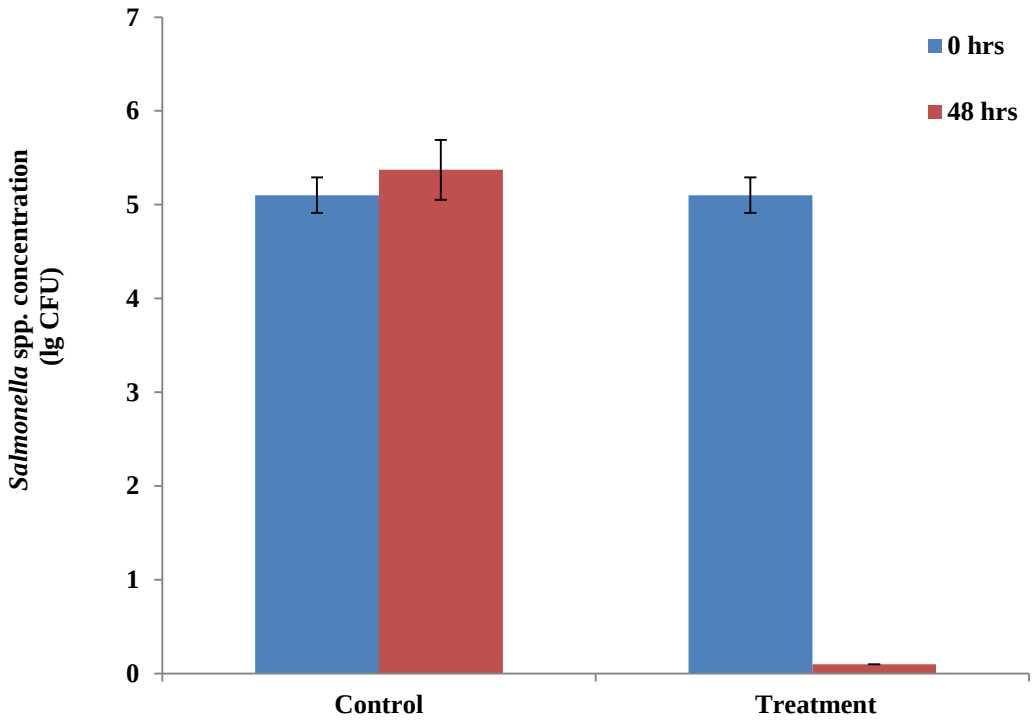


Figure 48. Microbial growth profiles of *Salmonella* spp. on fresh tomato after 48 hrs using PEO electrospun fiber

6 CONCLUSION

First of all, this research primarily demonstrated the hypothesis that CO_2 and moisture released from fresh produce during post-harvest stage is able to react with $NaClO_2$ to generate $ClO_{2(g)}$, inhibiting pathogen growth effectively without damaging the sensory profile. This hypothesis was primarily demonstrated through four consecutive experiments: (1) demonstrated that ClO_2 is able to be generated under ideal conditions, (2) demonstrated that $ClO_{2(g)}$ is able to be generated under practical conditions of fresh produce storage, (3) demonstrated that $ClO_{2(g)}$ generated under practical conditions of fresh produce storage is able to inhibit pathogen growth effectively, (4) demonstrated that $ClO_{2(g)}$ is able to be generated from respiration products of fresh produce (i.e. CO_2 and moisture) inhibiting pathogen growth effectively without damaging the sensory profiles.

Then, this research further investigated the basic science including chemistry and microbiology mechanisms related to the hypothesis. For the chemistry part, it demonstrated that only when the three components (i.e. CO_2, moisture and $NaClO_2$) existed in the system, $ClO_{2(g)}$ could be generated. Then the impact of each component (i.e. CO_2, moisture and $NaClO_2$), temperature and light in the release profiles of $ClO_{2(g)}$ was investigated and the results showed that increasing the $NaClO_2$ amount (from 0.05 g to 0.2 g) delayed $ClO_{2(g)}$ release but increased its maximum amount detected in headspace; increasing the CO_2 amount (from 7.5% to 15%) didn't create

any significant difference in $ClO_{2(g)}$ release profiles in headspace; increasing the RH content (from 45% to 90%) accelerated $ClO_{2(g)}$ release but didn't significantly affect its maximum amount detected in headspace; increasing the temperature (from 10 ºC to 35 ºC) accelerated $ClO_{2(g)}$ release and also increased its maximum amount detected in headspace; applying light environment accelerated the initial release of $ClO_{2(g)}$ but also accelerated its decomposition in headspace later on. Then the relationship between pH and ClO_2 generation profile in water solution was investigated and the results showed that lowering the pH (from 9.5 to 4) led to more ClO_2 generation. Finally the possible chemical reaction steps regarding ClO_2 generation were proposed. For the microbiology part, it demonstrated that only when the three components (i.e. CO_2, moisture and $NaClO_2$) existed in the system, *Salmonella* spp. (10^2 CFU on TSA agar) could be completely inhibited within 48 hrs. Then the impact of each component (i.e. CO_2, moisture and $NaClO_2$), temperature and light in the inhibition effects against *Salmonella* spp. (10^4 CFU on TSA agar) within 48 hrs was investigated and the results showed the level variations of all the factors including $NaClO_2$ content (0.05, 0.1 and 0.2 g), CO_2 amount (7.5 and 15%), RH content (45 and 90%), temperature (10, 22 and 35 ºC) and light environment (dark and light) didn't affect the inhibition effects against *Salmonella* spp. (10^4 CFU on TSA agar) within 48 hrs. Finally the "D value" and "Z value" were investigated and results showed that "D value" was 12.6, 6.4 and 3.5 hrs under 10, 22 and 35 ºC respectively and "Z value" was 45.5 ºC (between 10 ºC and 35 ºC).

To help practically apply this demonstrated hypothesis, this research developed two easy-to-use delivery systems for $NaClO_2$ as Tyvek sachet and gum arabic paste. For Tyvek sachet, the successful generation and release of $ClO_{2(g)}$ was confirmed through both analytical methods directly and microbial inhibition experiments indirectly under simulated conditions. Then the complete inhibition effects from Tyvek sachet against *Salmonella* spp. (10^2 and 10^4 CFU on TSA agar) using real fresh tomato as the source of CO_2 and moisture was observed within 12, 24 and 48 hrs for under 10, 22 and 35 ºC respectively. Finally the optimal amount of $NaClO_2$ in Tyvek sachet was determined, with which 10^5 CFU *Salmonella* spp. inoculated on the surface of fresh tomato was completely inhibited within 3 days and no bacteria grew back within the 21-day storage. Sensory evaluations have been conducted and the results showed that the $ClO_{2(g)}$ generated didn't place any significant impact in texture and color of fresh tomatoes utilized in the system though the typical tomato smell was scarified. For gum arabic paste, the successful generation and release of $ClO_{2(g)}$ was confirmed through both analytical methods directly and microbial inhibition experiments indirectly under simulated conditions. Then the complete inhibition effects from gum arabic paste against *Salmonella* spp. (10^4 CFU on TSA agar) using real fresh tomato as the source of CO_2 and moisture was observed within 12, 48 and 72 hrs for under 10, 22 and 35 ºC respectively. However significant sensory difference was detected between controlled and treated fresh tomatoes.

Besides the two easy-to-use delivery systems, this research also developed a promising delivery system for $NaClO_2$ as electrospun fibers. The physical properties (including pH, conductivity, surface tension and viscosity) of 2%, 3% and 4% PEO water solutions (containing 0%, 0.15% and 0.3% $NaClO_2$) were investigated and the results showed that as the concentration of PEO increased in the water solutions, the conductivity decreased while viscosity increased; as the concentration of $NaClO_2$ increased in PEO water solutions, the conductivity increased sharply while the viscosity slightly decreased. Then, the morphology and diameter distributions of fibers electrospun from 2%, 3% and 4% PEO water solutions (containing 0%, 0.15% and 0.3% $NaClO_2$) were investigated and the results showed as the concentration of PEO increased in water solution, the beads formation reduced while the average diameter of fibers produced increased; as the concentration of $NaClO_2$ increased in PEO water solution, the average diameter of fibers produced decreased. Then the successful loading of $NaClO_2$ onto the electrospun fiber was observed from SEM images and the loading efficiency was calculated to be 83.9% (±3.3%). Finally, the inhibition effects from electrospun fiber against 10^5 CFU *Salmonella* spp. was observed both on TSA agar under simulated conditions as well as on the surface of real fresh tomato within 48 hrs.

In conclusion, a hypothesis -- "CO_2 and moisture released from fresh produce during post-harvest stage is able to react with $NaClO_2$ to generate $ClO_{2(g)}$ inhibiting pathogen growth effectively without damaging the sensory profile", was formed from

reasonable speculation based on the understanding of ClO_2 generating mechanisms. Through the primary testing of the hypothesis and investigation of the basic science (i.e. chemistry and microbiology mechanisms) related to the hypothesis, the "technical feasibility" of this "novel gaseous chlorine dioxide generating method" was successfully demonstrated. Then through the development of the delivery systems for $NaClO_2$ (i.e. Tyvek sachet, gum arabic paste and electrospun fiber), the "practical feasibility" of this "novel gaseous chlorine dioxide generating method" was also successfully demonstrated. By combining the results from all parts of the research, it could be concluded that the "feasibility" of a "novel gaseous chlorine dioxide generating method" that "utilizes CO_2 and moisture released from fresh produce during post-harvest stage to react with $NaClO_2$ to generate $ClO_{2(g)}$ inhibiting pathogen growth effectively without damaging the sensory profile" was successfully demonstrated.

7 FUTURE WORK

7.1 Further Study for Electrospun Fiber Delivery System

This study has preliminarily demonstrated the great antimicrobial effectiveness of electrospun PEO fiber against $10^4/10^5$ CFU *Salmonella* spp. both on TSA agar under simulated conditions as well as on the surface of real fresh tomato within 48 hrs.

To continue this work, long term storage study (up to 21 days) with *Salmonella* spp. inoculated onto the surface of real fresh tomato will need to be performed to investigate whether *Salmonella* spp. will be able to grow back at the end of shelf life. Also, sensory evaluation including instrumental analysis (color and texture) as well as panel analysis will need to be performed to investigate whether any significant sensory impact could be detected from the treatment. Based on the experimental results of both microbial inhibition and sensory evaluation study, the optimal adding amount of $NaClO_2$ into PEO fiber could be determined.

For the electrospinning process, besides PEO, many other hydrophilic synthetic polymers could also be considered. These synthetic polymers may include polyacrylamide (PAM), polyethylenimine (PEI), polymethacrylate, polyvinyl alcohol (PVA) and etc. Many hydrophilic nature polymers with good mechanical properties may also be considered. These nature polymers may include starch, protein, chitosan, gelatin and etc [102].

7.2 System Effectiveness Verification

In this study, all three $NaClO_2$ delivery systems developed (including Tyvek sachet, gum arabic paste and electrospun fiber) exhibited great antimicrobial effectiveness against *Salmonella* spp. (as a representative of pathogens) on fresh tomato (as a representative of fresh produce), without damaging the sensory profiles of fresh tomato utilized.

As the next step, the microbial inhibition effectiveness of each developed delivery system will need to be verified by using other pathogens such as *E. coli* O157:H7 which is another common pathogen related with fresh produce outbreaks [21]. Also, the microbial inhibiting effectiveness of each developed delivery system will need to be verified by using other fresh produce with various respiration rates from "low" to "extremely high" (e.g. grape, green pepper, strawberry, snap bean and broccoli) [28]. Sensory evaluation including instrumental analysis (color and texture) as well as panel analysis will need to be performed to make sure that no significant sensory impact could be detected in fresh produce utilized under each delivery system.

From the experimental results of both microbial inhibition and sensory evaluation study, the optimal adding amount of $NaClO_2$ into each delivery system developed for different pathogens and fresh produce (combinations) could also be suggested.

7.3 Study on Shelf Life Extension Effectiveness

This study has shown the great antimicrobial effectiveness against *Salmonella* spp. (as a representative of pathogen) from all three $NaClO_2$ delivery systems developed (including Tyvek sachet, gum arabic paste and electrospun fiber).

Since ClO_2 has also been reported by many researchers to have great inhibition effectiveness against a variety of spoilage bacteria [103,104], as the next step, the potential shelf life extension effectiveness for fresh produce utilized in all three $NaClO_2$ delivery systems developed could be investigated. According to the preliminary experimental results (shown in Figure 49), the shelf life extension effectiveness using Tyvek sachet on fresh tomato was significant.

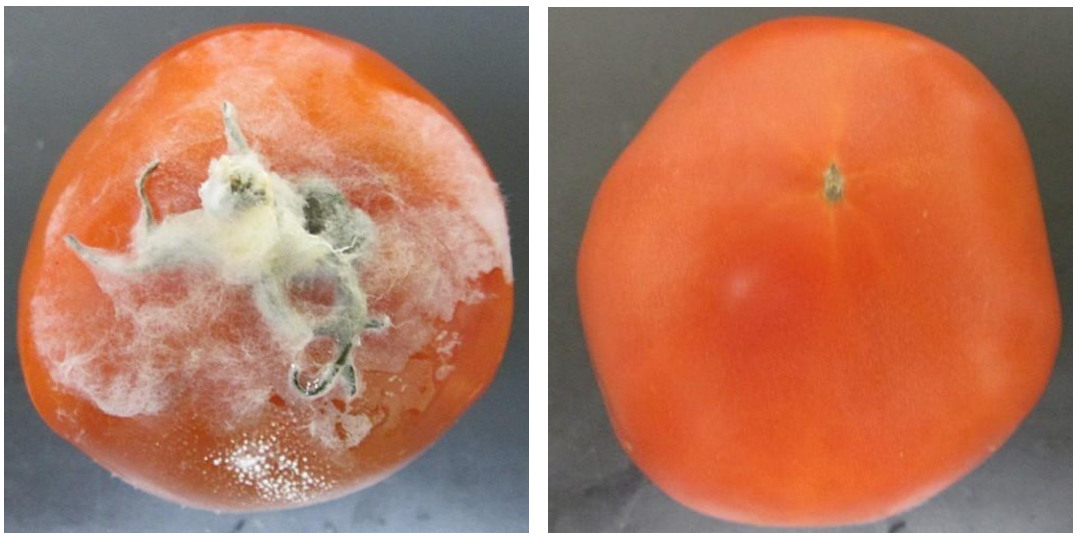


Figure 49. Appearance of controls (left) and treated samples using Tyvek sachet (right)

7.4 Model Development for Optimal System Design

This study systematically investigated the chemistry mechanism regarding the generation profile of $ClO_{2(g)}$ and the microbiology mechanism regarding the inhibition effectiveness against *Salmonella* spp. under different situations (different $NaClO_2$ contents, CO_2 contents, RH contents, temperatures and light conditions).

To better understand the functional relationship between these factors and their accurate impacts in the generation profile of $ClO_{2(g)}$ and the pathogen inhibiting effectiveness, model development (including both non-linear and linear models) will be necessary. Through model development, the integrated linkage between respiration profile of fresh produce, generation profile of $ClO_{2(g)}$ and pathogen inhibiting profile could be clearly revealed, helping achieve the optimal system design.

7.5 Other Delivery System Development

Besides Tyvek sachet, gum arabic paste and electrospun fiber, some other suitable $NaClO_2$ delivery systems (such as gelatin films) could also be developed. According to the preliminary experimental results, $NaClO_2$ incorporated gelatin solution-casting films exhibited great antimicrobial effectiveness against *Salmonella* spp. growth on TSA agar using fresh banana as the natural source of CO_2 and moisture. Similar inhibition effectiveness from $NaClO_2$ incorporated gelatin solution-casting films has also been observed by other researchers within the research group.

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