CHLORINE DIOXIDE EFFICACY AGAINST BOTRYTIS CINEREA IN STRAWBERRIES AND CHLORINE DIOXIDE ABSORPTION BY FRESH PRODUCE

By

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ABSTRACT

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Chlorine dioxide (ClO_2) is an effective sanitizer and strong bactericide with a broad spectrum disinfection capability. As a result of ClO_2 gas treatments on fresh produce, it is absorbed by the produce tissue and oxidized into different compounds such as chlorate (ClO_3^-) and chlorite (ClO_2^-) ions upon contact.

Quantification of chlorine dioxide exposure and consumption by fresh produce is critical for determining the target dose and assessing the residual species post treatment as they could affect the safety and organoleptic profile of the treated fresh produce. A new *in situ* approach to measure the total exposure to the produce as well as consumption of ClO_2 gas by produces was developed by using a system equipped with a UV-vis spectrophotometer. The equipment was used to evaluate the effect of ClO_2 gas on *Botrytis cinerea* present in strawberries. The impact of high-concentration-short-time and low-concentration-long-time of ClO_2 gas exposure on the efficacy of ClO_2 on *Botrytis cinerea as* well as absorption of ClO_2 by the strawberries was evaluated. It was found that both exposure time and concentration were significant factors in the ClO_2 consumption by fresh produce and very fast consumption kinetics in strawberry fruit were found.

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1. INTRODUCTION

An estimated of 9.4 million foodborne illnesses are caused by known pathogens annually in the United States. The proportion of illness outbreaks associated to fresh produce has increased in the last years (Bean and Griffin 1990), from <1% of all reported outbreaks with known food vehicle in the 1970s to 6% in the 1990s (Sumathi, Cindy et al. 2004), and the proportion of outbreak-associated cases accounted for by fresh produce increased from <1% to 12% of illnesses in that same time period (Lynch, Tauxe et al. 2009). Some of the reasons fresh produce associated outbreaks have increased include higher awareness of fresh fruits and vegetables' benefits on human health such as improvement of life quality and prevention of chronic and cardiovascular diseases (Bhagat 2010). Prove of this, is the increased per capita consumption of fresh produce in America which has jumped from 91.6 to 121.1 kg, an increase of 32% (Harris, Farber et al. 2003).

This issue brings up the question on how to prevent contamination of fresh produce in a first place, and one of the answers is to develop and implement more rigorous, effective sanitizing strategies along the food chain. Washing the produce with sanitizing solutions is the most common method used during production (Harris, Farber et al. 2003; Parish, Beuchat et al. 2003). After production, another sanitizing approach includes the use of packaging systems that contain antimicrobial agents that reduce the population of pathogens present in the produce and thus also prolong its shelf life. These sanitizing agents could be added as a coating layer in the packaging material, as a sachet that actively releases the agent inside the headspace until the reagents have

depleted, or by injecting different sanitizing agents inside the package to alter the gas composition and help extend the produce's shelf life (Appendini and Hotchkiss 2002).

One of the sanitizing agents that has caught the attention of researchers in the last decades is chlorine dioxide (ClO_2) thanks to its reported efficacy against a variety of microorganisms in fruits and vegetables such as *Listeria monocytogenes*, *Escherichia coli O157:H7*, and *Salmonella* among others (Han, Linton et al. 2000; Han, Floros et al. 2001; Han, Linton et al. 2001; Du, Han et al. 2002; Du, Han et al. 2003; Lee, Costello et al. 2004). Although the great majority of researchers have studied the efficacy of ClO_2 against pathogens, a few researchers have also investigated its effect against molds, yeasts and other aerobes (Spotts and Peters 1980; Roberts and Reymond 1994).

Chlorine dioxide is a strong bactericide that has replaced Cl_2 in several applications at both the drinking water and food industry, because it has approximately 2.5 times the oxidation capacity of Cl_2 (Benarde, Israel et al. 1965), and because it is approximately 10 times more soluble above 11 ^{0}C (United States Environmental Protection Agency (EPA) 1999).

Chlorine dioxide could be used at different stages throughout the food chain process. For example, it could be used during the processing of fresh produce as a sanitizing solution for either fruits and vegetables itself, or for the tools and equipment to prevent cross contamination. After processing, ClO_2 could also be applied inside a package in the gaseous form to extend the shelf life of fresh produce.

Therefore ClO_2 can be used in the processing line as a washing solution for fruits and vegetables, or can be applied inside a package in the gaseous form. There are two ways ClO_2 could be applied in the package, which are i) sachets that contain chemicals that react to continuously generate and build up ClO_2 gas concentration in the headspace (Ellis, Cooksey et al. 2006), and ii) direct injection of ClO2 inside the package by means of a commercially available gas generator using a reaction with sodium chlorite and chlorine gas

In the gaseous form, ClO_2 penetrates through irregularities and complex areas more efficiently than the aqueous form, making ClO_2 gas more effective than the aqueous form (Du, Han et al. 2002; Lee, Costello et al. 2004).

However, there are some concerns when it comes to sanitation of fruits and vegetables with ClO_2 gas that need to be properly addressed. First of all, during treatment (inside a package or during production), a good circulation of the gas must be ensured so the gas can envelop the produce and effectively reach those sites where microbes might be hiding (Staschower 2012). Improper circulation of the gas around the produce will make the treatment less effective.

Another concern with the use of ClO_2 gas as a sanitizer of fresh produce has to do with its toxicology characteristics. ClO_2 participates in a series of oxidative reactions that result in by products such as (ClO_3^-) and chlorite (ClO_2^-) ions, which can at the same time further transform into chloride (Cl^-) (United States Environmental Protection

Agency (EPA) 1999; Gómez-López, Rajkovic et al. 2009). When produce surfaces absorb ClO_2 during treatments, it then oxidizes and breaks down upon contact (Netramai 2011), leaving surface residues that could later cause the formation of chlorinated byproducts. For these reasons, although regulation 21 CFR 173.300 allows the use of ClO_2 to disinfect fruits and vegetables, it requires a potable rinse step after treatment in order to assure that there are no residues of concern for consumers' consumption (Trinetta, Vaidya et al. 2011).

Some of the currently available methods to quantify surface residues on produce after exposure to ClO_2 are the colorimetric method, amperometric titration, and chromatography. These methods have in common that i) they analyze a washing solution after treating the produce with the gas, and ii) they require a post-treatment technique in order to quantify the residues left on the produce's surface.

In light of this discussion, the goal of this study is to develop a continuous method to quantify ClO_2 in situ: exposure concentration and consumption of ClO_2 by fresh produce.

To achieve this goal, two specific objectives were set and they are described below:

To develop a new system and online method to quantify ClO_2 exposure concentration and consumption by fresh produce.

To assess the application of the new equipment by evaluating the ClO_2 gas efficacy against *Botrytis cinerea* on strawberries.

BIBLIOGRAPHY

BIBLIOGRAPHY

Appendini, P., Hotchkiss, J.H., (2002). Review of antimicrobial food packaging. Innovative Food Science & Emerging Technologies 3(2), 113-126.

Bean, N.H., Griffin, P.M., (1990). Foodborne disease outbreaks in the United States, 1973-1987: pathogens, vehicles, and trends. Journal of Food Protection 53.

Benarde, M.A., Israel, B.M., Olivieri, V.P., Granstrom, M.L., (1965). Efficiency of chlorine dioxide as a bactericide. Applied Microbiology 13(5), 776-780.

Bhagat, A.R., (2010). Modeling Critical Factors to Optimize the Treatment of Selected Fruits and Vegetables with Chlorine Dioxide Gas Using a Miniaturized Insustrial-Size Tunnel System. Purdue University, West Lafayette, Indiana.

Du, J., Han, Y., Linton, R.H., (2002). Inactivation by chlorine dioxide gas (ClO2) of Listeria monocytogenes spotted onto different apple surfaces. Food Microbiology 19(5), 481-490.

Du, J., Han, Y., Linton, R.H., (2003). Efficacy of chlorine dioxide gas in reducing Escherichia coli O157:H7 on apple surfaces. Food Microbiology 20(5), 583-591.

Ellis, M., Cooksey, K., Dawson, P., Han, I., Vergano, P., (2006). Quality of Fresh Chicken Breasts Using a Combination of Modified Atmosphere Packaging and Chlorine Dioxide Sachets. Journal of Food Protection 69, 1991-1996.

Gómez-López, V.M., Rajkovic, A., Ragaert, P., Smigic, N., Devlieghere, F., (2009). Chlorine dioxide for minimally processed produce preservation: a review. Trends in Food Science & Technology 20(1), 17-26.

Han, Y., Floros, J.D., Linton, R.H., Nielsen, S.S., Nelson, E., (2001a). Response Surface Modeling for the Inactivation of Escherichia coli O157:H7 on Green Peppers (Capsicum annuum L.) by Chlorine Dioxide Gas Treatments. Journal of Food Protection 64, 1128-1133.

Han, Y., Linton, R.H., Nielsen, S.S., Nelson, P.E., (2000). Inactivation of Escherichia coli O157:H7 on surface-uninjured and -injured green pepper (Capsicum annuum L.) by chlorine dioxide gas as demonstrated by confocal laser scanning microscopy. Food Microbiology 17(6), 643-655.

Han, Y., Linton, R.H., Nielsen, S.S., Nelson, P.E., (2001b). Reduction of Listeria monocytogenes on Green Peppers (Capsicum annuum L.) by Gaseous and Aqueous Chlorine Dioxide and Water Washing and Its Growth at 7C. Journal of Food Protection 64, 1730-1738.

Harris, L.J., Farber, J.N., Beuchat, L.R., Parish, M.E., Suslow, T.V., Garrett, E.H., Busta, F.F., (2003). Outbreaks Associated with Fresh Produce: Incidence, Growth, and Survival of Pathogens in Fresh and Fresh-Cut Produce. Comprehensive Reviews in Food Science and Food Safety 2, 78-141.

Lee, S.-Y., Costello, M., Kang, D.-H., (2004). Efficacy of Chlorine Dioxide Gas as a Sanitizer of Lettuce Leaves. Journal of Food Protection 67, 1371-1376.

Lynch, M., Tauxe, R., Hedberg, C., (2009). The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. Epidemiology and infection 137(3), 307.

Netramai, S., (2011). Utilization of chlorine dioxide gas in food packaging application. Michigan State University, East Lansing, Michigan.

Parish, M.E., Beuchat, L.R., Suslow, T.V., Harris, L.J., Garrett, E.H., Farber, J.N., Busta, F.F., (2003). Methods to Reduce/Eliminate Pathogens from Fresh and Fresh-Cut Produce. Comprehensive Reviews in Food Science and Food Safety 2, 161-173.

Roberts, R.G., Reymond, S.T., (1994). Chlorine dioxide for reduction of postharvest pathogen inoculum during handling of tree fruits. Applied and environmental microbiology 60(8), 2864-2868.

Spotts, R., Peters, B., (1980). Chlorine and chlorine dioxide for control of d'Anjou pear decay. Plant Dis 64(1), 2.

Staschower, F., (2012). Residues from Chlorine Dioxide Gas Treatment, Generated by Different Delivery Systems, on Fresh Produce. Michigan State University, East Lansing, Michigan.

Sumathi, S., Cindy, R.F., Linda, C., Robert, V.T., (2004). Fresh Produce: A Growing Cause of Outbreaks of Foodborne Illness in the United States, 1973 through 1997. Journal of Food Protection 67(10), 2342-2353.

Trinetta, V., Vaidya, N., Linton, R., Morgan, M., (2011). Evaluation of Chlorine Dioxide Gas Residues on Selected Food Produce. Journal of Food Science 76(1), T11-T15.

United States Environmental Protection Agency (EPA), (1999). Alternative Disinfectants and Oxidants Guidance Manual: Chapter 4. Chlorine Dioxide, EPA 815-R-99-014.

2. LITERATURE REVIEW

2.1. Framework

In recent years, an interest for healthier, fresher foods has become a common denominator in consumers across age, sex and ethnic backgrounds. In (Sloan 2013) article “Top 10 Food Trends”, listed healthier options in the second place out of the top 10 food trends in 2013.

Americans have increased their consumption of fruits and vegetables since 1979 in part due to an active promotion to include fruits and vegetables for a healthy diet. From 1982 to 1997, the consumption of fresh fruits and vegetables per capita in the U.S increased from 91.6 to 121.1 kg, an increase of 32% (Harris, Farber et al. 2003). During the same period, organizations such as U.S. Department of Agriculture, the U.S. Department of Health and Human Services, and the Centers for Disease Control and Prevention (CDC) used resources to raise awareness and developed strategies to combat obesity and other diseases (Plattner and Perez 2012). Research confirmed that the consumption of minimally processed produce improves the quality of life and prevents chronic and cardiovascular diseases (Bhagat 2010).

Minimally processed foods are those which do not generally contain preservatives or antimicrobial agents and which rarely undergo any heat processing prior to consumption (Seymour and Appleton 2001). Considering that the original purpose of minimal processing was to minimize the thermal treatments that reduce food quality, researchers were forced to develop non-conventional heat techniques to extend the shelf life of food products (Balla and Farkas 2007).

However, not only does minimal processing not completely inactivate all microorganisms present in the raw fruits and vegetables, but processing of raw materials also promotes a faster physiological deterioration, which at the same time cause microbial degradation. Therefore the microbiological safety during the shelf life of minimally processed foods is of great importance (O'Beirne and Francis 2003; Balla and Farkas 2007).

2.2. Food quality and safety

Foodborne disease outbreaks are a concern especially when it comes to minimally processed foods like fresh fruits and vegetables, which have been identified as vehicle for illnesses since they can carry both spoilage and pathogenic microorganisms (Bhagat 2010). From harvest to shelf, fresh produce encounters many different sites where possible contamination can occur including harvesting, processing, distribution, and storage. Some examples of these potential sources of contamination are suggested by Brackett (1999) as cross contamination with feces of domestic and wild animals, contaminated irrigation water, use of untreated manure or sewage, lack of sanitation in the field, irrigation and harvesting equipment and handlers at both the retails and consumer levels (Brackett 1999). Figure 1, taken from Beuchat (1996) shows the different mechanisms by which fresh produce becomes contaminated with pathogenic microorganisms such as viruses, bacteria and parasites.

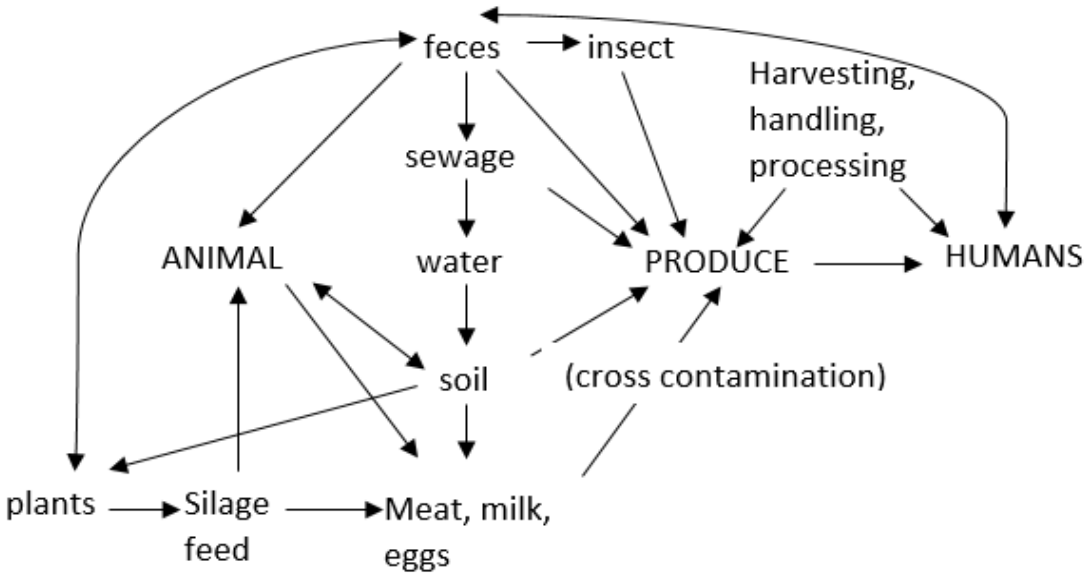

FIGURE 1. Mechanisms by which fresh produce becomes contaminated along the production chain (Beuchat 1999).

During the period of 2009 to 2010, a total of 1,527 foodborne disease outbreaks were reported resulting in 29,444 cases of illness, 1,184 hospitalizations, and 23 deaths (Centers for Disease Control and Prevention 2013). CDC defines a foodborne disease outbreak as the occurrence of two or more similar illnesses resulting from ingestion of a common food. An estimated of 9.4 million foodborne illnesses are caused by known pathogens annually in the United States. The proportion of illness outbreaks associated to fresh produce has increased in the last years (Bean and Griffin 1990), from <1% of all reported outbreaks with known food vehicle in the 1970s to 6% in the 1990s (Sumathi, Cindy et al. 2004), and the proportion of outbreak-associated cases accounted for by fresh produce increased from <1% to 12% of illnesses in that same time period (Lynch, Tauxe et al. 2009). The possible causes behind the increment of fresh produce related outbreaks are believed to go beyond the increased demand for these types of products, and include the changes in agronomic, processing, preservation, packaging,

distribution, and marketing technologies on a global scale which have enabled the raw fruit and vegetable industry to supply consumers with a wide range of high quality produce from different countries year round, which have implemented practices and technologies that may increase risk for human illness associated with pathogenic microorganisms (Beuchat 2002).

However as mentioned before, fruits and vegetables are also susceptible of contamination with spoilage type of microorganisms, which in most cases won't hurt humans health, but will cause food product losses and a reduction in the food availability. According to Kantor et al., the annual edible food available to the U.S is reduced by 27% due to food spoilage and waste at the retail and consumer level (Kantor, Lipton et al. 1997). In a different study carried out between 2010 to 2011 by The Swedish Institute for Food and Biotechnology (SIK) sponsored bythe Food and Agriculture Organization of the United Nations (FAO), was suggested that roughly one-third of the food produced for human consumption is lost or wasted globally, which amounts to about 1.3 billion tons per year (Gustavsson, Cederberg et al. 2011). In the fruits and vegetables commodity group, both agriculture and processing are the two major factors impacting total waste across all 7 regions studied (North America and Oceania, Industrialized Asia, Subsahara Africa, North Africa, West & Central Asia, South and Southeast Asia, and Latin America). The causes of food waste and losses around the world are not limited to their contamination with spoilage microorganisms, but leaving economic and political factors aside, this is one of the most relevant causes of the problem especially in some regions of the world where there are inadequate

storage facilities and infrastructure and poor harvesting practices (Gustavsson, Cederberg et al. 2011).

2.2.1. Pathogenic and spoilage microorganisms associated with fresh produce

The top five pathogens causing domestically acquired foodborne illnesses have been identified as Norovirus, *Clostridium perfringens*, *Campylobacter spp*., *Salmonella* nontyphoidal, and *Staphylococcus aureus* (Centers for Disease Control and Prevention 2011). In addition to the latter two pathogens listed, *Esterichia Coli* and *Listeria monocytogenes* are the most commonly found pathogens in fresh produce.

2.2.1.1. Salmonella

The genus *Salmonella* has over 2700 serotypes. Animals and birds are the natural reservoirs. Surveys of fresh produce have revealed the presence of several Salmonella serotypes capable of causing human infection, though poultry and other meat products, eggs and dairy products, are the most commonsources in salmonellosis outbreaks. Fresh fruits and vegetables are implicated less frequently, although outbreaks have been documented most notably in cantaloupe and sprouts. Laboratory studies revealed that the pathogen can grow in damaged, chopped, or sliced tomatoes (pH 4.1 – 4.5) stored at 20 to 30 ℃ (68 to 86 ℉) (Beuchat 1996; Harris, Farber et al. 2003). Produce items that were most frequently implicated in outbreaks of Salmonella infection include salad, sprouts, and melons (Sumathi, Cindy et al. 2004).

2.2.1.2. Staphylococcus aureus

According to the CDC, *staphylococcus aureus* (*S. aureus)* is a type of bacteria that most of the time does not cause any harm; however, sometimes it causes infections which can be fatal such as bacteremia, pneumonia, endocarditis, and osteomyelitis.

S. aureus has been detected on fresh produce and ready-to-eat vegetable salads, and is known to be carried by food handlers. However, *S. aureus* does not compete well with other microorganisms normally present on fresh produce, so incipient spoilage caused by nonpathogenic micro flora would likely precede the development of high populations of this pathogen (Harris, Farber et al. 2003).

2.2.1.3. Esterichia Coli

Escherichia coli is a common cause of travelers' diarrhea. Fresh produce comes in contact with this pathogen through cross-contamination from meat products, or through direct contamination in the field with feces of wild or domestic animals (Bhagat 2010). Contaminated raw vegetables are thought to be a common source of the pathogen which depending on the kind, may cause diarrhea, urinary tract infections, respiratory illness and pneumonia, and other illnesses. (Beuchat 1996). Items frequently implicated in outbreaks of Escherichia coli O157:H7 infection are lettuce and apple cider (Sumathi, Cindy et al. 2004).

2.2.1.4. Listeria monocytogenes

Listeria is a gram-positive foodborne intracellular human pathogen, which has been associated with serious food-borne outbreaks and has been responsible of a number of recalls of fresh produce (Bhagat 2010). Plants and plant parts used as salad

vegetables play a key role in disseminating the pathogen from natural habitats to the human food supply (Beuchat 1996). L. monocytogenes is one of the most common food-borne pathogens in ready to eat foods responsible for causing serious illness such as septicemia and meningitis in humans where fatality rate is very high (up to 75% in highly immunocompromised individuals) (Bhagat 2010).

On the other hand, spoilage bacteria are microorganisms that cause food to deteriorate and develop unpleasant odors, tastes, and textures. These microorganisms can cause fruits and vegetables to rot, get mushy or slimy, and to develop undesirable color changes and odors. Each vegetable possesses a unique set of intrinsic factors that can influence the survival and growth of different microorganisms such as the pH, water activity and temperature (Beuchat 2002; Dagnas and Membre 2013).

Mold spoilage results from a biological process that includes having a product contaminated with fungal spores which germinate and extend in the form of hyphae to form a visible mycelium over time before the end of the product's shelf life (Dantigny, Guilmart et al. 2005). Optimum conditions at which mold growth is favored vary with the type of microorganisms, but in general, food spoilage will occur at temperatures around 25^0C and aw above 0.85 provided that food substances are contaminated with spores and that these spores are able to germinate (Dantigny, Guilmart et al. 2005; Gougouli and Koutsoumanis 2012)

A common fungus attacking all parts of the shoots of strawberries and raspberries, causing the greatest losses on ripe or near-ripe fruit is *botrytis cinerea*.

2.2.1.5. Botrytis cinerea

Botrytis cinerea occurs abundantly throughout the year as a saprophyte and facultative parasite on a wide variety of plant materials in raspberry and strawberry plantations (Jarvis 1962). Figure 2 show an example of symptoms of *botrytis cinerea* in strawberries.

The majority of fruit infections are initiated from mycelium growing saprophytically in contiguous plant material or foam spores germinating in solutions trapped between the fruit surface (Jarvis 1962).

For these reasons, the infection of fruit with this fungus can be traced to the infection of floral parts in the field (Powelson 1960) or by contact with contaminated berries (Bhaskara Reddy, Belkacemi et al. 2000)The rot caused by *botrytis cinerea* usually occurs at the stem end of the fruit.

FIGURE 2. Ripe strawberries infected with botrytis cinerea. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

This was studied by Powelson et al. (1960), who demonstrated that the proportion of symptomless strawberries at maturity was always higher in the group of fruit where petals, stamens, and sepals were removed regardless of the environmental

conditions in the greenhouse (wet or dry) (Powelson 1960). Braun et al. (1988) found that the receptivity of strawberry leaves to infection by *botrytis cinerea* depended on the developmental stage of the leaf (Braun and Sutton 1988). This idea is in agreement with Williamson et al. (2007), who described that *botrytis cinerea* is most destructive on mature or senescent tissues of dicotyledonous hosts, even though it usually access such tissues at a much earlier stage in crop development and remains quiescent for a considerable period before rapidly rotting tissues when the environment is conducive and the host physiology changes (Williamson, Tudzynski et al. 2007).

Therefore, evidence of fruit rot may happen long after harvest of apparently healthy crops which deteriorate during transport and storage.

Botrytis cinerea has a variety of modes of attack, diverse hosts as inoculum sources, and it can survive as mycelia and/or conidia or for extended periods as sclerotia in crop debris (Williamson, Tudzynski et al. 2007).

For all these reasons, *botrytis cinerea* is difficult to control and normally one single control measure won't succeed.

One way science can help understand the interactions between the microorganisms and hosts, or the mechanisms, dynamics and kinetics of bacteria or fungal growth, is by employing predictive models for shelf life determination and to predict risk of specific diseases.

2.3. Microbial growth models

Predictive modelling of filamentous fungal growth has not received the same attention as that of bacterial development, maybe due to inherent complexities associated with the quantification of fungal growth (Garcia, Ramos et al. 2009). These complexities, or specificities described by Dantigny et al. include i) the reproductive differences of bacteria and fungi which occur by fission due to their ability of dividing therefore it is easier to enumerate, while in contrast fungal growth involves germination and hyphal extension forming mycelium whose weight does not increase exponentially except at early stages of growth, and the ii) factors affecting microorganism growth, which for bacteria temperature plays the major role, while for mold growth water activity is more important (Dantigny, Guilmart et al. 2005). However, the number of studies focusing on fungal development has increased, and Dantigny et al. (2006) listed standardizing techniques for the study of fungal germination after realizing that techniques, definitions and methods for assessing mold germination varied greatly between authors, making it hard to compare experimental data (Dantigny, Bensoussan et al. 2006). Apart from other definitions, Dantigny et al. (2006) also mentioned two of the primary germination models that are most commonly used by researchers: the Gompertz and the logistic function models.

2.3.1. Primary models

Primary models are those which describe the growth of a population of microorganisms over time from the basis of adopting strategies for food safety. Primary microbial models can further be classified as germination, growth and inactivation models (Garcia, Ramos et al. 2009). Growth models can be either empirical (set out

principally to describe) or mechanistic (attempt to give a description with understanding) (López, Prieto et al. 2004).

2.3.1.1. Gompertz Model

Increase of percentage of germinated spores over time can be modeled with the Gompertz equation:

$$P = \alpha e^{-e^{[\beta(t-\theta)]}} \quad (1)$$

Where *P* (%) is the percentage germination, *α* (%) is the asymptotic value of P when time goes to infinite (t → ∞), *β* in inverse time units, is the growth rate or slope term of the tangent line through the inflection point (*θ*), which in time units is the point at which the concavity of the curve changes (Dantigny, Marín et al. 2007; Garcia, Ramos et al. 2009).

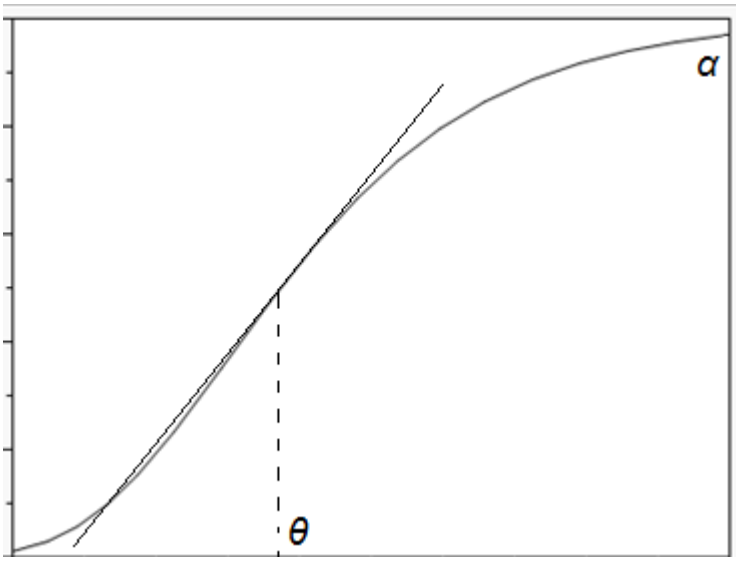

FIGURE 3. Gompertz model with three parameters *α*: Asymptote, *β*: Growth rate, and *θ*: inflection point.

2.3.1.2. Logistic function

$$P = \frac{P_{max}}{1+e^{[k(\tau-t)]}} \quad (2)$$

Where P (%) is the percentage germination, P_{max} (%) is the asymptotic P value at $t \rightarrow \infty$, τ (in time units) is the inflection point where P equals half of P_{max}, t is the time, and k (in inverse time units) is related to the slope of the tangent line through the inflection point (Dantigny, Guilmart et al. 2005; Dantigny, Marín et al. 2007).

Contrary to the Gompertz model, the logistic function is symmetric about the point of inflection, for this reason the Gompertz model may offer greater flexibility than the logistic (Gibson, Bratchell et al. 1987).

Gibson et al. (1987) used both Gompertz model and logistic equations to fit microbial growth data of *Clostridium botulinum* type A in pasteurized pork slurry with a reasonable good fit; therefore, it is possible then to estimate the growth parameters with physical meaning (Gibson, Bratchell et al. 1987). Declerck et al. (2001) compared the fit of the Schute, Gompertz and logistic models to fungal growth data and found that all three fitted the data very well with very similar residuals (Declerck, D'or et al. 2001).

Dantigny et al. (2007) studied mold germination and compared the two models; based on RMSE and the goodness of fit, they determined that both models were similar and and provided equivalent outcomes (Dantigny, Marín et al. 2007). Similarly, Gougouli et al. (2012) used the Gompertz equation to model the germination of *Penicilum expansum* and *Aspergillus niger* spores on malt extract agar finding a satisfactory description with acceptable goodness of fit for all parameters (Gougouli and Koutsoumanis 2012).

Although both the Gompertz model and the logistic equation are widely accepted and commonly used as means to predict fungal growth and development, there are several, simple statistical methods to evaluate similarities and differences between nonlinear models or to decide which one fits the data better. The main statistical procedures for model comparison are residual analysis and tests for goodness of fit (López, Prieto et al. 2004).

2.3.2. Secondary models

These type of models describe the influence of environmental factors such as temperature, water activity, or pH on key parameters of the primary model (Dantigny, Guilmart et al. 2005). Examples of secondary models are listed below

2.3.2.1. Ratkowski square-root model

Originally developed for bacteria, the model is as follows:

$$\sqrt{\mu_{max}} = b(T - T_{min})\{1 - e^{[c(T-T_{max})]}\} \quad (3)$$

Where μ is the fungal growth rate, T is temperature, and b, c, T_{max} and T_{min} are estimated constants (Garcia, Ramos et al. 2009).

2.3.2.2. Linear Arrhenius-Davey equation

$$\ln \mu_{max} = a_0 + \frac{a_1}{T} + \frac{a_2}{T^2} \quad (4)$$

Where μ is the fungal growth rate, T is absolute temperature (K), and a_0, a_1 and a_2 are constant to be estimated (Garcia, Ramos et al. 2009).

Other secondary models are also the Rosso cardinal model and the Gibson model.

2.3.3. Tertiary models

Tertiary models could be defined as the integration of primary and secondary models in a software using databases. Some tertiary model tools include the Pathogen Modelling Program (PMP) developed by the USDA, which was designed as a research and instructional tool for estimating the effects of multiple variables on the growth, inactivation or survival of food borne pathogens (United States Department of Agriculture (USDA) 2003), and the latest version of ComBase (www.combase.cc) that includes a modelling tool that uses its database to generate growth or inactivation curves (Garcia, Ramos et al. 2009).

In general, these models are used as tools that facilitate the decision process for example as a quality assessment in a food manufacturing operation or product development in order to determine the feasibility of a new formula, process or as a strategy for the inactivation of microbial growth. The application of these models provide an understanding of the inactivation mechanisms, the dynamics and the kinetics of fungal growth, which as explained before, vary with the external environments and with the type of crop, plant or fruit involved. All these insights bring to researchers and companies a foundation to develop innovative strategies to inhibit microbial growth while maintaining quality, freshness and safety. Some of such strategies that are currently being used are described next.

2.4. Strategies to prolong the shelf life and ensure safety of fresh produce

As explained before in section 1.2, there are a number of different sources of contamination for food which start early in the food chain process such as harvesting and production. The human interaction at this stage is significant, and for that reason the USDA proposed a plan in 2004 that aims to minimize foodborne illness associated with fresh produce consumption (United States Food and Drug Administration (FDA) 2004) which accounts for processes equipment and personal involved in the food production responsible for the control and elimination of microbial contamination of produce intended for consumers.

Another approach being implemented by producers and distributors is the HACCP (Hazard Analysis and Critical Control Point) which aims to prevent foodborne diseases by identification, evaluation and control of key points in the fresh produce supply chain (Netramai 2011). Simple practices such as education to growers about the importance of hygiene habits, and good agricultural practices also add up towards the collective goal of making sure fresh produce arrives in good quality and safe to consumers.

During distribution and storage it is vital to maintain fresh produce under adequate (cool) temperatures and relative humidity since they are the most important factors affecting spoilage and growth of pathogenic microorganisms in fruits and vegetables (Allende, Tomás-Barberán et al. 2006).

Other preservation techniques that are currently being implemented by fresh produce industry include the use of antioxidants, irradiation, modified atmosphere

packaging, antimicrobial packaging and sanitizing agents among others (Parish, Beuchat et al. 2003; Allende, Tomás-Barberán et al. 2006).

2.4.1. Modified atmosphere packaging (MAP)

The objective of a MAP technique applied to fresh produce is to extend its shelf life by reducing the produce respiration without completely stopping it. Failure to allow levels of oxygen inside the package could create opportunities for slower growing pathogenic bacteria, and for that reason MAP techniques must be designed with care (Allende, Tomás-Barberán et al. 2006). The packaged for a fresh produce is surrounded by a different gas mixture other than air which could include gases such as CO_2, N_2 or O_2 depending on the application (Phillips 1996). Packaging materials intended to use for this purpose must be designed with selective barrier properties that provide a controlled oxygen transmission rate (OTR) and they should be selected according to the specific produce's respiration rate (Exama, Arul et al. 1993). For decades MAP application has been successfully used in food products such as fish, meat, fruits and vegetables for decades (Phillips 1996).

2.4.2. Irradiation

Although in some cases irradiation has affected the quality attributes of fresh produce, irradiation with low-dose gamma is very effective in reducing bacterial, parasitic, and protozoan pathogens in raw foods (Beuchat 1998; Rico, Martín-Diana et al. 2007). The maximum dose approved by the FDA for fruits and vegetables is 1.0 kGy irradiation (Rico, Martín-Diana et al. 2007). Ultraviolet light acts as an antimicrobial agent due to DNA damage, and indirectly due to induction of resistance mechanisms in

different fruits and vegetables against pathogens (Allende, Tomás-Barberán et al. 2006; Rico, Martín-Diana et al. 2007). UV consist of beams that accelerate the ions to interact with microorganisms and inactivate them (El-Samahy, Youssef et al. 2000). Non-ionizing, artificial ultraviolet-C (UV-C) radiation is extensively used in a broad range of antimicrobial applications including water, air, food preparation surfaces and containers (Allende, Tomás-Barberán et al. 2006).

2.4.3. Antimicrobial packaging

Antimicrobial packaging is another type of active packaging which objective is to reduce, inhibit or retard the growth of microorganisms that may be present in the package system (Appendini and Hotchkiss 2002). Antimicrobial packaging could take several forms including i) addition of sachets/pads containing sanitizing agents into packages, ii) incorporation of antimicrobial agents directly into polymers, iii) application of surface coatings to polymers, or iv) use of polymers that are inherently antimicrobial (Appendini and Hotchkiss 2002). Some of the sanitizing agents that are used either in antimicrobial packaging or as a preservation technique during food processing are described below.

2.5. Common sanitizing agents

2.5.1. Chlorine

Chlorine (Cl_2) has been used for sanitation purposes in food processing for several decades, and its perhaps the most widely used sanitizer in the food industry (Parish, Beuchat et al. 2003). It can be used with water as a washing solution or as a spray to sanitize produce surfaces including the food package (Bhagat 2010). The

mode of inactivation of Cl_2 is through the bactericidal activity of hypochlorous acid produced by the breakdown of sodium hypochlorite (Bhagat 2010). The Food and Drug Administration (FDA) in the United States permits the use of Cl_2 as a disinfectant in wash, spray and fume waters in the raw fruit and vegetable industry (Seymour and Appleton 2001).

The main disadvantages of Cl_2 as a disinfectant are i) it can form chlorinated compounds such as trihaloethanes which are considered carcinogenic (Parish, Beuchat et al. 2003; Bhagat 2010) and that ii) its efficacy is affected by changes in pH, temperature, presence of organic matter, light, air and metals (Parish, Beuchat et al. 2003). Cl_2 is generally used in concentration between 50-200ppm for treatments of 1-2 minutes with reported reductions of less than 2 log CFU g-1 on vegetables and fruits (Staschower 2012).

2.5.2. Hydrogen peroxide

Hydrogen peroxide (H_2O_2) possesses bactericidal and inhibitory activity due to its properties as an oxidant agent, and due to its capacity to generate other cytotoxic oxidizing species such as hydroxyl radicals (Parish, Beuchat et al. 2003). Its rapid breakdown makes it a good sanitizing agent for food surfaces and packaging materials in aseptic filling operations (Parish, Beuchat et al. 2003; Rico, Martín-Diana et al. 2007). The main drawbacks of the use of H_2O_2 as sanitizer of fresh produce are that i) similarly to Cl_2, its effectiveness depends on temperature and pH, and that ii) treatments with this agent caused significant quality loss on fresh produce (Beuchat 1998).

2.5.3. Ozone

Ozone (O_3) is used in gaseous form or dissolved in water, it is a strong oxidizing agent able to destroy microbial cells due in part to its strong penetrability in produce surface (Rico, Martín-Diana et al. 2007). It is an effective treatment for drinking water and will inactivate bacteria, fungi, viruses and protozoa (Parish, Beuchat et al. 2003). Two main advantages of O_3 are that i) it will decompose into non-toxic product, and ii) its performance is not affected by pH (Rodgers, Cash et al. 2004; Rico, Martín-Diana et al. 2007). Treatment with ozonated water showed an increase in shelf life of apples, grapes, oranges, pears, raspberries, and strawberries by reducing microbial populations and by oxidation of ethylene to retard ripening (Beuchat 1998). However, some of the disadvantages of ozone for use as a sanitizing agent are that i) it can cause changes in fruit quality, specifically color and texture (Daş, Gürakan et al. 2006) and furthermore ii) due to its high corrosiveness and potential hazard, it has to be generated on site and cannot be transported (Beuchat 1998).

The list of available sanitizers and strategies that aim to reduce human health risks and food spoilage by preservation of fresh produce and other food products is much longer. There is not one universal solution for the diversity of challenges that the food industry encounters with different type of produce and microorganisms, affecting different parts of the world where availability of resources, or technology or infrastructure are different and unique. In many cases, a combination of two or more preservation techniques is the best approach given that multiple points for control and reduction of microorganism are necessary in order to minimize the possibilities for

outbreaks. However, there is one sanitizer agent that is being considered and assessed by the scientific community as one promising option to disinfect fresh produce efficiently. It is the case of chlorine dioxide.

2.6. Chlorine Dioxide

Discovered by Sir Humphrey Davy in the nineteenth century after acidifying potassium chlorate with sulfuric acid, chlorine dioxide came a hundred years later to be known as a powerful disinfectant and bleaching agent (Aieta and Berg 1986). Thanks to its broad spectrum disinfection capability, chlorine dioxide has a wide variety of applications (Knapp and Battisti 2001). The largest application of ClO_2 is in the paper industry as wood pulp bleaching where it is preferred over other types of oxidizing agents thanks to its i) high solubility in water, ii) commercial availability of sodium chlorite, and because iii) it does not compromise the mechanical integrity of the bleached pulp (Kaczur and Cawlfield 2000). In other applications, chlorine dioxide is used as a surface sanitizer of diverse kinds (Keskinen and Annous 2011), it is also used in the drinking water treatment to produce safe potable water (Aieta and Berg 1986), and most recently in the food and packaging industry for its usefulness as a treatment to minimize microbial cross contamination and to extend the shelf life of products (Aieta and Berg 1986).

2.6.1. Physical and Chemical Properties

Chlorine dioxide is a volatile, highly energetic molecule (United States Environmental Protection Agency (EPA) 1999) which contains two atoms of chlorine (Cl_2) and one atom of oxygen (Knapp and Battisti 2001). In the gas form, ClO_2 has a

yellow-green color with a pungent odor similar to chlorine and sodium hypochlorite; while in the liquid form, ClO_2 has a deep red color and is explosive at temperatures above -40 ^{0}C (Kaczur and Cawlfield 2000; Linton, Han et al. 2006). Two of the most important physical characteristics of ClO_2 are both that it exists almost exclusively as a free radical monomer even when diluted in aqueous solutions, and its high solubility in water where it forms a stable yellow greenish color solution provided that it is kept cool in a closed container and in the dark (United States Environmental Protection Agency (EPA) 1999). The free radical molecular structure of the gas is shown in Figure 3.

FIGURE 4 - Free radical molecular structure of chlorine dioxide (Knapp and Battisti 2001)

As a strong oxidizing agent, ClO_2 will participate in oxidation reduction reactions that involve one electron transfer mechanism forming chlorite (ClO_2^-), chloride (Cl^-) and chlorate (ClO_3^-) (United States Environmental Protection Agency (EPA) 1999; Keskinen and Annous 2011). The concentration at which these by-products are generated would depend on the reaction pH and light conditions. For example, in aqueous solutions at pH>10, chlorine dioxide will hydrolyze to form ClO_3- and ClO_2- ions, while in neutral or near neutral solutions (4< pH <10) ClO_2 will remain stable as a free radical (United States Environmental Protection Agency (EPA) 2006). Some of these key reactions

include (United States Environmental Protection Agency (EPA) 1999; Qingdong, Guangming et al. 2006):

$$ClO_{2\,(aq)} + e^- \rightarrow ClO_2^-$$

$$ClO_2^- + 2H_2O + 4e^- \rightarrow Cl^- + 4OH^-$$

$$ClO_2^- + 2OH^- \rightarrow ClO_3^- + H_2O + 2e^-$$

$$ClO_2 + H2O \rightarrow ClO_3^- + 2H+ + e^-$$

$$ClO_2 + 4H+ + 4e^- \rightarrow ClO_3^-$$

Due to its free radical natural state, ClO_2 will degrade with UV or fluorescent light breaking the chlorine oxygen bond, forming O- and ClO- which are considered reactive forms (Kaczur and Cawlfield 2000; Netramai 2010).

Table 1 provides a summary of additional ClO_2 properties.

Table 1. Additional ClO_2 physical and chemical properties (Windholz, Budavari et al. 1976; Keskinen and Annous 2011)

CAS Registry Number	10049-04-4
Molecular weight	67.46 g/mol
Water solubility	3.0 g/L at 25 C and 34 mmHg
Specific gravity	1.642 g/mL at 0 C (liquid); 3.09 g/L (gas)
Critical temperature	465 K
Critical pressure	8621.6 kPa

2.6.2. Antimicrobial Properties

ClO_2 disinfects by an oxidation mechanism without involving chlorination (United States Environmental Protection Agency (EPA) 1999). Research has been carried out in

order to determine the bactericide, fungicide and viricide efficacy of ClO_2 against multiple microorganisms present in different types of produce. Appendix 1 shows a summary of the main findings for fresh produce.

The attractiveness of ClO_2 use as a biocide has increased to the extent that it has replaced Cl_2 in several applications at both the drinking water and food industry because i) it has approximately 2.5 times the oxidation capacity of Cl_2 (Benarde, Israel et al. 1965), ii) it is approximately 10 times more soluble above 11 ^{0}C (United States Environmental Protection Agency (EPA) 1999), and iii) its efficacy is not affected by changes in pH as it does not ionize in water (Benarde, Snow et al. 1967).

However, it is has also been found that ClO_2 is less effective than ozone (United States Environmental Protection Agency (EPA) 1999).

In general, although appendix 1 shows that ClO_2 has been proved to be a powerful sanitizing agent, its efficacy in reducing populations of bacteria, protozoa, or virus will vary with factors such as produce, target microorganism, relative humidity (United States Environmental Protection Agency (EPA) 1999) and temperature (Benarde, Snow et al. 1967).

Another important factor that affects ClO_2 efficacy is the state at which it is used in a treatment; published data suggest that aqueous ClO_2 is less effective than gaseous when treating fruits and vegetables. For example, Han et al. explained that the greater penetration ability of gases could be responsible for this phenomenon when they proved the better efficiency of ClO_2 gas versus aqueous solution in injured and uninjured green

peppers (Han, Linton et al. 2001). Singh et al. found that a treatment with a higher concentration of aqueous ClO_2 for 5 minutes produced a lower (1.55-log) reduction of *E. coli O157:H7* on lettuce than a treatment with a lower concentration of aqueous ClO_2 for 30 seconds which resulted in a reduction of almost 5 times (5-log) when *E. coli ATCC 11229* was suspended in water. These results show that aqueous ClO_2 is not as effective at killing pathogens on vegetables such as lettuce as it is in killing pathogens in aqueous suspensions (Singh, Singh et al. 2002; Lee, Costello et al. 2004). At the same time, these results show that fruits or vegetables, just as the lettuce in the example above, could provide protection or sites for microorganisms to hide, as they can be attached to its surfaces in locations inaccessible to aqueous treatments such as hydrophobic pockets, irregular folds, or underneath leaves or calyxes (Lee, Costello et al. 2004; Staschower 2012).

Seo et al. also found that a liquid solution would be incapable of inactivating microorganisms attached to the produce's broken trichomes, cracks, stomata and cut edges after finding many live *E. coli O157:H7* in the stomata of lettuce after treatment with a 20 mg/L aqueous solution (Seo and Frank 1999).

On the other hand, treatments with gaseous ClO_2 proved to be efficacious in a wide number of examples such as: Du et al (2002) who demonstrated that ClO_2 gas inactivated *E. coli O157:H7* on apple surfaces, specifically on its calyx and cavities; Sy et al. who found significant reductions of *Salmonella* populations on blueberries, strawberries, and raspberries, as well as reductions of *Salmonella*, *E. coli O157:H7*, *L. monocytogenes*, yeasts and molds on cabbage, carrots, apples, tomatoes and peaches

(Sy, McWatters et al. 2005; Sy, Murray et al. 2005); Han et al. who found that treatments were highly effective in reducing *E. coli O155:H7* and *L. monocytogenes* on strawberry surfaces (Han, Selby et al. 2004); Lee et al. who showed efficacy of the gas against *E. coli O155:H7* on lettuce leaves (Lee, Costello et al. 2004); Du, Han, and Linton who found gaseous ClO_2 effective against both *E. coli O155:H7* and *L. monocytogenes* on apple surfaces (Du, Han et al. 2002; Du, Han et al. 2003); Han et al. who found that treatments were effective in the inactivation of both *E. coli O155:H7* and *L. monocytogenes* on green pepper surfaces (Han, Floros et al. 2001; Han, Linton et al. 2001) and many others as shown in Appendix 1.

2.7. Methods of Generating Chlorine Dioxide Gas

Being highly unstable and explosive under pressure, ClO_2 gas cannot be compressed or stored commercially due to safety reasons (United States Environmental Protection Agency (EPA) 1999). Thus, ClO_2 gas cannot be shipped and must be produced at the point of use (Keskinen and Annous 2011). Commercial generators of ClO_2 gas use sodium chlorite as the precursor feedstock chemical for the production of ClO_2 gas (Kaczur and Cawlfield 2000), although in the last two decades production of ClO_2 gas from sodium chlorate has been explored especially at water treatment plants (United States Environmental Protection Agency (EPA) 1999). The principal generation reactions that use sodium chlorite to produce ClO_2 gas are listed below (United States Environmental Protection Agency (EPA) 1999):

$NaClO_2 + Cl_{2\ (gas)} \rightarrow 2\ ClO_{2\ (gas)} + 2\ NaCl$

$2\ NaClO_2 + HOCl \rightarrow 2\ ClO_{2\ (gas)} + NaCl + NaOH$

$5\ NaClO_2 + 4\ HCl \rightarrow 4\ ClO_{2\ (gas)} + 5\ NaCl + 2H_2O$

This set of reactions show how generators can differ even if the same precursors are used.

2.7.1. Constant gas flow generators

The advantage of these type of generators is that they ensure the production of ClO_2 gas at constant flow rate and concentration. The generators are equipped with a control system that allow the reactions to occur continuously as needed in order to compensate for the depleted or consumed gas in the system. An example of a commercial generator of this type is the system manufactured by ChloriDiSys Solutions Inc. (Lebanon, NJ) which is shown in figure 5. Chlorine gas (2%) in nitrogen flows into three sodium chlorite cartridges to generate ClO_2 gas. Both the flow rate and the concentration are controlled by a set of sensors and the integrated UV-VIS photometric detection system which alert the system for more generation if needed (Czarneski and Lorcheim 2005).

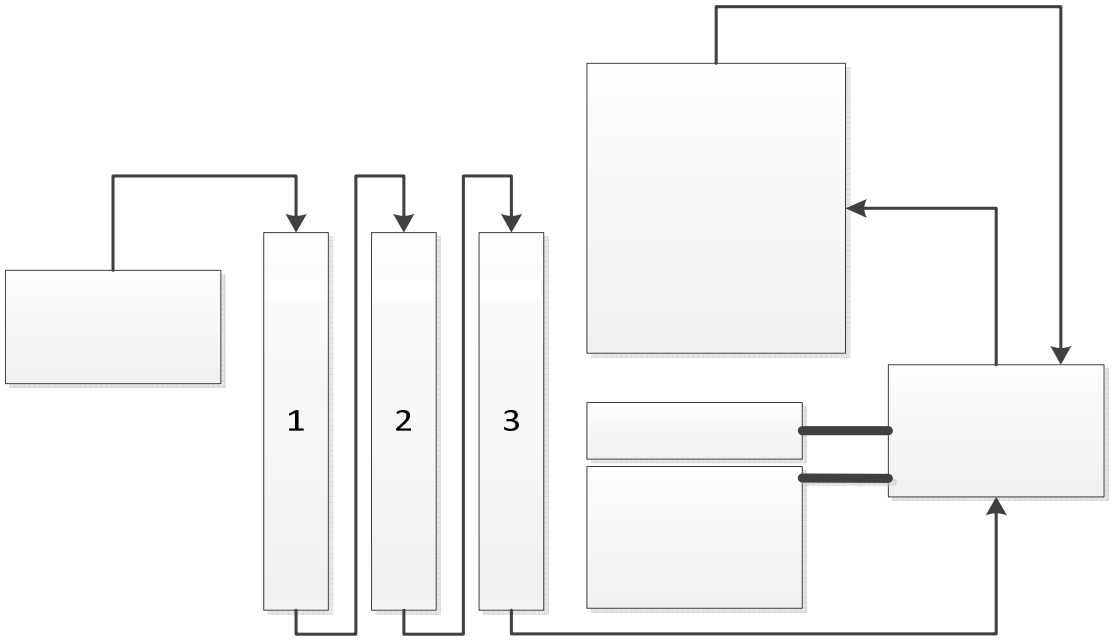

FIGURE 5. Schematic diagram of a Minidox-M from ChlorDiSys ClO_2 gas generation system, adapted from (Czarneski and Lorcheim 2005)

2.7.2. Sachet

This type of generation use a mix of solid precursors to initiate the reaction and as a result the concentration is variable over time. The concentration starts to build up until the target (maximum) concentration is reached where the concentration would start to decrease due to the depletion of precursors. This way to generate ClO_2 is done using a sachet and it is considered a non-continuous process (Staschower 2012). An example of a commercially available product are the Z-series technology developed by ICA TriNova (Newnan, GA) where ClO_2 gas is generated by mixing two dry solids where the precursor could either be sodium chlorite or sodium chlorate, and activator which is

an acid (HCl, H_2SO_4, citric, acetic, etc.), following the following reaction (Linton, Han et al. 2006; Staschower 2012):

$$4ClO_2^- + 4H^+ \rightarrow 2ClO_2 + ClO_3^- + Cl^- + 2H^+ + H_2O$$

To ensure that the gas generated is released into the required atmosphere, the components are mixed inside a permeable pouch which permits the gradual release of gas either into water or air.

2.7.3. Liquid solution

Due to its volatile nature, a concentrated ClO_2 solution will vaporize if it is maintained inside a sealed chamber, creating a ClO_2 atmosphere above the solution (Mahovic, Bartz et al.). Every time the gas is consumed, more ClO_2 will be generated from the solution (Staschower 2012).

A commercial example of this type of ClO_2 gas generation are the set of dry mixtures manufactured by ICA TriNova (Newnan, GA) which similarly to the sachet technology described above, are supposed to be mixed inside a permeable pouch and then submerged inside deionized water for the gas to dissolve in it. In this case, $NaClO_2$ is used as the precursor while a formulation of $FeCl_3$ serves as an acid activator (Mahovic, Bartz et al.)

2.8. Absorption of ClO_2 by fresh produce and available methods for its quantification

As previously discussed, ClO_2 participates in a series of oxidative reactions which result in its break down into chlorate (ClO_3^-) and chlorite (ClO_2^-) ions, which can at the same time further transform into chloride (Cl^-) (United States Environmental Protection Agency (EPA) 1999; Gómez-López, Rajkovic et al. 2009). This is a particularly important fact to consider when evaluating ClO_2 gas as a sanitizer of fruits and vegetables since the cell structures, pigments, and microflora existing on produce surfaces will absorb ClO_2 which will then oxidize and break down upon contact (Netramai 2011), leaving surface residues that could later cause the formation of chlorinated byproducts (Trinetta, Vaidya et al. 2011). A few researchers have investigated the absorption of chlorine dioxide by fresh produce, and they have found not only evidence of residues in the surface of the produce, but also other clues that lead to the hypothesis that ClO_2 and its residues actually permeate inside the tissue of various produce types. For example, using the amperometric titration method, Han et al. (2004) recovered residues of ClO_2 and ClO_2^- on strawberries after 1 week of treatment (Han, Selby et al. 2004); Trinetta (2011) found residues of ClO_2, ClO_2^-, and ClO_3^- up to two weeks after treatment with ClO_2 in surfaces of hydroponic tomatoes, navel oranges, apples, strawberries, lettuce, alfalfa sprouts and cantalopes (Trinetta, Vaidya et al. 2011); Netramai (2011) found residues of ClO_2 and ClO_2^- in lettuce after

treatments with ClO_2 at different concentrations and exposure times (Netramai 2011); and more recently Staschower (2012) recovered ClO_2 and ClO_2^- on romaine lettuce and cherry tomatoes after treatments ClO2 using sachet, concentrated solution and a commercial gas generator (Staschower 2012). Staschower (2012) also recovered significantly higher amounts of residues on the surface of lettuce than on tomatoes which suggest that differences in the plant tissue and protective layers influence the absorption of ClO_2 by fresh produce. Another factor that may affect absorption are bruises and cuts present in the produce being treated, which creates susceptible areas for easier deterioration and biochemical reactions (Allende, Tomás-Barberán et al. 2006). This was proven by Netramai (2011) who evaluated the difference between ClO_2 absorbed by shredded lettuce and whole lettuce, finding that shredded lettuce absorbed approximately 10 times more than whole lettuce did. After analyzing absorption amounts of washed versus not washed lettuce, she also found that the presence of water did not significantly affect the absorption of ClO_2. Finally, both Netramai (2011) and Staschower (2012) found higher amounts of residues with increased ClO_2 concentration and exposure time, which means that ClO_2 absorption is affected by the severity of the treatment (Netramai 2011; Staschower 2012).

Analytical methods currently available for the measurement of ClO_2 gas concentration are also used by researchers to measure ClO_2 gas absorption by fresh

produce after dissolving it in water (Keskinen and Annous 2011). These methods are described below.

2.8.1. Amperometric Titration Method

The Amperometric titration for quantification of ClO_2 and its by-products is based on the pH-dependent oxidation principle of potassium iodide (KI) to selectively distinguish the various oxychlorine species from each other (Kaczur and Cawlfield 2000). The reaction of chlorine species with KI at various pH buffered conditions are shown below (Aieta, Roberts et al. 1984):

$Cl_2 + 2I^- \rightarrow I_2 + 2Cl^-$ pH 7, 2, <0.1

$2ClO_2 + 2I^- \rightarrow I_2 + 2ClO_2^-$ pH 7

$2ClO_2 + 10I^- + 8H^+ \rightarrow 5I_2 + 2Cl^- + 4H_2O$ pH 2 <0.1

$ClO_2^- + 4I- + 4H^+ \rightarrow 2I_2 + Cl^- + 2H_2O$ pH 2, <0.1

$ClO_3^- + 6I^- + 6H^+ \rightarrow 3I_2 + Cl^- + 3H_2O$ pH<0.1

Common titrants for the determination of chlorine compounds are sodium thiosulfate ($Na_2S_2O_3$) and phenyl arsine oxide (C_6H_5AsO) which reactions with KI are shown below (Kaczur and Cawlfield 2000):

$C_6H_5AsO + I_2 + 2H_2O \rightarrow C_6H_5As(OH)_2 + 2H^+ + 2I^-$

$2Na_2S_2O_3 + I_2 \rightarrow Na_2S_4O_6 + 2Na^+ + 2I^-$

These titrations can be performed manually using either a starch indicator for end point or more accurately by amperometric methods. A practical method outlined for

titration with C_6H_5AsO is described by the standard method 4500-ClO2 C (Appendix 2) where free chlorine, chloramines, chlorite and chlorine dioxide are measured separately. The method for the titration with $Na_2S_2O_3$ is outlined by ICA Trinova and it is presented in Appendix 3.

2.8.2. Colorimetric Method

The colorimetric method is an analytical method commonly used for qualification and sometimes quantification of compounds. It correlates color with concentration, understanding that higher intensity will mean higher concentration of the specific compound.

Although different colorimetric methods vary in the use of the indicator (Greenberg, Clesceri et al. 1992), most of them follow the steps described in 4500-ClO_2 D standard method which involves the reaction between ClO_2 and *N,N*-diethyl-*p*-phenylenediamine (DPD) to form an oxidized product that is measured at 550 nm (Pepich, Dattilio et al. 2007). The method 4500-ClO_2 D is popular, but subject to interferences from other oxychlorine oxidizing species present (Kaczur and Cawlfield 2000). Another colorimetric method is the Environmental Protection Agency (EPA) Method 327.0 which uses lissamine B and horseradish peroxidase which show fewer interferences (Pepich, Dattilio et al. 2007).

2.8.3. Ion chromatography

The EPA describes the method in de document “Method 300.0 or 300.1 - Determination of inorganic anions in drinking water by Ion Chromatography” by which

common inorganic anions and inorganic disinfection by-products are determined in reagent water, surface water, ground water, and finished drinking water (Hautman, Munch et al. 1997). The technique is able to determine multiple anions in a single analysis and it involves eluding an IC-pack column (Dionex, Sunnyvale, CA.) with a solution of 2 mM Na_2CO_3/0.75 mM $NaHCO_3$ at a flow rate of 2 mL/min which is used as the carrier (Trinetta, Vaidya et al. 2011). Ions present in the sample solutions are then separated as they interact with the column content and detected at the exit with a conductivity cell (Hautman, Munch et al. 1997). It is a very sensitive method for measuring chlorite and chlorate by-products, with a detection limit of 0.01 mg/L for chlorate and 2 mg/L for chloride (Trinetta, Vaidya et al. 2011).

2.8.4. UV spectroscopy

UV spectroscopy is a common method used to measure both aqueous and gaseous ClO_2. Due to its efficiency and accuracy, this method is commercially used on process analyzers for the online control of ClO_2 gas generation systems (Kaczur and Cawlfield 2000). Due to its natural double-bond character, ClO_2 absorbs UV light, and so it can be detected (Robinson 2005). Researchers have studied ClO_2 spectrum and UV absorption at different wavelengths and temperatures. Kaczur et al (2000) found that its maximum absorption happens near 360 nm wavelength and its molar extinction coefficient is more accurately valued at 1250 $(M\ cm)^{-1}$ when using high resolution, narrow bandwidth spectrophotometers (Kaczur and Cawlfield 2000). Wahner et al. (1987) however, found that maximum absorption of ClO_2 happened in the vicinity of 351

nm wavelength and reported values of absorption cross section in a wavelength region of 240-480 nm at three different temperatures (Wahner, Tyndall et al. 1987). The values reported by Wahner were in agreement with those reported by Kromminga (2003) who used a Bruker IFS- 120 HR Fourier-transform spectrometer designed for operation in the UV-Vis spectral range with a beamsplitter made of UV-Quartz as the detector (Kromminga, Orphal et al. 2003).

The majority of the analytical methods mentioned above are approved by the US Environmental protection Agency (EPA), the National Institute for Occupational Safety and Health (NIOSH), Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA) (Agency for Toxic Substances and Disease Registry Division of Toxicology and Envirommental Medicine 2004)

2.9. Toxicity, Risks and Regulations of Chlorine Dioxide Gas

Considering that disinfection by ClO_2 is followed by the formation of ClO_2^- and ClO_3^-, when evaluating the safety of ClO_2 gas as disinfectant, the toxicity of both the sanitizer and its by-products should be taken into consideration.

ClO_2 gas is considered a hazard material because it is unstable and explosive at concentrations above 10.1 KPa partial pressure in air. If involved in a fire, chlorine dioxide would serve as source of oxygen thus allowing its propagation (Kaczur and Cawlfield 2000). For these reasons, the gas cannot be compressed or stored commercially (United States Environmental Protection Agency (EPA) 1999) and must be produced exclusively at the point of use (Keskinen and Annous 2011). At the same time, ClO_2 and its by-products are considered a hazard because there is evidence to

prove that exposure to large amounts of these substances may provoke several health problems to animals and humans.

According to the Agency for Toxic Substances and Disease Registry Division of Toxicology and Environmental Medicine, after exposure to a hazardous substance, it is important to consider the precautions to be taken and all the factors that would categorize the degree of the hazard such as the type of substance, the exposure dose, the exposure duration, the organs affected and the manner of exposure (e.g. if the substance was inhaled, spilled, ingested, etc…), the age, sex, and health situation of the exposed individual (Agency for Toxic Substances and Disease Registry Division of Toxicology and Envirommental Medicine 2004).

Animal tests conducted in rats showed that after treatments with chlorite in drinking water at concentrations of 100 mg/L and above resulted in decreased red blood cell counts and hemoglobin concentrations after 30 and 60 days of exposure (Heffernan, Guion et al. 1979). Abdel-Rahman et al. studied the effects of ClO_2, ClO_2^- and ClO_3^- in both rats and chickens for periods of up to 9 months and found signs of anemia especially in those exposed to drinking water with concentrations of 10 and 100 mg/L of ClO_3^- (Abdel-Rahman, Couri et al. 1979).

The Agency for Toxic Substances and Disease Registry Division of Toxicology and Environmental Medicine describes that ClO_2 gas acts primarily as a respiratory tract and ocular irritant, producing eye, throat, nose, and lung irritation. However, the greatest potential for human exposure to ClO_2 and ClO_2^- is via oral exposure by drinking water where if ingested in large amounts, can cause mouth, esophagus and

stomach irritation as well as it may result in increased levels of methemoglobin in the blood which reduces the ability of oxygen to bind with hemoglobin (Heffernan, Guion et al. 1979; Agency for Toxic Substances and Disease Registry Division of Toxicology and Envirommental Medicine 2004).

In a review of toxicological effects of ClO_2, ClO_3^- and ClO_2^-, Couri et al.(1982) showed the adverse effects of the substances in hematologic parameters such as alterations in erythrocyte morphology and osmotic fragility, as well as inhibition of DNA synthesis in several organs (Couri, Abdel-Rahman et al. 1982).

Qingdong (2006) tested the effect of ClO_2 and its by-products in water ingested by rats at a concentration 120 times higher than for humans (553 mg/L of ClO_2, ClO_2^- and ClO_3^-) during 90 days. The study showed that the ingestion did not affect weight gain, food utilization, indexes of blood and serum, liver/bodyweight, or kidney/bodyweight (Qingdong, Guangming et al. 2006).

Other studies used humans as experimental subjects where volunteers ingested water treated with 5 mg/L ClO_2 (0.036 mg/kg/day) for 84 days and no adverse health effects were detected (Condie 1986). Gomez-Lopez et al. didn't find health effects in populations living in areas where water was treated with ClO_2 for 12 weeks (Gómez-López, Rajkovic et al. 2009).

As reported by The Agency for Toxic Substances and Disease Registry Division of Toxicology and Environmental Medicine though, it is not likely that humans would be exposed to critical levels of ClO_2 or ClO_2^- in the drinking water, since an average

human male (70 Kg reference bodyweight) drinking 2 liters of water at a concentration of 1 mgClO_2^-/L (minimum level allowed by EPA), would be consuming less than 1% of what has been the lowest observed adverse-effect level (LOAEL) for repeated oral exposure to chlorite.

Additional studies are necessary to evaluate the carcinogenic effect of ClO_2, but previous studies have shown no dermal cancer risk (United States Environmental Protection Agency (EPA) 2006). Similarly, Condie et al.(1986), did not detect development of any tumors after exposure to ClO_2 in mice (Condie 1986). Thus, based on the limited information available , the International Agency for Research on Cancer determined that neither sodium chlorite or ClO_2 are classifiable as to human carcinogenicity (Gómez-López, Rajkovic et al. 2009).

For all these reasons, consumption and ingestion of ClO_2 and its by-products raise up concerns due to potential adverse health effects, and its use as a sanitizer is regulated by different organizations. Examples of those regulations are listed below.

The liquid form of ClO_2 was first registered as a surface disinfectant and sanitizer by the EPA under the Federal Insecticide, Fungicide, and Rodenticide Act in 1967 for use in livestock barns, bottling plants, food processing plants, and other manufacturing and storage facilities (United States Environmental Protection Agency (EPA) 2007). Then in 1988, the EPA registered ClO_2 gas as a sterilizing agent for use in manufacturing, laboratory equipment, environmental surfaces, tools, and clean rooms (United States Environmental Protection Agency (EPA) 2007), and ever since, ClO_2 gas

has been increasingly used by manufacturers and the pharmaceutical and medical device industry as a sterilizing agent to clean rooms and pharmaceutical equipment (Czarneski and Lorcheim 2005).

After collecting data from 29 water treatment plants using chlorine dioxide treatment, the EPA determined maximum contaminant levels in treated drinking water for chlorite ions as 0.8 mg/L and 1.0 mg/L for ClO_2 and ClO_2^- respectively (United States Environmental Protection Agency (EPA) 2006).

The expert committee from The Food and Agricultural Organization / World Health Organization (FAO/WHO) determined an acceptable daily intake of 0.03 mg/kg and 0.01 mg/kg of body weight for chlorite and chlorate respectively (Gómez-López, Rajkovic et al. 2009).

The Food and Drug Administration (FDA) approved the use of ClO_2 as an antimicrobial agent in water used in both poultry processing and fruits and vegetables washing in an amount not exceeding 3 ppm residual ClO_2. Treatments of fruits and vegetables with ClO_2, must be followed by a potable water rinse, blanching , cooking or canning (United States Food and Drug Administration (FDA) 2012).

In 2001, the FDA approved the incorporation of particles blended with in food packaging grade of low density polyethylene (LDPE) film for the purpose of delivering a controlled, time-released dose of ClO_2 in the headspace of packaged meats, poultry and seafood in order to extend their shelf life. The maximum allowed amount of the

additive was determined to be 17.5 micrograms chlorite/in^2 of package film (United States Food and Drug Administration (FDA) 2011).

BIBLIOGRAPHY

BIBLIOGRAPHY

Abdel-Rahman, M. S., D. Couri, et al. (1979). "Kinetics of Cl02 and effects of Cl02, Cl02-, and Cl03- in drinking water on blood glutathione and hemolysis in rat and chicken." Journal of environmental pathology and toxicology 3(1-2): 431-449.

Agency for Toxic Substances and Disease Registry Division of Toxicology and Enviromental Medicine. (2004). "Toxicological Profile for Chlorine Dioxide and Chlorite." Retrieved June 1st, 2013, from http://www.atsdr.cdc.gov/toxprofiles/tp160.pdf.

Aieta, E. M. and J. M. Berg (1986). "A Review of Chlorine Dioxide in Drinking Water Treatment." J. Am. Water Works Assoc. 78 (6): 62-72.

Aieta, E. M., P. V. Roberts, et al. (1984). "Determination of Chlorine Dioxide, Chlorine, Chlorite, and Chlorate in Water." J. Am. Water Works Assoc. 76: 64-70.

Allende, A., F. A. Tomás-Barberán, et al. (2006). "Minimal processing for healthy traditional foods." Trends in Food Science & Technology 17(9): 513-519.

Appendini, P. and J. H. Hotchkiss (2002). "Review of antimicrobial food packaging." Innovative Food Science & Emerging Technologies 3(2): 113-126.

Balla, C. S. and J. Farkas (2007). Minimally Processed Fruits and Fruit Products and their Microbiological Safety. Handbook of Fruits and Fruit Processing, Blackwell Publishing: 115-128.

Bean, N. H. and P. M. Griffin (1990). "Foodborne disease outbreaks in the United States, 1973-1987: pathogens, vehicles, and trends." Journal of Food Protection 53.

Benarde, M. A., B. M. Israel, et al. (1965). "Efficiency of chlorine dioxide as a bactericide." Applied Microbiology 13(5): 776-780.

Benarde, M. A., W. B. Snow, et al. (1967). "Chlorine Dioxide Disinfection Temperature Effects." Journal of Applied Microbiology 30(1): 159-167.

Beuchat, L. (1998). "Surface decontamination of fruits and vegetables eaten raw: a review."

Beuchat, L. R. (1996). "Pathogenic Microorganisms Associated with Fresh Produce." Journal of Food Protection 59(2): 204-216.

Beuchat, L. R. (1999). "Survival of Enterohemorrhagic Escherichia coli O157:H7 in Bovine Feces Applied to Lettuce and the Effectiveness of Chlorinated Water as a Disinfectant." Journal of Food Protection 62: 845-849.

Beuchat, L. R. (2002). "Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables." Microbes and Infection 4(4): 413-423.

Bhagat, A. R. (2010). Modeling Critical Factors to Optimize the Treatment of Selected Fruits and Vegetables with Chlorine Dioxide Gas Using a Miniaturized Insustrial-Size Tunnel System. Doctor of Philosophy, Purdue University.

Bhaskara Reddy, M. V., K. Belkacemi, et al. (2000). "Effect of pre-harvest chitosan sprays on post-harvest infection by Botrytis cinerea and quality of strawberry fruit." Postharvest Biology and Technology 20(1): 39-51.

Brackett, R. E. (1999). "Incidence, contributing factors, and control of bacterial pathogens in produce." Postharvest Biology and Technology 15(3): 305-311.

Braun, P. and J. Sutton (1988). "Infection cycles and population dynamics of Botrytis cinerea in strawberry leaves." Canadian Journal of Plant Pathology 10(2): 133-141.

Centers for Disease Control and Prevention. (2011). "CDC Estimates of Foodborne Illness in the United States." Retrieved 9 April, 2013, 2013, from http://www.cdc.gov/foodborneburden/PDFs/FACTSHEET_A_FINDINGS_updated4-13.pdf.

Centers for Disease Control and Prevention. (2013). "Surveillance for Foodborne Disease Outbreaks — United States, 2009–2010." Retrieved June 1st, 2013, from http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6203a1.htm?s_cid=mm6203a1_w.

Condie, L. W. (1986). "Toxicological Problems Associated With Chlorine Dioxide." Journal (American Water Works Association) 78(6): 73-78.

Couri, D., M. S. Abdel-Rahman, et al. (1982). "Toxicological Effects of Chlorine Dioxide, Chlorite and Chlorate." Environmental Health Perspectives 46: 13-17.

Czarneski, M. A. and P. Lorcheim (2005). "Isolator decontamination using chlorine dioxide gas." Pharmaceutical technology: 124-133.

Dagnas, S. and J.-M. Membre (2013). "Predicting and Preventing Mold Spoilage of Food Products." Journal of Food Protection 76(3): 538-551.

Dantigny, P., M. Bensoussan, et al. (2006). "Standardisation of methods for assessing mould germination: A workshop report." International journal of food microbiology 108(2): 286-291.

Dantigny, P., A. Guilmart, et al. (2005). "Basis of predictive mycology." International journal of food microbiology 100(1-3): 187-196.

Dantigny, P., S. Marín, et al. (2007). "Mould germination: Data treatment and modelling." International journal of food microbiology 114(1): 17-24.

Daş, E., G. C. Gürakan, et al. (2006). "Effect of controlled atmosphere storage, modified atmosphere packaging and gaseous ozone treatment on the survival of Salmonella Enteritidis on cherry tomatoes." Food Microbiology 23(5): 430-438.

Declerck, S., D. D'or, et al. (2001). "Modelling the sporulation dynamics of arbuscular mycorrhizal fungi in monoxenic culture." Mycorrhiza 11(5): 225-230.

Du, J., Y. Han, et al. (2002). "Inactivation by chlorine dioxide gas (ClO2) of Listeria monocytogenes spotted onto different apple surfaces." Food Microbiology 19(5): 481-490.

Du, J., Y. Han, et al. (2003). "Efficacy of chlorine dioxide gas in reducing Escherichia coli O157:H7 on apple surfaces." Food Microbiology 20(5): 583-591.

El-Samahy, S., B. M. Youssef, et al. (2000). "Microbiological and chemical properties of irradiated mango." Journal of food safety 20(3): 139-156.

Exama, A., J. Arul, et al. (1993). "Suitability of Plastic Films for Modified Atmosphere Packaging of Fruits and Vegetables." Journal of Food Science 58(6): 1365-1370.

Garcia, D., A. J. Ramos, et al. (2009). "Predicting mycotoxins in foods: A review." Food Microbiology 26(8): 757-769.

Gibson, A. M., N. Bratchell, et al. (1987). "The effect of sodium chloride and temperature on the rate and extent of growth of Clostridium botulinum type A in pasteurized pork slurry." Journal of Applied Microbiology 62(6): 479-490.

Gómez-López, V. M., A. Rajkovic, et al. (2009). "Chlorine dioxide for minimally processed produce preservation: a review." Trends in Food Science & Technology 20(1): 17-26.

Gougouli, M. and K. P. Koutsoumanis (2012). "Modeling germination of fungal spores at constant and fluctuating temperature conditions." International journal of food microbiology 152(3): 153-161.

Greenberg, A. E., L. S. Clesceri, et al. (1992). Standard methods for the examination of water and wastewater. Washington, D.C., American Public Health Association: 4-56 - 54.81.

Gustavsson, J., C. Cederberg, et al. (2011). Global Food Losses and Food Waste. International Congress SAVE FOOD!, Dusseldorf, Germany.

Han, Y., J. D. Floros, et al. (2001). "Response Surface Modeling for the Inactivation of Escherichia coli O157:H7 on Green Peppers (Capsicum annuum L.) by Chlorine Dioxide Gas Treatments." Journal of Food Protection 64: 1128-1133.

Han, Y., R. H. Linton, et al. (2001). "Reduction of Listeria monocytogenes on Green Peppers (Capsicum annuum L.) by Gaseous and Aqueous Chlorine Dioxide and Water Washing and Its Growth at 7C." Journal of Food Protection 64: 1730-1738.

Han, Y., T. L. Selby, et al. (2004). "Decontamination of Strawberries Using Batch and Continuous Chlorine Dioxide Gas Treatments." Journal of Food Protection 67: 2450-2455.

Harris, L. J., J. N. Farber, et al. (2003). "Outbreaks Associated with Fresh Produce: Incidence, Growth, and Survival of Pathogens in Fresh and Fresh-Cut Produce." Comprehensive Reviews in Food Science and Food Safety 2: 78-141.

Hautman, D. P., D. J. Munch, et al. (1997). "US EPA Method 300.1, Determination of Inorganic Anions in Drinking Water by Ion Chromatography, EPA/600/R-98/118, NIST PB-98-169196INZ." (Revision 1.0).

Heffernan, W., C. Guion, et al. (1979). "Oxidative damage to the erythrocyte induced by sodium chlorite, in vivo." Journal of environmental pathology and toxicology 2(6): 1487.

Jarvis, W. R. (1962). "The infection of strawberry and raspberry fruits by Botrytis cinerea Fr." Annals of Applied Biology 50(3): 569-575.

Kaczur, J. J. and D. W. Cawlfield (2000). Chlorine Oxygen Acids and Salts, Chlorous Acid, Chlorites, and Chlorine Dioxide. Kirk-Othmer Encyclopedia of Chemical Technology. John Wiley and Son. New York. NY, John Wiley & Sons, Inc.: 1-26.

Kantor, L. S., K. Lipton, et al. (1997). "Estimating and addressing America's food losses." Food Review 20(1): 2-12.

Keskinen, L. A. and B. A. Annous (2011). Chlorine Dioxide (Gas). Nonthermal Processing Technologies for Food. H. Q. Zhang, G. V. Barbosa-Cánovas, V. M. Balasubramaniamet al. Oxford, UK, Wiley-Blackwell: 359 - 365.

Knapp, J. E. and D. L. Battisti (2001). Chlorine Dioxide. Disinfection, Sterilization, and Preservation. S. S. Block. Philadelphia, Lippincott Williams & Wilkins: 215-227.

Kromminga, H., J. Orphal, et al. (2003). "New measurements of OClO absorption cross-sections in the 325–435 nm region and their temperature dependence between 213 and 293 K." Journal of Photochemistry and Photobiology A: Chemistry 157(2–3): 149-160.

Lee, S.-Y., M. Costello, et al. (2004). "Efficacy of Chlorine Dioxide Gas as a Sanitizer of Lettuce Leaves." Journal of Food Protection 67: 1371-1376.

Linton, R., Y. Han, et al. (2006). "Gas-/vapor-phase sanitation (decontamination) treatments." Microbiology of fruits and vegetables: 401-435.

López, S., M. Prieto, et al. (2004). "Statistical evaluation of mathematical models for microbial growth." International journal of food microbiology 96(3): 289-300.

Lynch, M., R. Tauxe, et al. (2009). "The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities." Epidemiology and infection 137(3): 307.

Mahovic, M., J. A. Bartz, et al. "Chlorine Dioxide Gas from an Aqueous Solution: Reduction of Salmonella in Wounds on Tomato Fruit and Movement to Sinks in a Treatment Chamber." Journal of Food Protection® 72(5): 952-958.

Netramai, S. (2011). Utilization of chlorine dioxide gas in food packaging application. Doctor of Philosophy Ph.D., Michigan State University.

O'Beirne, D. and G. Francis (2003). "Reducing the pathogen risk in MAP-prepared produce." Novel food packaging techniques 12: 231-275.

Parish, M. E., L. R. Beuchat, et al. (2003). "Methods to Reduce/Eliminate Pathogens from Fresh and Fresh-Cut Produce." Comprehensive Reviews in Food Science and Food Safety 2: 161-173.

Pepich, B. V., T. A. Dattilio, et al. (2007). "An improved colorimetric method for chlorine dioxide and chlorite ion in drinking water using lissamine green B and horseradish peroxidase." Analytica Chimica Acta 596(1): 37-45.

Phillips, C. A. (1996). "Review: Modified Atmosphere Packaging and its effects on the microbiological quality and safety of produce." International Journal of Food Science & Technology 31(6): 463-479.

Plattner, K. and A. Perez. (2012). "United States Department of Agriculture (USDA) Report: Fruit and Tree Nuts Outlook." from http://www.ers.usda.gov/media/970458/fts354.pdf.

Powelson, R. (1960). "Initiation of strawberry fruit rot caused by Botrytis cinérea." Phytopathology 50: 491-494.

Qingdong, X., Z. Guangming, et al. (2006). "Study on Subchronic Toxicity of Chlorine Dioxide and By-Products in Water." Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering 41(7): 1347 - 1353.

Rico, D., A. B. Martín-Diana, et al. (2007). "Extending and measuring the quality of fresh-cut fruit and vegetables: a review." Trends in Food Science & Technology 18(7): 373-386.

Robinson, J. W., Skelly Frame, Frame II (2005). Undergraduate Instrumental Analysis. New York.

Rodgers, S. L., J. N. Cash, et al. (2004). "A Comparison of Different Chemical Sanitizers for Inactivating Escherichia coli O157:H7 and Listeria monocytogenes in Solution and on Apples, Lettuce, Strawberries, and Cantaloupe." Journal of Food Protection 67(4): 721-731.

Seo, K. H. and J. F. Frank (1999). "Attachment of Escherichia coli O157:H7 to Lettuce Leaf Surface and Bacterial Viability in Response to Chlorine Treatment as Demonstrated by Using Confocal Scanning Laser Microscopy." Journal of Food Protection 62: 3-9.

Seymour, I. J. and H. Appleton (2001). "Foodborne viruses and fresh produce." Journal of Applied Microbiology 91(5): 759-773.

Singh, N., R. K. Singh, et al. (2002). "Effect of inoculation and washing methods on the efficacy of different sanitizers against Escherichia coli O157:H7 on lettuce." Food Microbiology 19(2-3): 183-193.

Sloan, A. E. (2013). "Top 10 Food Trends." from http://sloantrend.com/attachments/article/9/2013-04IFTTop10Trends.pdf.

Staschower, F. (2012). Residues from Chlorine Dioxide Gas Treatment, Generated by Different Delivery Systems, on Fresh Produce. Master of Science, Michigan State University.

Sumathi, S., R. F. Cindy, et al. (2004). "Fresh Produce: A Growing Cause of Outbreaks of Foodborne Illness in the United States, 1973 through 1997." Journal of Food Protection 67(10): 2342-2353.

Sy, K. V., K. H. McWatters, et al. (2005). "Efficacy of Gaseous Chlorine Dioxide as a Sanitizer for Killing Salmonella, Yeasts, and Molds on Blueberries, Strawberries, and Raspberries." Journal of Food Protection 68: 1165-1175.

Sy, K. V., M. B. Murray, et al. (2005). "Evaluation of Gaseous Chlorine Dioxide as a Sanitizer for Killing Salmonella, Escherichia coli O157:H7, Listeria monocytogenes, and Yeasts and Molds on Fresh and Fresh-Cut Produce." Journal of Food Protection 68: 1176-1187.

Trinetta, V., N. Vaidya, et al. (2011). "Evaluation of Chlorine Dioxide Gas Residues on Selected Food Produce." Journal of Food Science 76(1): T11-T15.

United States Department of Agriculture (USDA). (2003). "Pathogen modelling program, version 7.0." Retrieved June 1st, 2013, from http://ars.usda.gov/services/docs.htm?docid=6786.

United States Environmental Protection Agency (EPA) (1999). "Alternative Disinfectants and Oxidants Guidance Manual: Chapter 4. Chlorine Dioxide, EPA 815-R-99-014."

United States Environmental Protection Agency (EPA) (2006). Registration eligibility decision for Chlorine Dioxide and Sodium Chlorite (Case Number 4023). http://www.epa.gov/oppsrrd1/REDs/chlorine_dioxide_red.pdf.

United States Environmental Protection Agency (EPA). (2007). "Anthrax spore decontamination using chlorine dioxide." Retrieved June 1st, 2013, from http://www.epa.gov/pesticides/factsheets/chemicals/chlorinedioxidefactsheet.htm.

United States Food and Drug Administration (FDA). (2004). "Produce Safety From Production to Consumption: 2004 Action Plan to Minimize Foodborne Illness Associated with Fresh Produce Consumption." Retrieved June 1st, 2013, from

http://www.fda.gov/Food/FoodborneIllnessContaminants/BuyStoreServeSafeFood/ucm129487.htm.

United States Food and Drug Administration (FDA). (2011). "Agency Response Letter GRAS Notice No. GRN 000062." Retrieved June 1st, 2013, from http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm153971.htm.`

United States Food and Drug Administration (FDA). (2012). "Secondary direct food additives permitted in food for human consumption. Chlorine dioxide. CFR Title 21, part 173.300." Retrieved 9 Apr 2013, 2013, from http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=173.300.

Wahner, A., G. S. Tyndall, et al. (1987). "Absorption cross sections for symmetric chlorine dioxide as a function of temperature in the wavelength range 240-480nm." The Journal of Physical Chemistry 91(11): 2734-2738.

Williamson, B., B. Tudzynski, et al. (2007). "Botrytis cinerea: the cause of grey mould disease." Molecular Plant Pathology 8(5): 561-580.

Windholz, M., S. Budavari, et al. (1976). The Merck index. An encyclopedia of chemicals and drugs. Rahway, New Jersey, Merck & Co.

3. IN SITU QUANTIFICATION OF CHLORINE DIOXIDE CONSUMPTION BY FRESH PRODUCE USING UV-VISIBLE SPECTROSCOPY

3.1. Introduction

Foodborne disease outbreaks originating from contamination of minimally processed foods like fresh fruits and vegetables have been identified as a main vehicle for illnesses because they can carry different microorganisms (Bhagat 2010). The Centers for Disease Control and Prevention (CDC) estimate that at least 12 percent of the foodborne outbreak associated illnesses in the 1990s were linked to fresh produce items (United States Food and Drug Administration (FDA) 2004). Therefore, produce quality assurance is critical to the effort of minimizing foodborne illnesses and diseases linked to produce contamination. One of the current alternatives available to assure quality and safety of fresh produce, is the use of sanitizers such as ozone, hydrogen peroxide, chlorine or chlorine dioxide (ClO_2) among others (Allende, Tomás-Barberán et al. 2006). The use of ClO_2 has become more attractive than chlorine as a disinfectant due to its superior sanitizing efficacy (Akin, Hoff et al. 1982), and because it minimizes changes in the odor and taste of the disinfected product (Couri, Abdel-Rahman et al. 1982). The efficacy of ClO_2 against several microorganisms in different produce has been extensively documented (Han, Guentert et al. 1999; Han, Floros et al. 2001; Han, Linton et al. 2001; Du, Han et al. 2003; Han, Selby et al. 2004; Sy, McWatters et al. 2005; Mahmoud, Bhagat et al. 2007; Keskinen, Burke et al. 2009). In most cases, gaseous ClO_2 has proven to be more effective than aqueous ClO_2 due to its higher capacity to reach cavities and to penetrate produce with irregular surfaces, which

results in higher inactivation of microorganisms (Han, Linton et al. 2001; Du, Han et al. 2002; Lee, Costello et al. 2004). Moreover, the aqueous form of ClO_2 leaves moisture on the surface of the produce which promotes fungal growth (Trinetta, Vaidya et al. 2011).

To expand the use of ClO_2 as a sanitizing agent for fresh produce, a thorough analysis of the by-products that result upon the interaction between gaseous ClO_2 and the organic matter as well as its implications in human health are needed (Nieuwenhuijsen, Toledano et al. 2000). ClO_2 primary by-products include chlorite, chlorate, chloride (United States Environmental Protection Agency (EPA) 1999). Some organizations have established limits for the use of ClO_2 for example, the U.S Food and Drug Administration allowed the use of ClO_2 not exceeding 3 mg/L in water as an aqueous antimicrobial agent to sanitize fresh fruits and vegetables (United States Food and Drug Administration (FDA) 2012), the EPA determined a maximum ingestion level of 0.8 mg/L and 1.0 mg/L for ClO_2 and chlorite respectively in treated drinking water (United States Environmental Protection Agency (EPA) 2006), and the Food and Agricultural Organization / World Health Organization (FAO/WHO) determined acceptable daily intake values of body weight per day to 0.03 mg/kg and 0.01 mg/kg chlorite and chlorate respectively (Gómez-López, Rajkovic et al. 2009).

Industrial applications of ClO_2 oxidation technology exist in the paper processing and water treatment industries. Therefore, several analytical methods have been developed for quantification of ClO_2 and its by-products in a solution in order to meet

the requirements of these industries. These methods include amperometric titration, colorimetric method, ion and gas chromatography, and UV-vis spectroscopy (Aieta, Roberts et al. 1984; Hautman, Munch et al. 1997; Kaczur and Cawlfield 2000; Trinetta, Vaidya et al. 2011). Using some of these techniques, researchers have been able to recover and quantify residues of ClO_2 and by-product ions after exposing fresh produce such as tomatoes, lettuce, alfalfa sprouts, and strawberries to gaseous ClO_2 (Kim, Marshall et al. 1999; Tsai, Huxsoll et al. 2001; Netramai 2011; Trinetta, Vaidya et al. 2011). However, these post-exposure quantification techniques mostly are able to recover readily removed residual by-products left on the surface of the fresh produce by analyzing a rinsed solution. Furthermore, these methodologies include several preparation steps before the extracted solutions can be measured, which is not ideal for reactive or unstable species such as ClO_2, ClO_2^-, or ClO_3^-. Considerable levels of ClO_2 and by-products were found on different produce even 14 days after exposure (Trinetta, Vaidya et al. 2011) indicating initial washing is either inefficient or that ClO_2 gas may penetrate deeply into some fresh produce tissue, which may lead to erroneous quantification of ClO_2 and its by-products. Therefore, it is conceivable that consumers may ingest unacceptably high quantities unreacted ClO_2 and its by-products from treated produce. Thus, the main goal of this study was to develop a new approach to quantify total ClO_2 gas consumption by fresh produce that would minimize sample preparation. The specific objectives were to develop a system that accurately quantifies ClO_2 concentration while the fresh produce is being treated throughout a wide range of

concentrations and to apply the newly developed system to two specific applications by determining the ClO_2 consumption as function of exposure concentration, time and the surface area, mass, and type of produce.

3.2. Materials and Methods

3.2.1. The Equipment.

Figure 6 shows the schematic of the developed equipment. The system can be graphically divided into three main parts: (Part I) ClO_2 gas generation of concentrations between 0.01 to 5 mg/L at a constant flow rate of 500 mL/min; (Part II) exposure chamber that was designed to promote a uniform ClO_2 atmosphere for exposed produce, and (Part III) a ClO_2 detection system which can monitor and record ClO_2 concentration throughout the desired treatments.

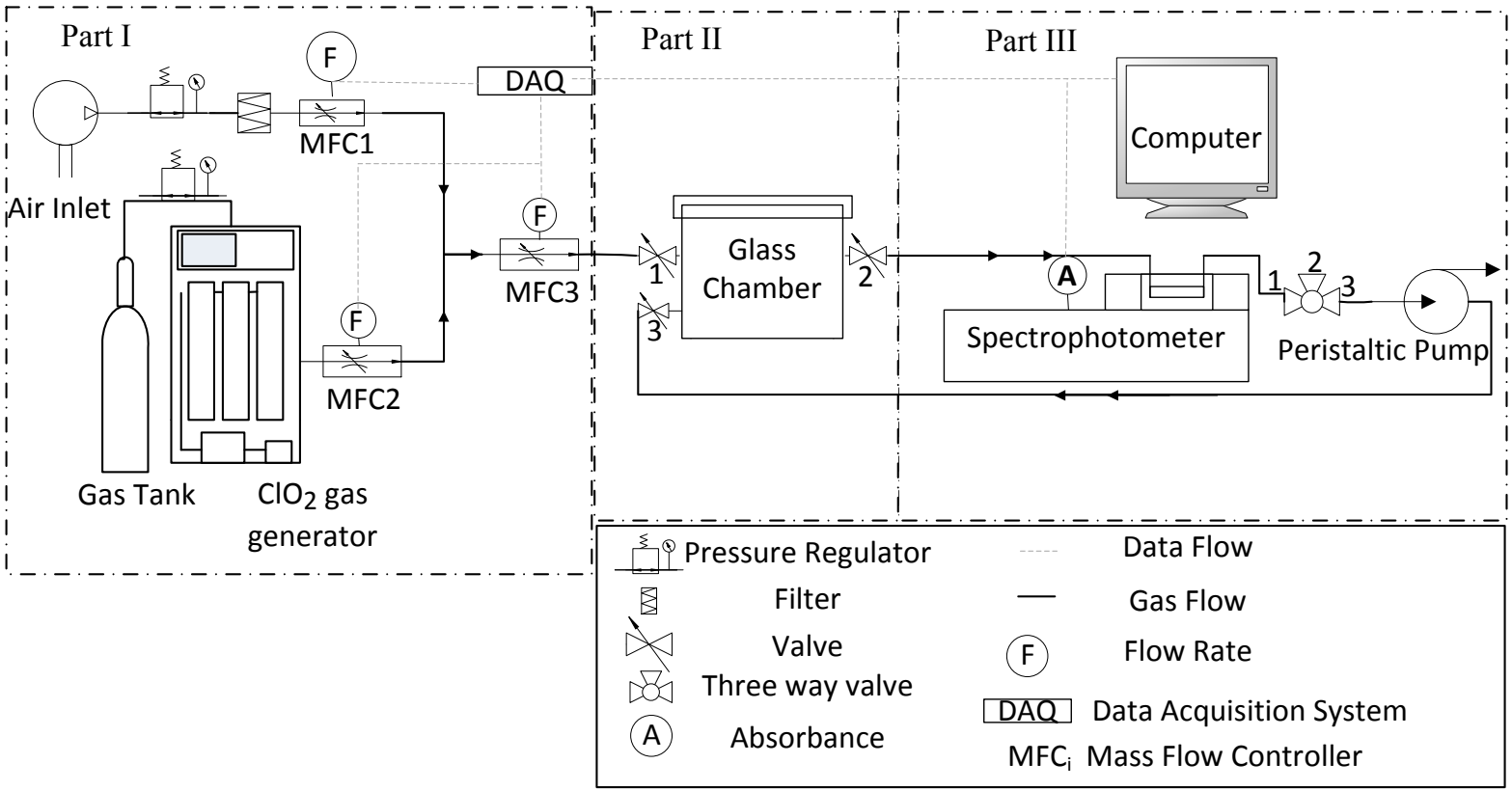

FIGURE 6. Schematic diagram of the three-component system for quantification of total chlorine dioxide absorption by fresh produce

3.2.2. Part I: Chlorine dioxide generation system

This system includes a Minidox-M ClO_2 gas generator from ClorDiSys Solutions Inc (Lebannon, NY) where chlorine (Cl_2) gas (2%) in nitrogen flows into three sodium chlorite ($NaClO_2$) cartridges to generate ClO_2 gas, an airline with filter and pressure regulator, and three Aalborg GFC (Models: 3NC-07-SS, 3AB-10-SS, and 3AB-06-SS) mass flow controllers (Orangeburg, NY) with volumetric flow rates of 1 L/min (used for ClO_2 flow), 10 L/min (used for air flow) and 500 mL/min (used for diluting ClO_2 gas in air) respectively. The flow rates of the mass flow controllers were controlled from a laptop computer using both a data acquisition (DAQ) device from National Instruments (Austin, TX) which serves as interface, and an in-house written program using LabView software from National Instruments (Austin, TX).

Filtered clean air at a pressure of 0.13 MPa (20 psi) flows into the first mass flow controller (MFC1) and simultaneously, ClO_2 gas generated by the Minidox-M at a desired ClO_2 concentration flows into the second mass flow controller (MFC2). Although the ClO_2 concentration generated by the Minidox-M is controlled by an integrated UV-vis photometric detection system (Czarneski and Lorcheim 2005), its accuracy at generating the desired concentration was evaluated and described in section 2.4. Both gases (air and ClO_2) are then combined into the third mass flow controller (MFC3),

which transports the gas mixture at the exposure ClO_2 concentration into the approximately 11 L glass chamber where the fresh produce is exposed.

At the beginning of each treatment, the chamber was filled with air that needs to be displaced to build up the targeted ClO_2 gas concentration inside. Introducing the ClO_2 gas into the chamber at constant flow rate and concentration could result in a lag time to build the targeted ClO_2 concentration. During this time period, the fresh produce would be exposed to an undesirably low concentration level. So, a two-step process was designed to speed up the filling of ClO_2 gas and reach the target concentration in the chamber during the first minute and a half for most treatments while keeping a constant flow rate. The first step consisted on injecting ClO_2 gas at a higher concentration without air dilution gas to build up a concentration beyond the target level by about 30 to 40%. In the second step, pure air flowed through MFC1 diluting the ClO_2 until the target concentration was achieved. At the end of this two-step process, the concentration which is monitored by UV-vis spectroscopy over time, reaches equilibrium.

During the first step, both MFC2 and MFC3 are fully open while MFC1 is closed. Table 2 shows a summary of the settings of the second step with its respective mass flow controllers flow rate, and set points of the ClO_2 generator for each treatment. Treatment 0 (Purge) was used every time between treatments to clean the tubes, chamber and spectrophotometer's cuvette.

Table 2. Mass Flow controllers set points and ClO_2 generator concentration for all treatments

Treatment	Concentration [mg/L]	Injection Time[a] [s]	ClO_2 Generator[b] [mg/L]	Duration time[c] [min]	MFC1 [mL/min]	MFC2 [mL/min]	MFC3 [mL/min]
0	Purge	-	-	-	1,000	0	500
1	0.22	12	4.5	84	9,000	500	500
2	0.63	15	12.6	7	9,500	500	500
3	0.41	60	4	17	9,000	1,000	500
4	5	170	20	7	1,500	500	500

[a] Time duration of first step filling process described in section 2.1.1

[b] Concentration at which ClO_2 generator was manually set.

[c] Duration of experiment

3.2.3. Part II: Exposure chamber

An approximately 11 L custom built glass chamber (Dow Chemical, Midland, MI) of circular base with a diameter of 0.30m (12 in) and height of 0.15 m (6 in) was equipped with four inlet/outlet ports, a glass lid and a PTFE O-ring; a metallic clamp made to order by Clampco (Wadsworth, OH) provided a preferred means of sealing; a stainless steel platform and a set of stainless steel three-legged stands were custom made by the mechanical shop at the Department of Physics & Astronomy, MSU. Both the stainless steel platform and stands were designed to hold the fresh produce and facilitate the circulation of the gas around them ensuring that the whole surface of the fresh produce was exposed to the ClO_2 gas (Figures 7 and 8). Either device could be used depending on the shape and size of the produce.

FIGURE 7. Picture of stainless steel platform holding strawberries inside the glass chamber.

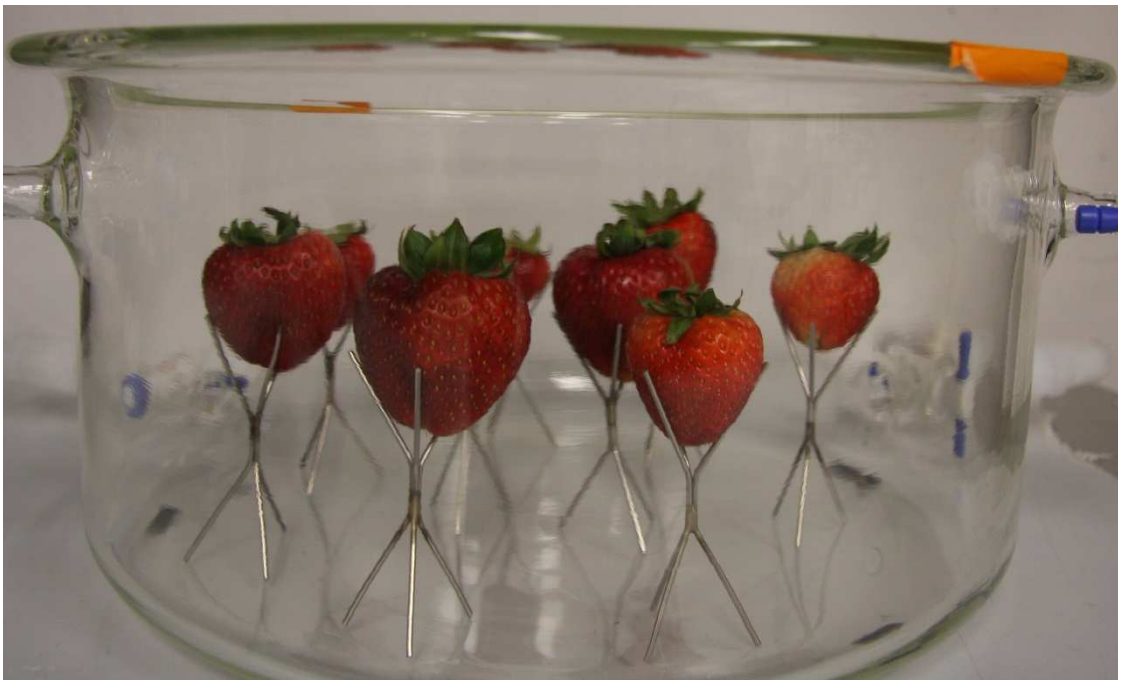

FIGURE 8. Picture of three legged stands holding strawberries inside the glass chamber.

To determine if a high flow rate of 500 mL/min had detrimental effects on produce such as excessive dehydration that may confound results from ClO_2 exposure, a small set of experiments were conducted to determine moisture loss. The tests were run including exposing fresh produce to three different conditions: in presence of static air, using air only at 500 mL/min flow rate for a duration of 1000 min, and using ClO_2 at 500 mL/min flow rate and a concentration of 0.01 mg/L for the same duration to determine if this maximum flow rate of MFC3 would not dry and damage the strawberries, which was the most sensitive of the tested produce. The introduction of ClO_2 gas at 500 mL/min did not statistically affect the strawberry (less than 3% total moisture loss in 1000 min). Also it was necessary to map the gas flow in the interior of the chamber because some strawberries could be dried faster in front of the gas port. Therefore, for the applications outlined in this research (*e.g.*, California strawberries), a

flow rate of 500mL/min was appropriated as long as the fruit was placed at least one inch away from the entrance port regardless of the support used (platform or three legged stands).

3.2.4. Part III: Chlorine Dioxide's detection system

The detection system consists of a Shimadzu UV 1800 spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD) and a 100 mm cylindrical quartz flow cell with two windows (Part # 34-Q-100, Starna Cells, Atascadero, CA). As the mixed gas exits the chamber, it is directed into the spectrophotometer for detection of ClO_2 where the head space concentration is quantified and recorded as a function of time.

Length of pipes and tubes were kept at short as possible to minimize ClO_2 interaction with system components and always covered with 1.27 cm (½ in) thick black foam to reduce the effect of light that may cause decay of the gas during the experiments. The glass chamber was also covered with a thick black cloth on every trial.

3.3. Gas exposure to fresh produce

The system was set to provide two different ClO_2 gas exposure profiles. In Route 1, a constant ClO_2 concentration was flowed to the chamber throughout the treatment after an initial high concentration of ClO_2 injected to reach the target concentration. In this case ports A and B of the three-way valve were open such that the system was open to a well-ventilated atmosphere after the gas flowed through the spectrophotometer. For Route 2, the system was designed to achieve a target ClO_2

concentration inside the chamber and then stop ClO_2 production while recirulating the existing gas inside the chamber. This was accomplished by closing valve B and opening valve C of the three-way valve, creating a closed-loop by switching the three-way to flow from the spectrophotometer to the peristaltic pump and back into the chamber through valve 3.

Route 1 simulates a disinfection process where a constant concentration is steadily applied on fresh produce for a fixed period of time while Route 2 simulates the kinetics inside a package that has been flushed with a known/target quantity of ClO_2 gas, which is depleted over time as it interacts with the fresh produce and other elements of the packaging environment.

3.4. Wavelength selection for ClO_2 detection with Uv-vis spectrophotometer

Primarily, to ensure an accurate determination of the concentration inside the chamber, it was critical to select the appropriate wavelength at which ClO_2 would follow a linear relationship of the Beer-Lambert law, described in Eq. (5), along the range of desired concentrations,

$$A = \varepsilon bc \quad (5)$$

where A is absorbance (O.D), ε is the molar absorptivity (M-1 cm-1), b is the path length (cm), and c is concentration (mol/L).

Spectra of ClO_2 were obtained by scanning known concentrations of ClO_2 at 0, 1.0, 2.5, 5.0, 7.5 and 10 mg/L with the UV-vis spectrophotometer over a wavelength range from 500 to 300 nm. Calibration curves were built at lambda maxima (λmax)

found from the spectra and at three additional wavelengths selected to compare the experimental values of ClO_2 absorption cross section (σ) and molar absorptivity (ε) with values found in the literature and ultimately verify how accurate the Minidox-M ClO_2 generation was.

3.5. Method for quantification of ClO_2 consumption by fresh produce.

For each treatment listed in Table 2, curves of the concentration of ClO_2 gas in the empty chamber (control) as function of time were recorded and then compared with the kinetic curves of the gas when the chamber contains fresh produce. The treatments in the list were randomly chosen but ensuring combinations of low concentrations with long exposure times, and high concentrations with short exposure times since it was expected that ClO_2 consumption by fresh produce exposed to a short treatment with a low ClO_2 concentration would be minimum, while exposure to a long treatment with a high ClO_2 concentration would probably damage the produce.

In order to quantify the total ClO_2 consumption by the fresh produce, the area under each curve was determined by numerical integration and the percent differences between the paired experiments (control vs. full) were multiplied by the total amount of ClO_2 injected into the chamber using Eq.(6):

$$Total\ Cl0_2\ Absorption\ [mg] = \left(\frac{A_e - A_f}{A_f}\right) * c * t * fr \quad (6)$$

A_e is the numerical integral under the curve when the chamber is empty (control), A_f is the numerical integral under the curve when the chamber contains produce, c is the ClO_2 concentration of exposure (mg/L), t is the time of exposure (min) and f_r is the flow rate at which the gas enters the chamber (set at 500 mL/min).

Under the same conditions of temperature, hardware, exposure time and ClO_2 concentration, the percentage difference between the two curves was attributed to the ClO_2 consumption by the fresh produce and not to degradation, oxidation or other reactions as these phenomena were accounted for with the control.

3.6. Application of the new system to quantify ClO_2 consumption by fresh produce

A set of experiments were designed to evaluate and validate the new system by quantifying total ClO_2 consumption when the fresh produce was exposed to different concentrations and exposure times using Route 1 and 2 settings.

3.6.1. Impact of concentration and exposure on chlorine dioxide consumption using Route 1

With the system set for Route 1, the new method to quantify consumption described in section 2.4 was applied using fresh California strawberries previously inspected for bruises, physical damage, mold, firmness and overall appearance. Strawberries were exposed to the different treatments shown in Table 2. Each treatment was repeated 4 times and 18 strawberries were used in each replication. Recorded

curves of concentration over time, for both the empty and the full chamber, were used to calculate ClO_2's consumption by the strawberries.

3.6.2. ClO_2 consumption by fresh produce at different concentrations using Route 2

As explained before, Route 2 simulates the behavior of ClO_2 gas injection inside a package, which depletes over time as it interacts with the interior environment of the package, including produce. With the system set to Route 2, three different concentrations: 1, 3 and 5 mg/L were generated both in the presence and in the absence of the selected California strawberries. The gas was left to recirculate until the concentration detected was negligible, and the curves were compared. Then, first order exponential decay reaction kinetics for ClO_2 consumption of the form $A=A_oe^{(-kt)}$ were fitted and are proposed. These equations were corrected for losses in the system.

1.1. Statistical Analysis

All data were analyzed using SAS 9.3 (SAS Institute Inc., Cary, NC). Analyses of variance were performed using ANOVA. Tukey's HSD test was used to determine differences ($p \leq 0.05$) among data groups.

3.7. Results and Discussion

3.7.1. Wavelenght selection for ClO_2 detection with Uv-vis spectrophotometer

Figure 9 shows a spectra of ClO_2 for concentrations 1, 2.5, 5, 7.5 and 10 mg/L at 22 ℃ scanned at a wavelength range from 500 to 300 nm. Maximum ClO_2 UV light

absorption (λ_{max}) occured at approximately 360 nm for all concentrations, which is in agreement with literature data (Wahner, Tyndall et al. 1987). However, at λ_{max} absorbance values overpass 1.5 a.u. for concentrations 5 mg/L and above suggesting that using this peak for calculation of molar absorptivity could end up in errors in the measurement due to very high light absorption by the analyte (low transmittance).

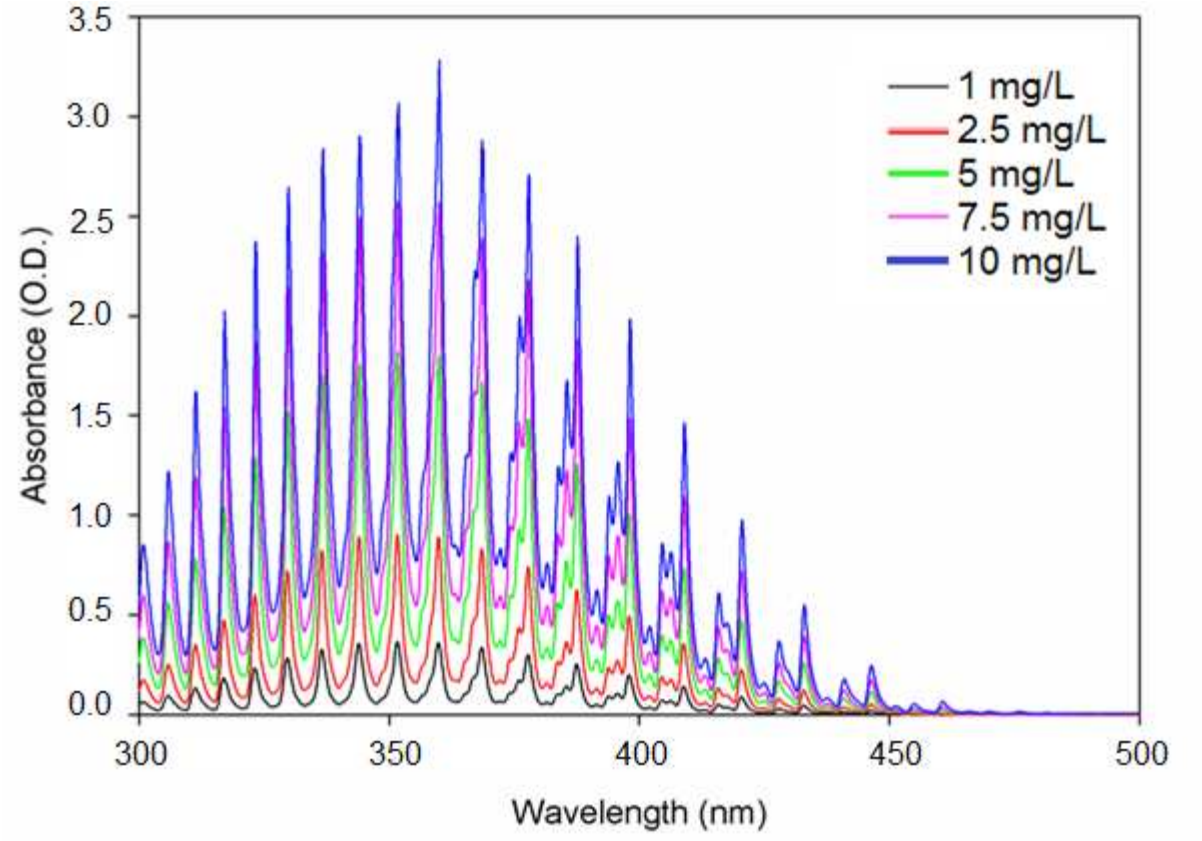

FIGURE 9. Spectra of chlorine dioxide at 1, 2.5, 5, 7.5 and 10 mg/L in a wavelength range from 500 to 300 nm at 22℃

Figure 9 also shows that lower wavelengths (range from 350 to 300 nm) provide lower absorbance values across the different concentrations. For that reason, a plot of absorbance versus concentration in units mol/L was constructed not only at λ_{max} (360 ± 0.5 nm), but also at 305 ± 0.5, 311 ± 0.5 and 351 ± 0.5 nm (Figure 10). As expected, the

linearity of the Beer's Law was compromised at λ_{max} and at 351nm, while scanning ClO_2 at 305 and 311 nm provided a good linear fit for the entire range of concentrations (r^2 0.998), and their respective linear slopes were 8139.7 and 11004.58 M^{-1} respectively.

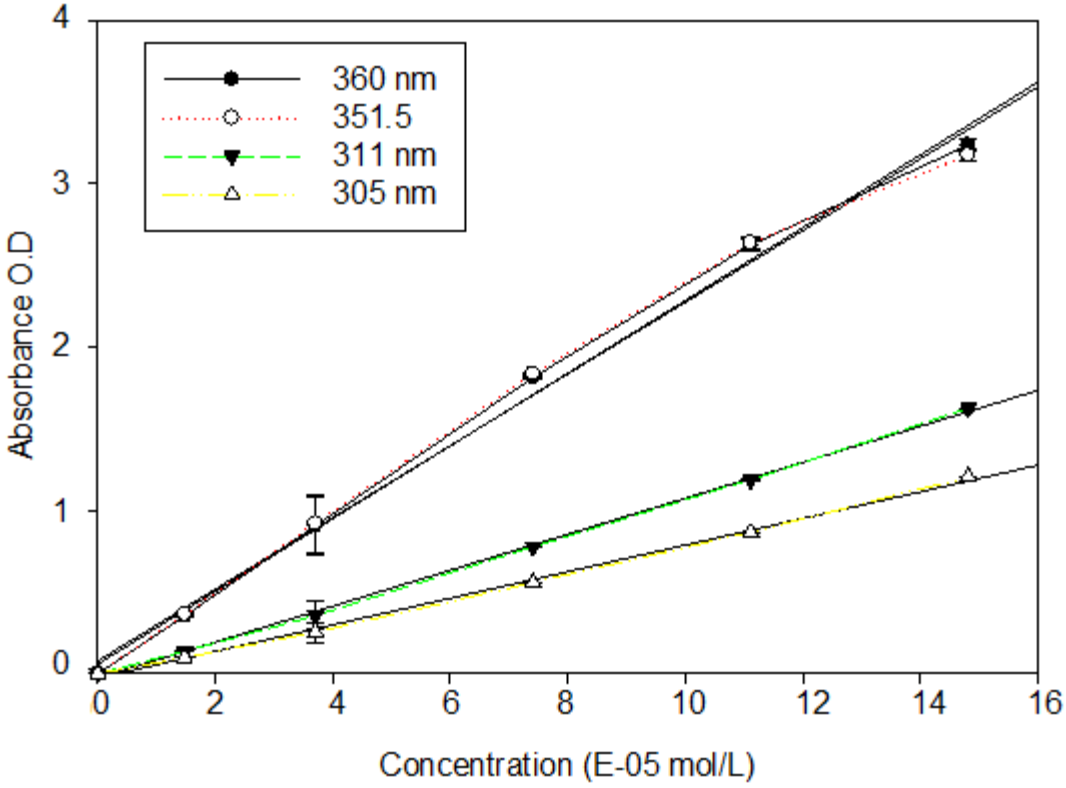

FIGURE 10. Absorbance (O.D) versus Concentration (mol/L) at 351±0.5, 360±0.5, 311±0.5 and 305±0.5 nm.

These values were used to calculate ClO_2 absorption cross section at 22 ^{0}C and the results are presented in Table 3 along with reference values reported by Wahner et al. 1987 (Wahner, Tyndall et al. 1987; Kromminga, Orphal et al. 2003) where it can be observed that at both 311 and 305 nm, the percent difference was less than 4 and 0.5% respectively, indicating a good agreement. For these reasons, the peak at 305 nm was

used to scan ClO_2 throughout the experiments. Additional experiments were carried out to determine the lower detection limit for ClO_2 gas at this wavelength. Concentrations below 0.01mg/L were at noise level.

Table 3. Chlorine dioxide's absorption cross section (σ) at 4 different wavelengths found experimentally and compared with reference values.

Wavelength [nm]	Reference [1] $\sigma x10^{-17}$ [cm^2]	Measured $\sigma \times 10^{-17}$ [cm^2]	Percent Difference, %
~360 (λ_{max})	1.219	0.850	-30.23
~351	1.275	0.839	-34.23
~311	0.435	0.420	-3.36
~305	0.312	0.311	-0.34

(Wahner, Tyndall et al. 1987)

3.8. Applications of the new system to quantify ClO_2 consumption by fresh produce

3.8.1. Impact of concentration and exposure time on chlorine dioxide consumption using Route 1

Figure 11 shows an example of a plot of ClO_2 headspace concentration versus time for treatment 0.22 mg/L for 84 minutes. The blue solid lines represent the 4 replicates of the ClO_2 concentration in the head space of the chamber while it was filled with 18 strawberries, and the red dash line represents the concentration of ClO_2 when the chamber was empty (control). The control curve indicates that the concentration peaks to maximum levels at the beginning of the treatment (step 1 described in section 2.1.1), and then few seconds later equilibrated (step 2 described in section 2.1.1). The

concentration equilibrates to the reach target concentration before 1.5 min except treatment 4, which takes 3 min. The curves describing ClO_2 concentration of the headspace while the chamber was filled with fruit (blue curves) also showed a maximum level at the beginning, sometimes peaking even higher than the control curves (empty chamber) due to a higher apparent concentration resulting from the lower volume inside the chamber taken up by the fresh produce itself. The final equilibrium was slowly reached due to the constant interaction between the ClO_2 and the strawberries. Figure 11 also shows that with the exception of the initial system fill of ClO_2, the concentration in the headspace was lower when strawberries were present, which is consistent with the consumption of ClO_2 gas by organic matter.

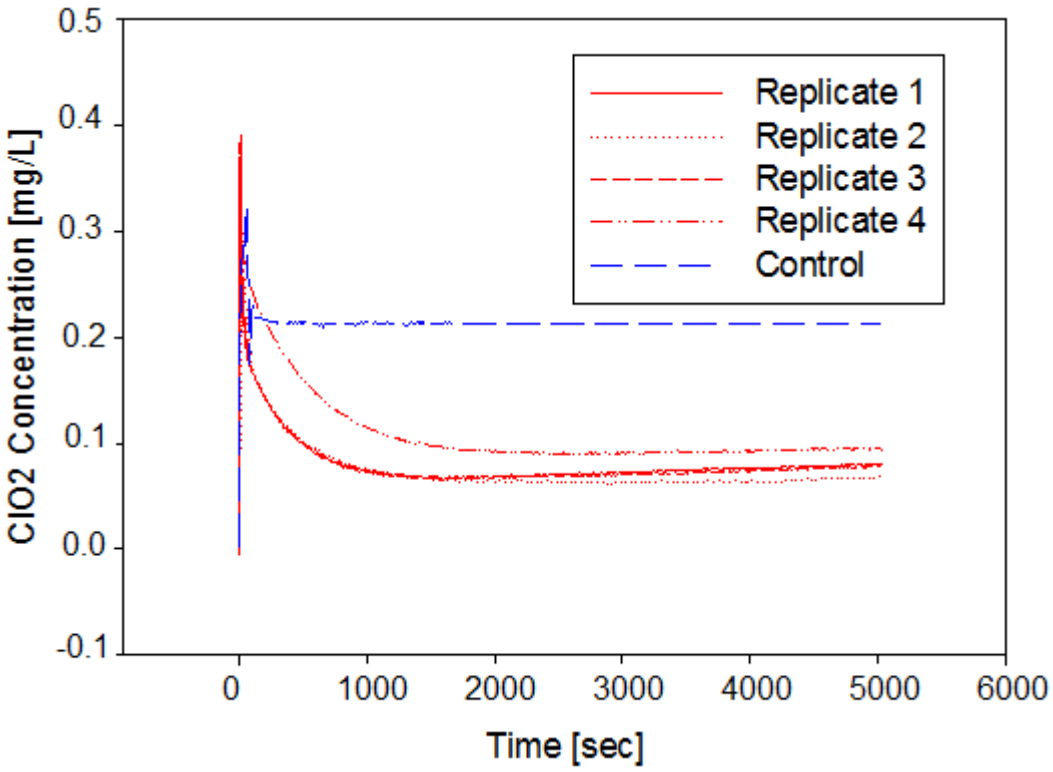

FIGURE 11. Absorbance versus time for treatment 1: concentration 0.22mg/L and 84 minutes of exposure

Table 4 shows that the average total ClO_2 absorbed by strawberries is significantly different among treatments. A maximum absolute ClO_2 consumption occurs in treatment 1 (0.22 mg/L for 84 min), the treatment with lowest concentration and highest exposure time. Treatment 4, having the highest concentration and lower time, reaches almost 62% of what was absorbed on treatment 1 but in just a fraction of the time. This implies that both factors (concentration and exposure time) contribute to ClO_2 consumption by fresh produce.

Table 4. Quantification of total chlorine dioxide absorbed by strawberries on each treatment

Treatment	Concentration [mg/L]	Exposure time [min]	Total ClO_2 generated [mg]	Average total ClO_2 absorbed [mg]	Average ClO_2 absorbed [$x10^{-3}$ $mgClO_2$/kg fruit]	Percent ClO_2 absorbed[a]
1	0.22	84	9.24	4.18±1.15 (a)	11.30 ±2.69E-06	45.2 (a)
2	0.41	17	3.49	1.56±0.09 (b)	4.54 ±5.42E-07	44.7(a)
3	0.63	7	2.21	0.41±0.13 (c)	1.18 ±4.74E-07	18.8 (b)
4	5	7	17.50	2.61±0.29 (d)	7.67 ±8.98E-07	14.9 (b)

[a] average total ClO_2 absorbed divided by total ClO_2 generated.

Note. Data of average total and absorbed ClO_2 are given as mean ± standard deviation. Different letters indicate significant differences among data in the column ($P<0.05$ adjusted - Tukey test).

Table 4 also shows that treatments 1 and 2 the longer experiments absorbed around 45% of the injected ClO_2 gas, and it was statistically different from treatments 3 and 4 which are the shorter treatments with less than 20% of the injected gas absorbed. This effect is explained by the kinetics of achieving absorption equilibrium, since treatments 3 and 4 are suspended before reaching this state, while treatments 1 and 2 are maintained at this steady state for most of the treatment.

3.8.2. ClO_2 dioxide consumption by fresh produce using Route 2 at different concentrations.

The decay of ClO_2 inside the chamber when the gas circulated both in the presence and absence (control) of the strawberries was recorded. For the control, ClO_2 decayed until the concentration was zero or almost zero after approximately 24, 33 and 10 h for the concentrations 5, 3 and 1 mg/L, respectively. This decay could be due to baseline system consumption or leaks. In the presence of strawberries, the ClO_2 was absorbed after approximately 11, 4 and 1 h after the chamber was supplied with the gas to reach the target concentrations of 5, 3 and 1 mg/L, respectively. The difference was attributed to the consumption of ClO_2 by the strawberries. This implies a very fast ClO_2 consumption kinetics in strawberry fruit. Figure 12 shows the fitted curves for the decay of the gas both in presence of strawberries and the control for the concentration 3 mg/L.

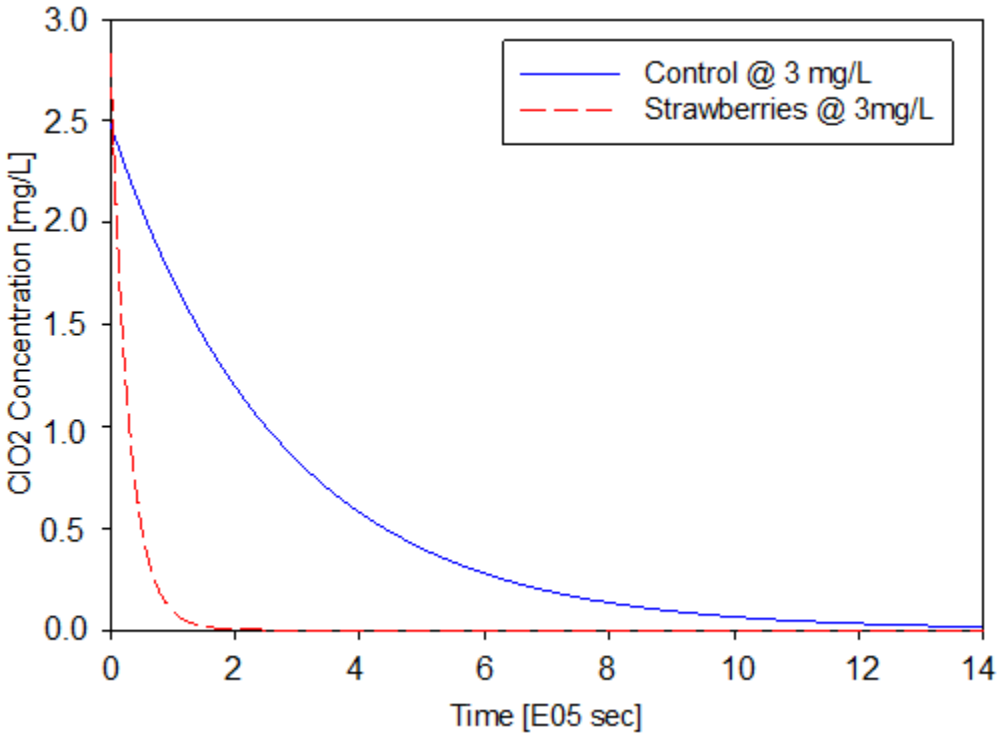

FIGURE 12. Chlorine dioxide concentration versus time for control and strawberries after supplying the chamber with 3 mg/L concentration using Route 2.

Table 5 shows the estimated parameters for the first order reaction equations fitted for ClO_2 consumption in the empty and full chamber. In this first order reaction equation with everything else being equal, smaller value of *k* yield higher absorbance values which in turn means that there is greater availability of ClO_2 in the headspace, hence smaller consumption of the gas. For concentrations of 3 and 5 mg/L, the reaction rate were similar estimated at 1.14E-04 and 3.06E-04, while for the controls, the estimate of the rate reaction parameter for the three curves (concentrations 5, 3 and 1 mg/L) was significantly smaller at 3.81E-05 ± 3.31E-06.The order of magnitude difference between the reaction rates for the control and the treatments are again explained by the consumption of ClO_2 by the strawberries

Table 5. First order reaction parameters of chlorine dioxide absorption using Route 2

Parameters	Concentrations, mg/L 5	3	1	0, Control[a]
A_o	4.14 ± 0.04	2.82 ± 0.01	0.83 ± 0.01	2.4 ± 1.62
$kx10^{-4}$	1.14 ± 2.21x10^{-6}	3.06 ± 1.14 x10^{-6}	9.78 ± 8.50 x10^{-6}	0.38 ± 3.31 x10^{-6}
R^2	0.9364	0.9978	0.9974	0.995

[a]Average and standard deviation calculated for control across concentrations

3.9. Conclusions

A novel method was developed to quantify in situ consumption of ClO_2 using an UV-visible spectroscopy equipment. The equipment could be used for a variety of applications, and two examples were described. This unique online system configuration is accurate, easy to use and fast in determining ClO_2 consumption by fresh produce. In the future it could be correlated with other valid quantification methods such as amperometric titration to establish a calibration curve to compare results in both systems. Other potential applications include the determination if surface area or mass

BIBLIOGRAPHY

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Aieta, E.M., Roberts, P.V., Hernandez, M., (1984). Determination of Chlorine Dioxide, Chlorine, Chlorite, and Chlorate in Water. J. Am. Water Works Assoc. 76, 64-70.

Akin, E.W., Hoff, J.C., Lippy, E.C., (1982). Waterborne outbreak control: which disinfectant? Environmental Health Perspectives 46, 7-12.

Allende, A., Tomás-Barberán, F.A., Gil, M.I., (2006). Minimal processing for healthy traditional foods. Trends in Food Science & Technology 17(9), 513-519.

Bhagat, A.R., (2010). Modeling Critical Factors to Optimize the Treatment of Selected Fruits and Vegetables with Chlorine Dioxide Gas Using a Miniaturized Insustrial-Size Tunnel System. Purdue University, West Lafayette, Indiana.

Couri, D., Abdel-Rahman, M.S., Bull, R.J., (1982). Toxicological Effects of Chlorine Dioxide, Chlorite and Chlorate. Environmental Health Perspectives 46, 13-17.

Czarneski, M.A., Lorcheim, P., (2005). Isolator decontamination using chlorine dioxide gas. Pharmaceutical technology, 124-133.

Du, J., Han, Y., Linton, R.H., (2002). Inactivation by chlorine dioxide gas (ClO2) of Listeria monocytogenes spotted onto different apple surfaces. Food Microbiology 19(5), 481-490.

Du, J., Han, Y., Linton, R.H., (2003). Efficacy of chlorine dioxide gas in reducing Escherichia coli O157:H7 on apple surfaces. Food Microbiology 20(5), 583-591.

Gómez-López, V.M., Rajkovic, A., Ragaert, P., Smigic, N., Devlieghere, F., (2009). Chlorine dioxide for minimally processed produce preservation: a review. Trends in Food Science & Technology 20(1), 17-26.

Han, Y., Floros, J.D., Linton, R.H., Nielsen, S.S., Nelson, E., (2001a). Response Surface Modeling for the Inactivation of Escherichia coli O157:H7 on Green Peppers (Capsicum annuum L.) by Chlorine Dioxide Gas Treatments. Journal of Food Protection 64, 1128-1133.

Han, Y., Guentert, A.M., Smith, R.S., Linton, R.H., Nelson, P.E., (1999). Efficacy of chlorine dioxide gas as a sanitizer for tanks used for aseptic juice storage. Food Microbiology 16(1), 53-61.

Han, Y., Linton, R.H., Nielsen, S.S., Nelson, P.E., (2001b). Reduction of Listeria monocytogenes on Green Peppers (Capsicum annuum L.) by Gaseous and Aqueous Chlorine Dioxide and Water Washing and Its Growth at 7C. Journal of Food Protection 64, 1730-1738.

Han, Y., Selby, T.L., Schultze, K.K., Nelson, P.E., Linton, R.H., (2004). Decontamination of Strawberries Using Batch and Continuous Chlorine Dioxide Gas Treatments. Journal of Food Protection 67, 2450-2455.

Hautman, D.P., Munch, D.J., Pfaff, J.D., (1997). US EPA Method 300.1, Determination of Inorganic Anions in Drinking Water by Ion Chromatography, EPA/600/R-98/118, NIST PB-98-169196INZ. (Revision 1.0).

Kaczur, J.J., Cawlfield, D.W., (2000). Chlorine Oxygen Acids and Salts, Chlorous Acid, Chlorites, and Chlorine Dioxide, in: John Wiley and Son (Ed.), Kirk-Othmer Encyclopedia of Chemical Technology, 5th Edition ed. John Wiley & Sons, Inc., New York. NY, pp. 1-26.

Keskinen, L.A., Burke, A., Annous, B.A., (2009). Efficacy of chlorine, acidic electrolyzed water and aqueous chlorine dioxide solutions to decontaminate Escherichia coli O157:H7 from lettuce leaves. International journal of food microbiology 132(2-3), 134-140.

Kim, J., Marshall, M.R., Du, W., Otwell, W.S., Wei, C., (1999). Determination of Chlorate and Chlorite and Mutagenicity of Seafood Treated with Aqueous Chlorine Dioxide. Journal of Agricultural and Food Chemistry 47(9), 3586-3591.

Kromminga, H., Orphal, J., Spietz, P., Voigt, S., Burrows, J.P., (2003). New measurements of OClO absorption cross-sections in the 325–435 nm region and their temperature dependence between 213 and 293 K. Journal of Photochemistry and Photobiology A: Chemistry 157(2–3), 149-160.

Lee, S.-Y., Costello, M., Kang, D.-H., (2004). Efficacy of Chlorine Dioxide Gas as a Sanitizer of Lettuce Leaves. Journal of Food Protection 67, 1371-1376.

Mahmoud, B.S.M., Bhagat, A.R., Linton, R.H., (2007). Inactivation kinetics of inoculated Escherichia coli O157:H7, Listeria monocytogenes and Salmonella enterica on strawberries by chlorine dioxide gas. Food Microbiology 24(7–8), 736-744.

Netramai, S., (2011). Utilization of chlorine dioxide gas in food packaging application. Michigan State University, East Lansing, Michigan.

Nieuwenhuijsen, M.J., Toledano, M.B., Elliott, P., (2000). Uptake of chlorination disinfection by-products; a review and a discussion of its implications for exposure assessment in epidemiological studies. Journal of exposure analysis and environmental epidemiology 10(6 Pt 1), 586-599.

Sy, K.V., McWatters, K.H., Beuchat, L.R., (2005). Efficacy of Gaseous Chlorine Dioxide as a Sanitizer for Killing Salmonella, Yeasts, and Molds on Blueberries, Strawberries, and Raspberries. Journal of Food Protection 68, 1165-1175.

Trinetta, V., Vaidya, N., Linton, R., Morgan, M., (2011). Evaluation of Chlorine Dioxide Gas Residues on Selected Food Produce. Journal of Food Science 76(1), T11-T15.

Tsai, L.S., Huxsoll, C.C., Robertson, G., (2001). Prevention of Potato Spoilage During Storage by Chlorine Dioxide. Journal of Food Science 66(3), 472-477.

United States Environmental Protection Agency (EPA), (1999). Alternative Disinfectants and Oxidants Guidance Manual: Chapter 4. Chlorine Dioxide, EPA 815-R-99-014.

United States Environmental Protection Agency (EPA), (2006). Registration eligibility decision for Chlorine Dioxide and Sodium Chlorite (Case Number 4023), http://www.epa.gov/oppsrrd1/REDs/chlorine_dioxide_red.pdf.

United States Food and Drug Administration (FDA), (2004). Produce Safety From Production to Consumption: 2004 Action Plan to Minimize Foodborne Illness Associated with Fresh Produce Consumption.

United States Food and Drug Administration (FDA), (2012). Secondary direct food additives permitted in food for human consumption. Chlorine dioxide. CFR Title 21, part 173.300.

Wahner, A., Tyndall, G.S., Ravishankara, A.R., (1987). Absorption cross sections for symmetric chlorine dioxide as a function of temperature in the wavelength range 240-480nm. The Journal of Physical Chemistry 91(11), 2734-2738.

4. EVALUATION OF CHLORINE DIOXIDE AS AN ANTIMICROBIAL AGAINST *BOTRYTIS CINEREA* IN CALIFORNIA STRAWBERRIES

4.1. Introduction

Food spoilage is both a sustainability and a commercial issue since as a result, visible mold and undesirable odors lead to consumer rejection, which in turn causes significant economic losses and food waste. According to Kantor et al. (1997), the annual edible food available to the U.S is reduced by about 27% due to food spoilage and waste at the retail and consumer level (Kantor, Lipton et al. 1997). This percentage is especially significant in the context of a life cycle assessment (LCA) study for food systems where input factors, including energy, water irrigation, packaging and transportation, are considered (Heller and Keoleian 2000; Peano, Girgenti et al. 2012).This is such a relevant issue that organizations like the EPA and the USDA have built their efforts to promote their initiatives “U.S. Food Waste Challenge” and the “EPA's Food Recovery Challenge” where their objectives are to both raise awareness about food waste management and to help entities of the U.S food chain to meet their food-waste goals respectively. Within the food production chain, fruits and vegetables may be contaminated at different stages since they are exposed to multiple sources of bacteria, parasites and viruses such as insects, irrigation water or rain, manure based fertilizers, manual handling by workers during the harvest and packaging process, food processing facilities and transportation among others (Wei, Huang et al. 1995; Yuk, Bartz et al. 2006). All these factors directly influence their mode of failure and the time to reach the end of shelflife. Specifically, mold spoilage of food results from a biological process that begins with contamination by fungal spores which germinate and extend

into hyphae to form a visible mycelium over time before the end of the product's shelf life (Dantigny, Guilmart et al. 2005). Mold growth is affected by factors such as water activity (a_w), pH and temperature, and is also affected by food constituents like proteins, carbohydrates, lipids and organic acids (Garcia, Ramos et al. 2009; Dagnas and Membre 2013). Optimum conditions for mold growth depends on the type of microorganism, but in general, food spoilage will occur at temperatures above 25℃ and a_w above 0.85 as long as the food system is contaminated with spores and that these spores are able to germinate (Dantigny, Guilmart et al. 2005; Gougouli and Koutsoumanis 2012). Therefore, to improve quality of fruits and vegetables, and to help prolong the shelf life of produce, it is necessary to both understand the behavior of fungal growth and to identify methods that effectively reduce the population of microorganisms. Researchers have proposed models to describe microorganism population growth as a function of time, and to simulate and predict the behavior of different types of fungi growth at different environmental conditions and at different growth stages (germination and hyphae extension) such as the logistic, the Gompertz and the Baranyi model among others (Declerck, D'or et al. 2001; López, Prieto et al. 2004; Dantigny, Bensoussan et al. 2006; Dantigny, Marín et al. 2007).

Understanding how molds behave and what factors influence their growth could, among others, help identify suitable sanitation technologies that would effectively reduce the fungi population that cause food spoilage. Chlorine dioxide (ClO_2) gas for example, has been extensively studied for its proven efficacy as a potent sanitizer and disinfectant capable of reducing populations of pathogenic microorganisms that compromise food safety (Reina, Fleming et al. ; Benarde, Snow et al. 1967; Costilow,

Uebersax et al. 1984; Abdul-Raouf, Beuchat et al. 1993; Han, Linton et al. 2000; Han, Sherman et al. 2000; Du, Han et al. 2002; Netramai 2011), and while less research has focused on studying its effect on spoilage molds, the few results found in the literature are promising and worth exploring. Spotts et al. (1980) investigated the effect of ClO_2 on fungi for the control of d'Anjou pear decay and found that ClO_2 did not affect the germination of *Botrytis cinerea* when concentrations lower than 5 mg/L were used even when held for 10 minutes; in fact ClO_2 was effective only when pears were treated with 10 mg/L concentration for 10 minutes (Spotts and Peters 1980). Roberts et al. (1994) for example studied the percentage spore mortality of four fungal species after in vitro exposure to different ClO_2 concentrations and times and found that *Botrytis cinerea* was one of the most resistant species, sensitive only to high concentrations of 3 µg/mL and 5 µg/mL within 1 minute of exposure (Roberts and Reymond 1994). This information seems to suggest that i) contrary to what has been documented for pathogenic microorganisms, it has been challenging to reduce populations of *Botrytis cinerea* using treatments with low concentrations of ClO_2; and that ii) despite Spotts et al. and Roberts et al. agreement on the concentration of the effective treatment (5 mg/L), the difference in exposure times (1 versus 10 minutes) implies that the biological subject where *Botrytis cinerea* is present has an effect on the efficacy of ClO_2 treatments. Specifically, strawberries are a unique fruit with irregular shape, seed-studded surfaces, and high organic acid content (Kallio, Hakala et al. 2000; Han, Selby et al. 2004) which shelf life is short due to susceptibility to rot-causing pathogens and fast ripening due to high

respiration rates (Kim, Kim et al. 2010). Kaye et al. (2005) found that yeast and mold populations (not specified what type of population) on strawberries were significantly reduced when treated with gaseous ClO_2 at concentrations of 8 mg/L for 120 minutes (Sy, McWatters et al. 2005). More recently, Aday et al. (2010) found that treatments with ClO2 on strawberries are capable of extending the quality of the fruit; and Vardar et al. (2012) found that the incidence of decay of *Botrytis cirenea* on strawberries was significantly reduced by treatments with ClO_2 with little impact on sensorial characteristics (Aday, Buyukcan et al. 2012; Vardar, Ilhan et al. 2012).

However, as a result of ClO_2 treatments, it and its by-products may persist in the fruit (United States Environmental Protection Agency (EPA) 1999) requiring knowledge of the quantities of these toxic materials, particularly in the case of fruits and vegetables because they are often consumed raw. Therefore a holistic approach to assess the feasibility of use of ClO2 as a sanitizing technology to reduce *Botrytis cinerea* on strawberries should focus on its efficacy, impact on quality, and ClO_2 consumption by the strawberries. For these reasons, the main goal of this study was to determine the efficacy of ClO_2 gas in the delay of onset of *Botrytis cinerea*, the most common microorganism responsible for spoilage of strawberries, meanwhile quantifying the concentration range that does not produce visual damage to strawberries. The specific objectives of this work were to (*i*) expose strawberries to different ClO_2 treatments to determine its efficacy while maintaining the color and moisture content of the fruit, (*ii*) to

quantify total ClO_2 absorption by the fruits and (*iii*) to correlate total ClO_2 absorption with concentration and exposure time.

4.2. Materials and Methods

4.2.1. Selection of California strawberries.

California strawberries purchased from a local supermarket store harvested about 1-week prior to experiments and shipped in refrigerated trucks to Michigan. There were differences between strawberry batches in terms of the suppliers and cultivars based on size and shape of the berries. All strawberries were thoroughly inspected. Only fruits similar in size, color and ripeness that were free of bruises and other damage were chosen and were randomly assigned to treatments. Raw strawberries were weighed and conditioned at 22 ± 2^0C for an hour prior to treatments in order to avoid condensation during treatments and to maintain a constant temperature inside the treatment chamber.

4.2.2. Design of experiments.

A set of three completely randomized block designs (RCBD) (hereafter experiment) was conducted in order to determine appropriate ClO_2 sanitizing treatments on California strawberries for delaying the growth of *Botrytis cinerea* without affecting the visual quality of the fruit in terms of bleaching and dryness of the tissue or calyx. Table 6 shows the different treatments in pairs of concentration and time of exposure selected for each experiment. It is important to mention that the order of

treatments was randomized to account for variability as treatments were performed sequentially.

Table 6. Summary of treatments 1, 2, and 3

Experiment 1				
Concentration [mg/L]	Exposure Time [min]	Replicates	No. strawberries per treatment	
0.01	191	1	12	
0.01	1000	1	12	
0.04	21	1	12	
0.08	1000	1	12	
0.22	84	2	12	
0.63	7	1	12	
1.26	332	2	12	
5	37	1	12	
5	7	1	12	
Control		10	12	
Experiment 2				
Concentration [mg/L]	Exposure Time [min]	Replicates	No. strawberries per treatment	
0.04	21	4	18	
0.22	84	4	18	
0.41	17	4	18	
0.63	7	4	18	
5	7	4	18	
Control		20	360	
Experiment 3				
Concentration [mg/L]	Exposure Time [min]	Replicates	No. strawberries per treatment	Incubation temperature [^{0}C]
0.63	7	16	9	22 ± 2
Control		16	9	22 ± 2
0.63	7	16	9	4 ± 2
Control		16	9	4 ± 2

[A] There is a set of control for each replicate of each treatment, therefore there are 4 replicates*5 treatments*18 strawberries per treatment. Note: Incubation temperature for experiments 1 and 2 was 22 ± 2^{0}C. Temperature numbers are reported as mean ± standard deviation during the treatment

The first experiment consisted of a 10-point response surface design covering low and high ClO_2 concentrations for short and long periods of time. Intentionally, combinations of high concentration with long exposure times, and low concentrations with short exposure times were left excluded because it was anticipated that in the first case the quality of the produce would be significantly diminished (*e.g.,* excessive bleaching and dessication), and that in the second case the sanitizing effect of the gas would be minimized.. Mold growth conditions were 22 ± 2^0C and approximately 100% relative humidity.

A second experimental design consisted of four of the conditions from the above design plus an additional center point treatment of 0.41 mg ClO_2/L for 17 minutes. In this case, 4 replicates of each condition with 18 berries per replicate were used to increase sample size and improve power level of the experiment A total of 360 strawberries were treated and 360 untreated berries were used as a control.. Mold growth conditions were 22 ± 2^0C and approximately 100% relative humidity.

A third experimental design, treatment 0.63 mg/L for 84 minutes was replicated 16 times and mold growth conditions were at 23 ℃ a nd 4 ℃ and approximately 100% relative humidity. The additional temperature variable was added in order to both avoid the growth of *rhizopus* in the strawberries and to delay the rate of fungal growth in both treated and control samples in an attempt to better distinguish differences between the treatments and onset of *Botrytis* growth. A total of 280 strawberries were selected and treated in batches of 18 strawberries per replication. Another set of 280 strawberries were selected as the control group.

4.2.3. Incubation tubs.

After each ClO_2 treatment on experiments 1, 2, and 3, the fruits were assessed by incubation tests consisting of arranging the fruit on 0.5-cm mesh screen in aluminum pans (26 x 32 cm) making sure the fruit was equidistantly spaced and separated from one another (see Figure 13). The tubs were filled with approximately 100 mL of water below the screen level, and covered with plastic film to maintain a relative humidity equal or close to 100%.

FIGURE 13. Position and separation of strawberry fruits inside incubation tubs at approximately 100% relative humidity.

This approach is similar to that used by Schilder et al. (2012) in evaluation of fungicide efficacy in fruit rot control in strawberries (*16*). Both mesh screens and

aluminum tubs used were properly sterilized by autoclaving prior to experiments. For all experiments, the incubation tubs were analyzed for fungal growth which was rated daily (see rating of fungal growth section).

4.2.4. Evaluating efficacy of ClO_2 against the growth of *Botrytis cinerea*.

California strawberries for all experiments were rated individually by establishing the percent area of each fruit that was infected on a daily basis. The continuous data collected for experiments 1 and 2 ranged from 0 to 1, where 0 represents no signs of mold and 1 represents 100% of the area covered with the fungus. Due to the size of experiment 3, strawberries were rated every other day using a binary code where 0 represents no sign of mold, and 1 represents at least 10% of area covered with the fungus (the amount of fungal growth that would be visible to a consumer and might lead to rejection of the fruit – an economically important level of infection).. In other words, the data collected in experiments 1 and 2 represents the percent growth of fungus while data collected from experiment 3 represents the probability of a strawberry to be infected. Rating was stopped when the majority of the strawberries reached approximately 90% infection.

4.2.5. Fruit quality assessment after treatment.

Photos were taken before and after treatments in order to identify the location and assess the magnitude of the discoloration or desiccation that could result after exposure with ClO_2. At the same time, pictures were taken daily during rating to record the fungal growth process. A professional Canon EOS 40D camera equipped with a zoom lens was placed and fixed on top of the exposure chamber at a distance of

approximately 70 cm from the platform on which the berries were placed. The glass chamber was fixed to the table with Velcro pieces in order to avoid any movement during exposure. Camera settings, contrast background color, amount of light, amount of flash lights used and distances to the chamber were fixed and unchanged throughout treatments to ensure that any changes in the photos from before and after ClO_2 exposure were only due to the effect of treatments in strawberries.

4.2.6. Continuous ClO_2 gas treatment.

The system for treating strawberries was described by Arango et al. (2013)(Arango, Rubino et al. 2013). Figure 6 from previous chapter shows a schematic diagram of the system developed for (i) the continuous ClO_2 exposure and (ii) the quantification of ClO_2 absorption by fresh produce (e.g., strawberries). In summary, the equipment could be divided into three main components. Component 1 consists of a Minidox-M ClO_2 gas generator from ClorDiSys Solutions Inc (Lebannon, NY) equipped with a 98% nitrogen and 2% chlorine gas tank, an airtube with filter and pressure regulator, and a set of three Aalborg GFC mass flow controllers (Orangeburg, NY). This component continuously generates and dilutes ClO_2 gas in air to reach gas concentrations below 1 mg/L (minimum ClO_2 concentration of the Minidox-M ClO_2 gas generator). The equipment is calibrated so that any ClO_2 gas concentration from 0.01 to 5 mg/L could be produced. Component 2 consists of an 11 liter glass chamber with three inlet/outlet ports, a glass lid, a polytetrafluoroethylene O-ring, a metallic clamp to

provide sealing and closure respectively and a stainless steel platform to hold the produce inside the chamber. The chamber is connected to the mass flow controller 3 (MFC3 from Figure 6) from Component 1 and its main function is to hold the strawberries and the gas during treatments. Component 3 consists of a Shimadzu UV 1800 spectrophotometer (Scientific Instruments, Columbia, MD) and a 100 mm cylindrical quartz flow cell with two windows (Quartz spectrophotometer cell, cylindrical, Starna Cells, Atascadero, CA). This component continuously detects the headspace concentration inside the chamber before and during treatments upon the gas exit from the chamber.

At the beginning of each treatment, the chamber has an air atmosphere part of which was displaced in order for the concentration of ClO_2 to build up. For this reason, a two-step process was designed to speed up the chamber filling. The first step consisted on injecting only ClO_2 gas at a higher concentration than desired until the concentration in the chamber headspace was past set point. In the second step, air started flowing and diluting the ClO_2 gas to achieve the desired concentration. At the end of the two-step process, the concentration entering the chamber and the concentration in the chamber headspace equilibrated to the desired concentration and the UV-vis detector recorded a steady value over time.

4.2.7. Quantification of ClO_2 absorption by fresh produce

Total ClO_2 absorption was calculated by equation (6), as discussed on previous chapter, after recording curves of the headspace concentration over time for both the empty and the full chamber (*e.g.*, with strawberries).

$$\text{Total } ClO_2 \text{ absorption} = \left(\frac{A_e - A_f}{A_e}\right) * c * t * fr \quad (6)$$

where A_f is the area under the curve with chamber containing produce (or full), A_e is the area under the curve with the empty chamber, c is the ClO_2 concentration of exposure (mg/L), t is the time of exposure (min) and fr is the flow rate at which the gas enters the chamber (always set at 500mL/min).

Under the same conditions of temperature, materials used, exposure time and concentration, the percentage difference between the curves was attributed to the ClO_2 absorption by fresh produce and not to degradation, oxidation or other reactions in the system since they were already accounted while running the system with the empty chamber (control).

4.2.8. Statistical Analysis.

To evaluate ClO_2 efficacy, statistical analyses were performed using the repeated measures methodology considering that the same strawberries were subjected to quality assessment as a function of time. With the repeated measures methodology, it was possible to compare all treatments with the control independently at

each day of rating. Simultaneously, a mathematical growth model was used to fit the data: the non-linear Gompertz model as described by equation (1):

$$y = \alpha e^{-e^{-\beta(t-\theta)}} \quad (1)$$

Where y is fungal growth, and α, β, and θ are estimates of parameters asymptote, growth rate, and inflection point simultaneously (Dantigny, Marín et al. 2007; Garcia, Ramos et al. 2009). To assess the effect of exposure time and concentration on ClO_2 absorption, a surface response analysis was conducted using JMP (SAS Institute Inc., Cary, NC) and Matlab (The MathWorks, Inc., Natick, MA). All other statistical analyses were done using SAS 9.3 (SAS Institute Inc., Cary, NC).

4.3. Results and Discussion

4.3.1. Quality Assessment.

Visual inspection of the exposed California strawberries showed that exposure to either prolonged treatments such as 0.22 mg ClO_2/L for 84 min, 0.01 and 0.08 mg ClO_2/L for 1000 min and 1.26 mg ClO_2/L for 332 min, or exposure to high concentration such as 5 mg ClO_2/L for 7 and 37 min discolored strawberries by changing the red pigment of the fruit from red to pale yellow as well as it dried and bleached the calyx evidenced by a texture and color change from fresh green to an ashy yellow color (Figure 14). Furthermore, the degree and area of bleaching increased as the concentration and/or time increased.

FIGURE 14. From left to right quality decay of calyx in strawberries before and after chlorine dioxide treatments at 5 mg/L concentration and 7 minutes of exposure

Figures 15 and 16 show and example of strawberries before and after exposure to treatment 5 mg ClO_2/L for 7 minutes, where the quality of the fruit was moderately affected as i) every single treated strawberry had a lighter overall color, ii) some parts of the tissue were more evidently discolored and damaged, and iii) every single calyx showed evident color changes and dryness after exposure. On the other hand, discoloration of strawberries exposed to treatments 0.63 mg ClO_2/L for 7 minutes, 0.04 mg ClO_2/L for 21 min and 0.01 mg ClO_2/L for 191 min was minimum and almost unnoticeable.

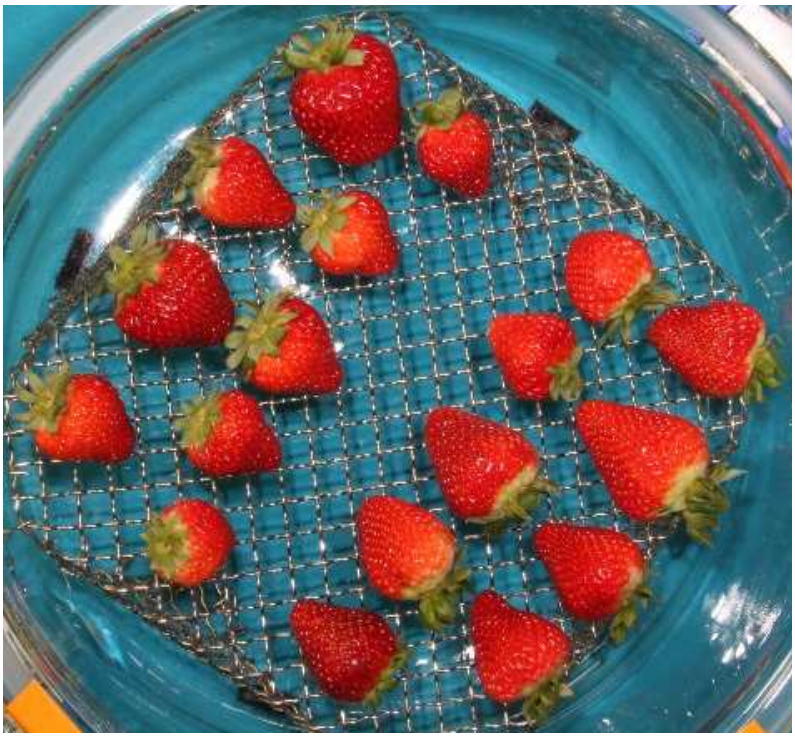

FIGURE 15. Strawberries inside treatment chamber before exposure to chlorine dioxide looking fresh, and with bright tissue color

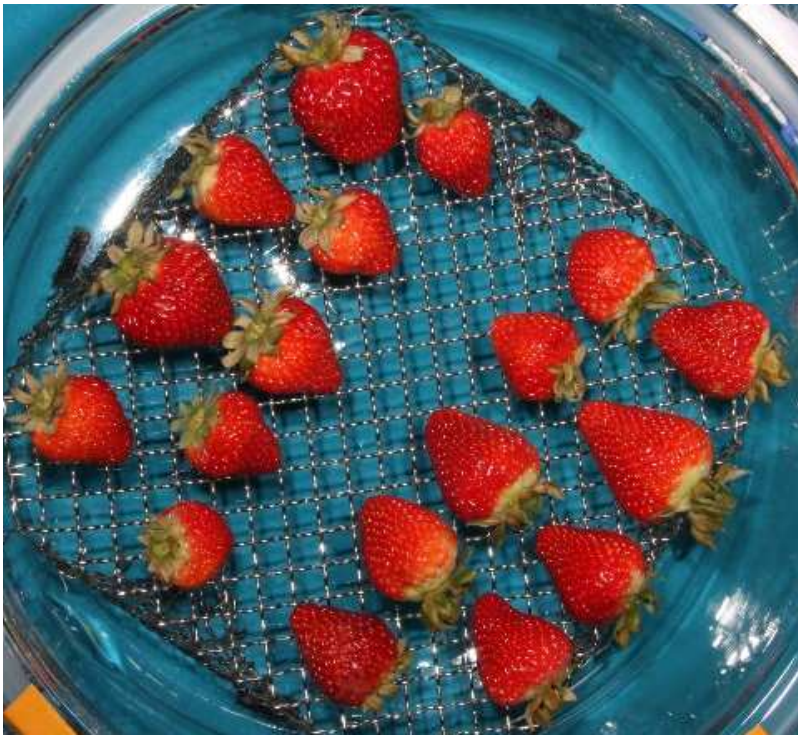

FIGURE 16. Strawberries inside treatment chamber after exposure to 5 mg/L chlorine dioxide gas for 7 minutes looking dull, color changed.

4.3.2. Quantification of ClO_2 absorption by fresh produce.

Total ClO_2 absorption by strawberries was calculated for each treatment using equation 1 and the results are shown in Table 7. Combining this information with the results from the visual evaluation of the fruit, it can be demonstrated that the quality of the fruit after exposure (discoloration or bleaching) is in agreement and positively correlated to ClO_2 absorption by the fruit as presented on Figure 17. The data fits an exponential growth model with 2 parameters and r^2=0.89 shown in equation (7):

$$Q = 100e^{0.11A} \quad (7)$$

Where *Q* is the percentage of exposed strawberries presenting discoloration between 0 and 1, and *A* is the total ClO_2 absorbed by the strawberries in each treatment.

Table 7. Summary of total chlorine dioxide absorption by California strawberries after exposure to the different treatments considered in experiments 1, 2, and 3.

Concentration [mg/L]	Exposure time [min]	ClO_2 absorption [mgClO_2/g fruit]	Percent Strawberries with discoloration [%]
0.01	191	1.83E-03[A]	0
0.01	1000	10.9477[A]	100
0.04	21	0.5483[A]	5
0.08	1000	73.6638[A]	100
0.22	84	10.2231 ± 0.7831	48.5
0.63	7	1.3675 ± 0.1688	5
1.26	332	234.8402 ± 1.254	100
5	7	7.8815 ± 0.2618	65
0.41	17	4.5404 ± 0.1564	11

Note. Data in column 3 is given as mean ± standard error. [A] Treatments not replicated, therefore only means are presented.

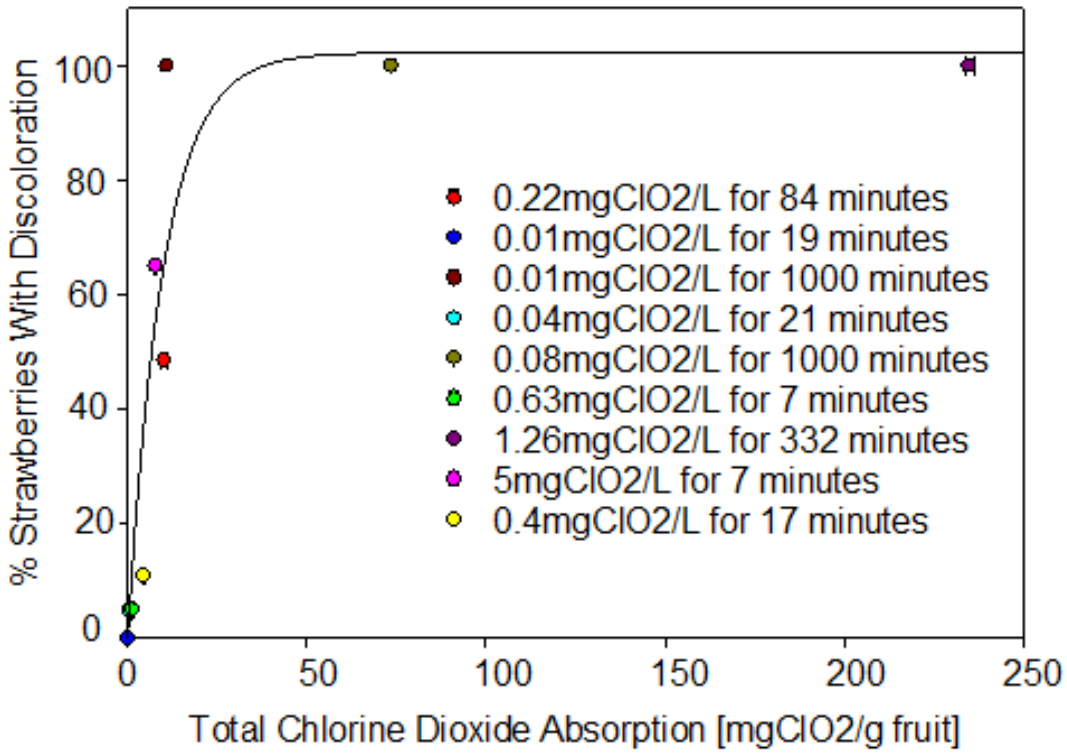

FIGURE 17. Relation between percent of strawberries discolored versus total chlorine dioxide absorption in mgClO_2/g fruit

The steep behavior of the graph shows a fast quality deterioration of the strawberries with small changes in ClO_2 absorption. This fact is of important concern because it leaves a small window for treatments that could potentially be used without affecting the fruit's quality. In fact, according to this set of experiments, significant bleaching fraction of strawberries occur at levels of absorption greater than at least 7.9 mgClO_2/g of fruit, and an acceptable limit for ClO_2 absorption would be at least 4.5 mgClO_2/g of fruit.

A mathematical model that predicts ClO_2 absorption by fresh produce in terms of concentration and exposure time was developed using a response surface method (RSM). For the response variable, the analysis of variance indicated that the model was

significant ($p<0.01$) with significant linear and quadratic effects at a significance level lower than 0.01. The prediction model is described by equation 7 and the respective plot bounded by the limits of concentrations and exposure times considered in the set of experiments is shown in figure 18.

$$Abs_{ClO_2} = 16.62 - 41.27X_1 - 44.24X_2 + 45.34X_1X_2 + 17.32X_1^2 + 26.06X_2^2 \quad (3)$$

Where Abs_{ClO2} is the response variable absorption of ClO_2 in mg ClO_2, X_1 is the logarithm of concentration in mg/L, and X_2 is the logarithm of time in minutes.

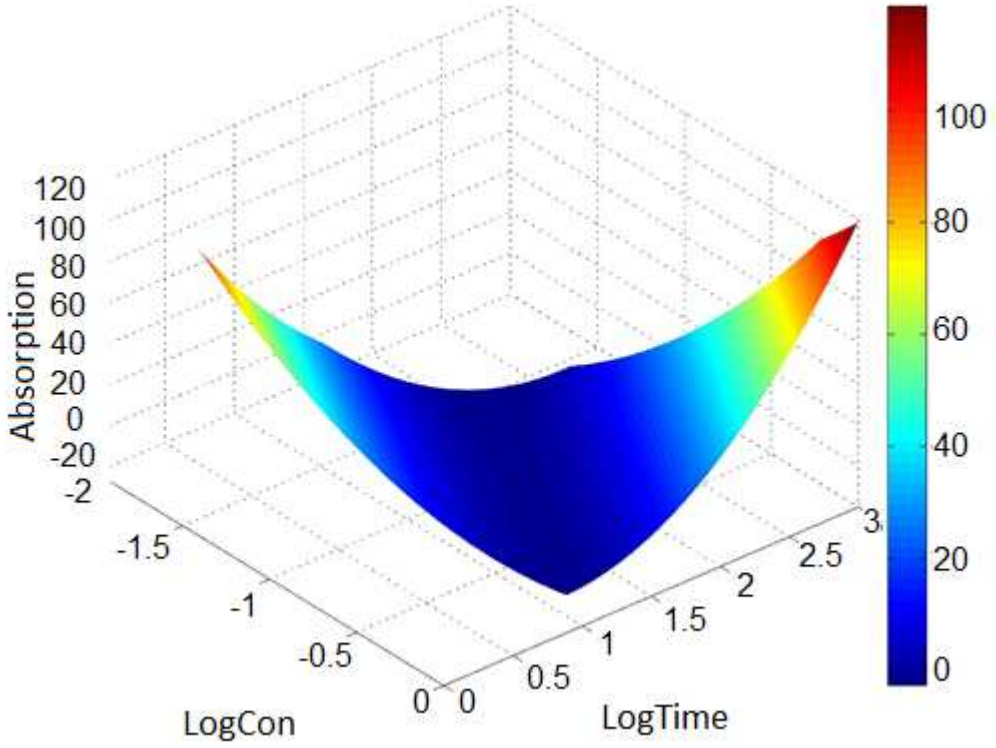

FIGURE 18. Response Surface plot for ClO_2 absorption by fresh produce in terms of concentration and time

4.3.3. Fungal growth.

To simulate commercial conditions and fungal loads present on strawberries in marketing channels as they reach retail stores and consumers, strawberries used in these experiments were treated as they came from the store, and no inoculation, pre-wash or calyx removal was performed. Preliminary experiments had also indicated that inoculation of strawberries with *Botrytis cinerea* made no difference in the amount of mold growth that developed as most strawberries tend to be already infected in the field prior to harvest ((Maas 1995)).

For these reasons, the variability in the degree of infection between strawberries was high. Therefore, there were cases when treated or untreated strawberries would remain intact after days of incubation in the aluminum tubs while other strawberries in the same tray would be completely covered with fungal growth. Furthermore, even though strawberries within experiments were all from the same lot and same producer, strawberries between experiments were not, and so the variation in the rate of decay of strawberries between experiments 1, 2 and 3 was also wide. Evidence of this is that rating in experiment 1 lasted only for 4 days, after which the majority of strawberries were rotted, while experiment 2 was suspended after 7 days of incubation, and rating of strawberries from experiment 3 incubated at 22^0C was suspended after 13 days. Differences in cultivar susceptibility, harvest timing, and fungicide programs can influence the incidence and rate of decay of strawberries by *Botrytis cinerea* ((Maas 1995; Wise, Gut et al. 2012; Schilder, Gillett et al. 2013)

4.3.4. Quality and efficacy assessment of ClO_2.

To model repeated measures over time, the fungal growth data collected from experimental designs 1, 2, and 3 was fitted to a first-order autoregressive model because in this case, observations more adjacent to each other in time might be more highly correlated with each other that those observations farther apart in time within each strawberry. In experiment 1 it was found that both the effect of the treatments ($p<0.0150$) and the interaction of treatment with rating day ($p<0.0217$) was significant. From Table 8 and Figure 19, it can be observed that the efficacy of the ClO_2 gas against *Botrytis cinerea* was proven on treatments 5 mg ClO_2/L for 7 minutes, 0.22 mgClO_2/L for 84 minutes, 1.26 mgClO_2/L for 332 minutes and 0.08 mgClO_2/L for 1000 minutes, all which as of the second day post treatment showed significantly lower fungal growth than the control. Other treatments such as 0.63 mg ClO_2/L for 7 minutes and 5 mg ClO_2/L for 37 minutes showed significant differences from the control but only in some rating days.

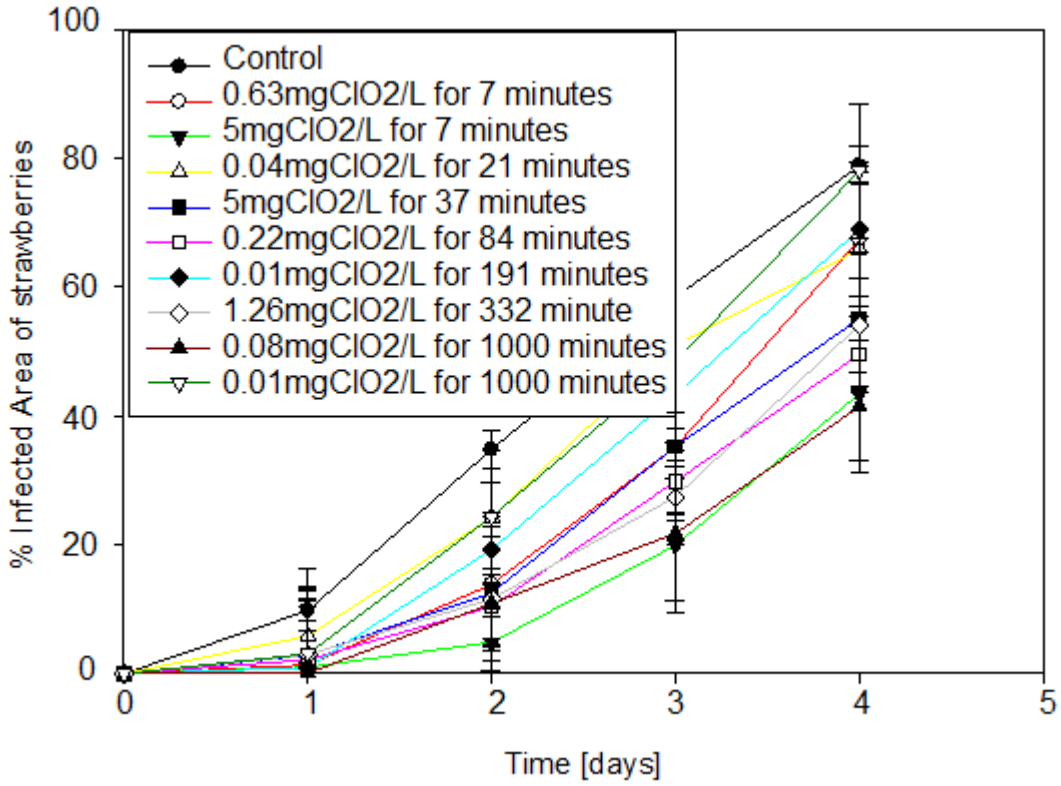

FIGURE 19. Repeated measures model over time for all treatments in experiment 1.

Because the objective of these series of experiments was to find treatments where both efficacy and quality were met, treatments 5 mgClO_2/L for 7 minutes and 0.22 mgClO_2/L for 84 minutes (both with moderate quality damage after exposure), and treatments 0.04 mg ClO_2/L for 21 minutes, and 0.63 mg ClO_2/L for 7 minutes were repeated in experiment 2. Although the last two treatments were not efficacious, they were selected because they did not affect the quality of the strawberries after exposure and replicating them with increased sample size to increase confidence in the efficacy response was warranted (Figure 17). Additionally, as explained in the design of

experiments section, treatment 0.41 mg ClO_2/L for 17 min was selected because it was calculated with equation (7) that it would not damage the fruit after exposure (Q=16.36).

In experiment 2 it was found that the effect of the treatments was significant at $p<0.0585$, while the interaction of treatment with rating day was significant at $p<0.0101$. From Table 9 and Figure 20, it can be observed that treatments 0.63 mg ClO_2/L for 7 minutes, 0.22 mg ClO_2/L for 84 minutes and 5 mgClO_2/L for 7 min are significant after rating day 4 at 95% confidence level. These results showed that both criteria ClO_2 gas efficacy and quality of the strawberries after exposure were met with the treatment 0.63 mgClO_2/L for 7 min, which was then run alone versus the control in experiment 3. Further analysis assuming a power of 0.8 and the observed variation within strawberries to test for significant differences at $p<0.05$, resulted in a required minimum sample size of 144 strawberries per treatment.

Table 8. Repeated measures results for treatments in experiment 1.

	Rating Day			
Treatment	1	2	3	4
Control	9.78 ± 2.99^{A}	34.77 ± 2.99^{A}	58.45 ± 2.99^{A}	78.79 ± 2.99^{A}
0.63 mg/L for 7 minutes	1.33 ± 10.39^{A}	13.833 ± 10.39^{A}	35.17 ± 10.39^{B}	67.33 ± 10.39^{A}
5 mg/L for 7 minutes	1.00 ± 10.39^{A}	4.83 ± 10.39^{B}	19.92 ± 10.39^{B}	43.42 ± 10.39^{B}
0.04 mg/L for 21 minutes	5.83 ± 10.39^{A}	24.25 ± 10.39^{A}	51.00 ± 10.39^{A}	65.83 ± 10.39^{A}
5 mg/L for 37 minutes	2.83 ± 10.39^{A}	12.50 ± 10.39^{A}	35.25 ± 10.39^{B}	55.08 ± 10.39^{B}
0.22 mg/L for 84 minutes	2.17 ± 10.39^{A}	10.39 ± 10.39^{B}	29.83 ± 10.39^{B}	49.50 ± 10.39^{B}
0.01 mg/L for 191 minutes	0.92 ± 10.39^{A}	19.25 ± 10.39^{A}	43.33 ± 10.39^{A}	68.92 ± 10.39^{A}
1.26 mg/L for 332 minutes	2.92 ± 7.35^{A}	11.58 ± 7.35^{B}	27.42 ± 7.35^{B}	54.08 ± 7.35^{B}
0.08 mg/L for 1000 minutes	0.08 ± 10.39^{A}	10.83 ± 10.39^{B}	21.75 ± 10.39^{B}	41.42 ± 10.39^{B}
0.01 mg/L for 1000 minutes	3.08 ± 10.39^{A}	24.33 ± 10.39^{A}	48.50 ± 10.39^{A}	78.00 ± 10.39^{A}

Note. Data in columns are given as mean ± standard error. ^{A,B} Within columns, means followed by the same letter are not significantly different (α=0.05).

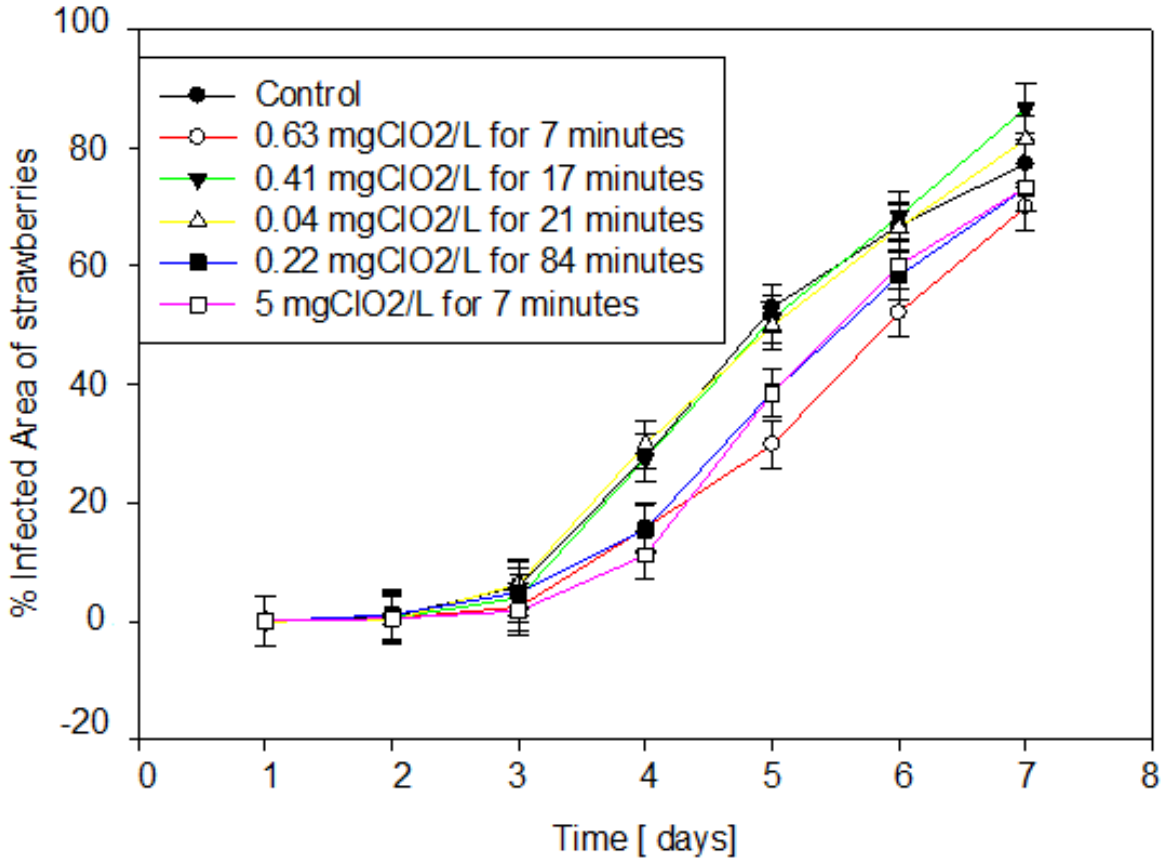

FIGURE 20. Repeated measures model over time for all treatments in experiment 2

Table 9. Repeated measures results for treatments in experiment 2.

Treatment	Rating Day						
	1	2	3	4	5	6	7
Control	-2.84E-14 ± 3.80^{A}	0.76 ± 3.80^{A}	5.90 ± 3.80^{A}	27.71 ± 3.80^{A}	52.99 ± 3.80^{A}	66.82 ± 3.80^{A}	77.28 ± 3.80^{A}
0.63 mg/L for 7 minutes	-2.84E-14 ± 3.80^{A}	0.46 ± 3.80^{A}	2.36 ± 3.80^{A}	15.78 ± 3.80^{B}	29.85 ± 3.80^{B}	52.08 ± 3.80^{B}	64.00 ± 4.09^{B}
0.41 mg/L for 17 minutes	-9.24E-14 ± 3.80^{A}	0.49 ± 3.80^{A}	3.99 ± 3.80^{A}	27.50 ± 3.80^{A}	51.08 ± 3.80^{A}	68.42 ± 3.80^{A}	86.61 ± 3.80^{A}
0.04 mg/L for 21 minutes	-4.26E-14 ± 3.80^{A}	0.15 ± 3.80^{A}	6.28 ± 3.80^{A}	29.93 ± 3.80^{A}	49.89 ± 3.80^{A}	66.49 ± 3.80^{A}	81.39 ± 3.80^{A}
0.22 mg/L for 84 minutes	-5.68E-14 ± 3.80^{A}	1.08 ± 3.80^{A}	4.85 ± 3.80^{A}	15.49 ± 3.80^{B}	38.68 ± 3.80^{B}	58.33 ± 3.80^{A}	73.17 ± 3.80^{C}
5 mg/L for 7 minutes	-1.38E-12 ± 3.80^{A}	0.49 ± 3.80^{A}	1.74 ± 3.80^{A}	11.14 ± 3.80^{B}	38.49 ± 3.80^{B}	60.13 ± 3.80^{A}	73.28 ± 3.80^{C}

Note. Data in columns are given as mean ± standard error. ^{A,B} Within columns, means followed by the same letter are not significantly different (α=0.05).

In experiment 3, 576 strawberries were divided into 4 groups: control at 4 ℃ incubation temperature, control at 22 ℃ incubation temperature, treated at 4 ℃ incubation temperature, and treated at 22 ℃ incubation temperature. The rating of strawberries stored at 4 ℃ was suspended after 24 days, while the rating of those stored at 22 ℃ was suspended after 13 days. Fungal growth in these strawberries took almost twice as much than experiment 2, and 3 times as much than experiment 1, which could be interpreted as resulting from an inherently more disease-resistant batch of strawberries, or a significantly less contaminated one among other considerations. Figure 21 shows the results of experiment 3 for both incubation temperatures. It was found that the simple effects of rating day and temperature were significant while the effect of the treatment 0.63 mgClO_2/L for 7 minutes was not ($p<0.3458$). However, the interaction of the treatment across rating days was significant at $p<0.0529$ and for strawberries stored at 4 ℃, it was found that the treatment was significantly better than the control between days 17 through 20 with $p<0.0586$, and between 17 through 21 with $p<0.0702$. To further understand how ClO_2 was being effective at reducing the growth of *Botrytis cinerea* in strawberries, the Gompertz model as described in equation (1) for mold growth was employed.

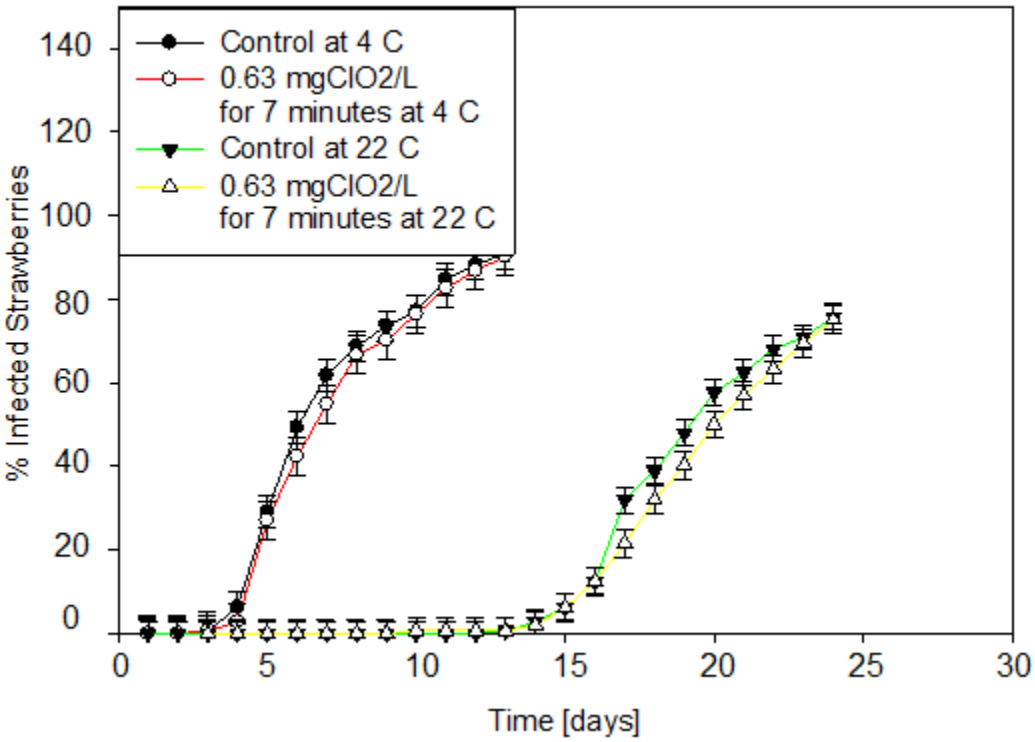

FIGURE 21. Repeated measures model over time for treated strawberries in experiment 3 stored at 4 ℃ and 22 ℃

Figures 22, 23 and 24 show the plots for experiments 1, 2 and 3 fitted to the Gompertz model, and Table 10 summarizes the results for the three parameters α, β, and θ for all treatments. One of the advantages of using a parametric model such as the Gompertz model to understand the effect of the treatments is that the parameters provide physical meaning to the behavior of the fungal growth, and therefore they allow for better understanding of the ClO_2 gas efficacy. For example, from Table 10 shows that treatments 0.63 mgClO2/L for 7 min, 5 mgClO2/L for 7 min, 1.26 mgClO2/L for 332 min, and 0.08 mgClO2/L for 1000 min from experiment 1, as well as treatments 0.63 mgClO2/L for 7 min, 5 mgClO2/L for 7 min, and 0.22 mgClO2/L for 84 min from experiment 2, show the lowest β and highest θ values.

Table 10. Gompertz model parameters for treatments in experiments 1, 2, and 3.

Experiment 1			
Treatment	Asymptote (α)	Growth Rate (β)	Inflection Point (θ)
Control	1.05 ± 0.15	0.7 ± 15E-04	2.2 ± 25E-04
0.63 mgClO2/L for 7 minutes	1.73 ± 3.41	0.5 ± 68E-04	3.9 ± 0.04
5 mgClO2/L for 7 minutes	1.11 ± 2.89	0.6 ± 11E-03	3.9 ± 0.04
0.04 mgClO2/L for 21 minutes	0.81 ± 0.34	0.9 ± 70E-04	2.2 ± 64E-04
5 mgClO2/L for 37 minutes	0.88 ± 0.90	0.7 ± 81E-04	2.9 ± 0.02
0.22 mgClO2/L for 84 minutes	0.81 ± 0.52	0.7 ± 49E-04	3.0 ± 0.01
0.01 mgClO2/L for 191 minutes	1.05 ± 0.87	0.7 ± 68E-04	2.8 ± 0.01
1.26 mgClO2/L for 332 minutes	1.39 ± 2.08	0.5 ± 52E-04	3.9 ± 0.03
0.08 mgClO2/L for 1000 minutes	1.02 ± 2.73	0.5 ± 96E-04	3.8 ± 0.05
0.01 mgClO2/L for 1000 minutes	1.35 ± 1.21	0.6 ± 54E-04	3.0 ± 0.02
Experiment 2			
Treatment	Asymptote (α)	Growth Rate (β)	Inflection Point (θ)
Control	0.82 ± 0.06	0.9 ± 17E-04	4.1 ± 14E-04
0.04 mgClO2/L for 21 minutes	0.93 ± 0.10	0.7 ± 14E-04	4.3 ± 21E-04
0.22 mgClO2/L for 84 minutes	0.97 ± 0.17	0.6 ± 14E-04	4.9 ± 36E-04
0.41 mgClO2/L for 21 minutes	1.02 ± 0.11	0.7 ± 14E-04	4.5 ± 20E-04
0.63 mgClO2/L for 7 minutes	1.13 ± 0.34	0.5 ± 16E-04	5.5 ± 69E-04
5 mgClO2/L for 7 minutes	0.83 ± 0.08	0.9 ± 19E-04	4.7 ± 15E-04
Experiment 3 - Temp 4 ℃			
Treatment	Asymptote (α)	Growth Rate (β)	Inflection Point (θ)
Control	0.8 ± 0.04	0.4 ± 0.05	17.2 ± 0.20
0.63 mgClO2/L for 7 minutes	0.9 ± 0.08	0.3 ± 0.05	18.3 ± 0.41
Experiment 3 - Temp 22 ℃			
Treatment	Asymptote (α)	Growth Rate (β)	Inflection Point (θ)
Control	0.87 ± 0.03	0.6 ± 0.07	5.3 ± 0.14
0.63 mgClO2/L for 7 minutes	0.9 ± 0.04	0.5 ± 0.08	5.6 ± 0.20

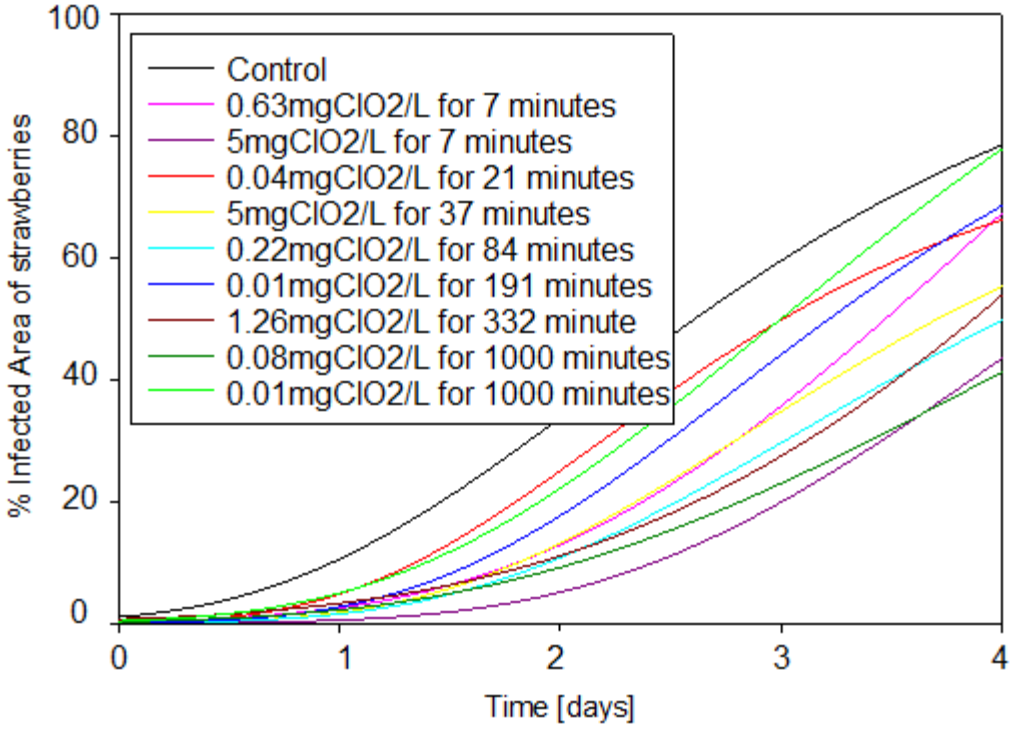

FIGURE 22. Gompertz model fit for treatments in experiment 1.

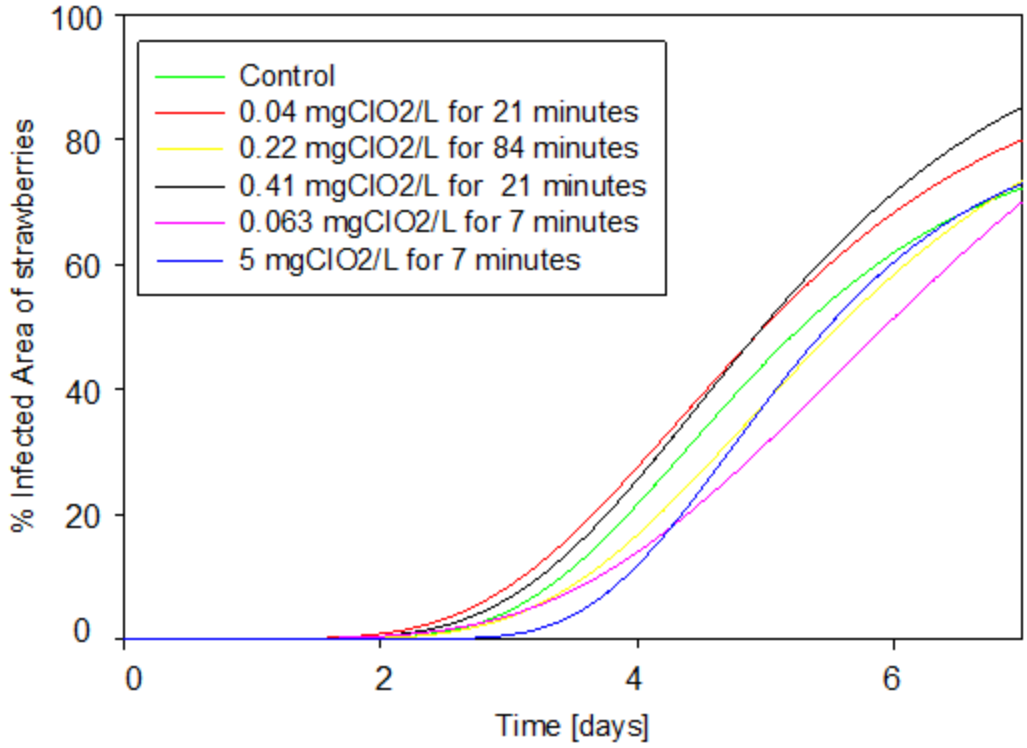

FIGURE 23. Gompertz model fit for treatments in experiment 2.

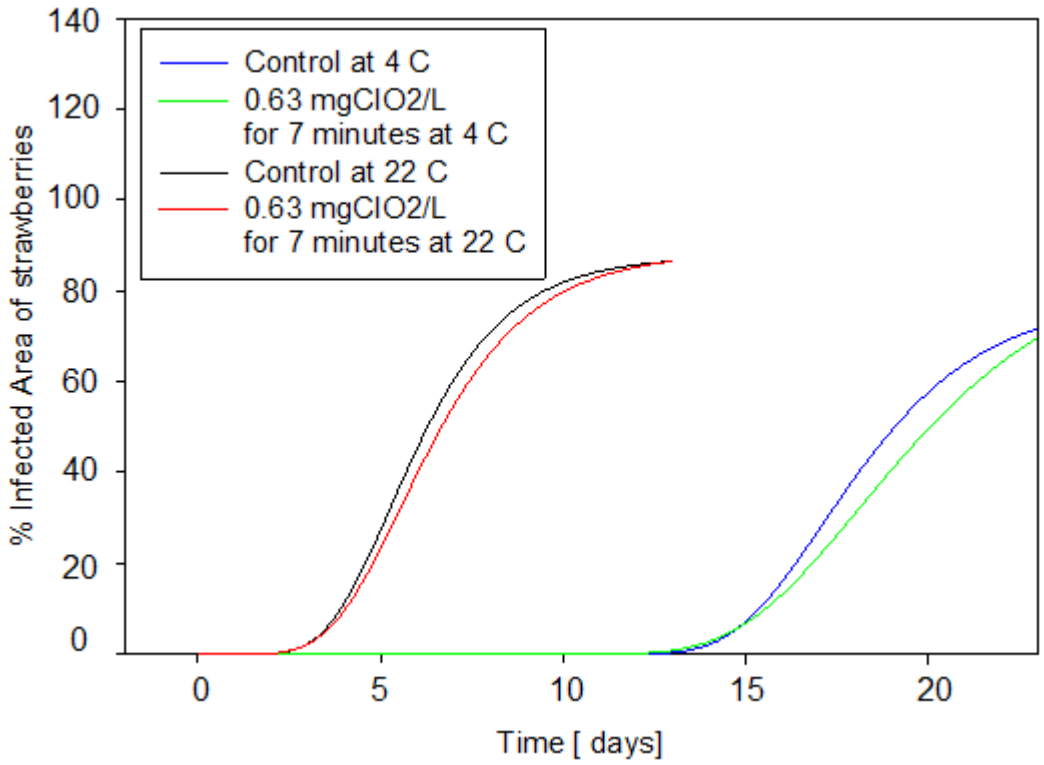

FIGURE 24. Gompertz model fit for treatments in experiment 3 both at 4 ℃ and 22℃

This indicates that the infection growth rate is slower for these treatments and that as a result, the onset of *Botrytis cinerea* is delayed. Therefore, in cases where ClO_2 gas was found to be effective, it acted as both an inhibitor of initial growth (delay of onset) and as a regulator along time since the speed at which the disease grew was significantly slower. These results are in good agreement with the findings from the repeated measures modeling since the treatments that were selected for their better efficacy are the same across experiments. Equally, the results from the Gompertz model show that the treatment selected for experiment 3 behave similarly to the control especially at incubation temperature 22 ^{0}C, which means that ClO_2 was not effective at delaying the onset and growth of *Botrytis cinerea* on the strawberries.

One of the main differences in studies of effects of ClO_2 on human-pathogenic microbes on fruit compared to fungal decay of field-grown strawberries naturally infected with *Botrytis cinerea* may be that the former are usually surface contaminants or applied to the fruit surface by researchers, whereas *Botrytis cinerea* is a fungal plant pathogen that infects fruit in the field prior to harvest, such that the fungus may already be present within plant tissues by the time the ClO_2 treatment is applied. This reduces the capacity of ClO_2 to reach and interfere with fungal growth. Furthermore, ClO_2 treatment on the berries after shipping across the United States for least 5-7 days, even though under refrigerated conditions, may be too late to achieve strong and consistent efficacy of ClO_2 as *Botrytis cinerea* may continue to grow, albeit slowly, during the shipping process. In addition, regardless of our careful selection process, bruising of berries during shipping was inevitable, thus increasing the susceptibility of fruit tissue to fungal decay.

4.4. Conclusions

ClO_2 absorption by fresh produce as a function of concentration and exposure time, the information related to the description of their effects and interactions were presented by a mathematical model using a response surface methodology design (RSM. Experiments 1 and 2 showed the efficacy of ClO_2 gas treatments on the onset delay of *Botrytis cinerea* on strawberries though natural variability was high even when statistical improvements were observed. However, when the treatment that met both quality standards and efficacy against the mold was tested again for validation on the

final experiment, the results were inconclusive. The differences in the contaminated proportion of strawberries across treatments, as well as the differences in fungal resistance of strawberries across experiments are important sources of variation that could have affected the results and its interpretation. Future work should focus on minimizing sources of variation before treatments like for example removing the calix from the fruit in order to ensure that all parts of the strawberry are exposed to the gas, using strawberries of the same cultivar, field, and harvest period to ensure at least a more homogeneous degree of contamination across samples. While sequential application of treatments is unavoidable if a single ClO_2 chamber is used for the experiments, treating fresh strawberries immediately after harvest would eliminate confounding factors that leave questions about the efficacy of the gas against *Botrytis cinerea*.

BIBLIOGRAPHY

BIBLIOGRAPHY

Abdel-Rahman, M. S., D. Couri, et al. (1979). "Kinetics of Cl02 and effects of Cl02, Cl02-, and Cl03- in drinking water on blood glutathione and hemolysis in rat and chicken." Journal of environmental pathology and toxicology 3(1-2): 431-449.

Abdul-Raouf, U. M., L. R. Beuchat, et al. (1993). "Survival and growth of Escherichia coli O157:H7 on salad vegetables." Appl. Environ. Microbiol. 59(7): 1999-2006.

Aday, M. S., M. B. Buyukcan, et al. (2012). "MAINTAINING THE QUALITY OF STRAWBERRIES BY COMBINED EFFECT OF AQUEOUS CHLORINE DIOXIDE WITH MODIFIED ATMOSPHERE PACKAGING." Journal of Food Processing and Preservation: no-no.

Agency for Toxic Substances and Disease Registry Division of Toxicology and Envirommental Medicine. (2004). "Toxicological Profile for Chlorine Dioxide and Chlorite." Retrieved June 1st, 2013, from http://www.atsdr.cdc.gov/toxprofiles/tp160.pdf.

Aieta, E. M. and J. M. Berg (1986). "A Review of Chlorine Dioxide in Drinking Water Treatment." J. Am. Water Works Assoc. 78 (6): 62-72.

Aieta, E. M., P. V. Roberts, et al. (1984). "Determination of Chlorine Dioxide, Chlorine, Chlorite, and Chlorate in Water." J. Am. Water Works Assoc. 76: 64-70.

Akin, E. W., J. C. Hoff, et al. (1982). "Waterborne outbreak control: which disinfectant?" Environmental Health Perspectives 46: 7-12.

Allende, A., F. A. Tomás-Barberán, et al. (2006). "Minimal processing for healthy traditional foods." Trends in Food Science & Technology 17(9): 513-519.

Appendini, P. and J. H. Hotchkiss (2002). "Review of antimicrobial food packaging." Innovative Food Science & Emerging Technologies 3(2): 113-126.

Arango, J., M. Rubino, et al. (2013). Quantification of Total Chlorine Dioxide Absorption by UV-visible Spectroscopy. Michigan, US, Michigan State University.

Balla, C. S. and J. Farkas (2007). Minimally Processed Fruits and Fruit Products and their Microbiological Safety. Handbook of Fruits and Fruit Processing, Blackwell Publishing: 115-128.

Bean, N. H. and P. M. Griffin (1990). "Foodborne disease outbreaks in the United States, 1973-1987: pathogens, vehicles, and trends." Journal of Food Protection 53.

Benarde, M. A., B. M. Israel, et al. (1965). "Efficiency of chlorine dioxide as a bactericide." Applied Microbiology 13(5): 776-780.

Benarde, M. A., W. B. Snow, et al. (1967). "Chlorine Dioxide Disinfection Temperature Effects." Journal of Applied Microbiology 30(1): 159-167.

Beuchat, L. (1998). "Surface decontamination of fruits and vegetables eaten raw: a review."

Beuchat, L. R. (1996). "Pathogenic Microorganisms Associated with Fresh Produce." Journal of Food Protection 59(2): 204-216.

Beuchat, L. R. (1999). "Survival of Enterohemorrhagic Escherichia coli O157:H7 in Bovine Feces Applied to Lettuce and the Effectiveness of Chlorinated Water as a Disinfectant." Journal of Food Protection 62: 845-849.

Beuchat, L. R. (2002). "Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables." Microbes and Infection 4(4): 413-423.

Bhagat, A. R. (2010). Modeling Critical Factors to Optimize the Treatment of Selected Fruits and Vegetables with Chlorine Dioxide Gas Using a Miniaturized Insustrial-Size Tunnel System. Doctor of Philosophy, Purdue University.

Bhaskara Reddy, M. V., K. Belkacemi, et al. (2000). "Effect of pre-harvest chitosan sprays on post-harvest infection by Botrytis cinerea and quality of strawberry fruit." Postharvest Biology and Technology 20(1): 39-51.

Brackett, R. E. (1999). "Incidence, contributing factors, and control of bacterial pathogens in produce." Postharvest Biology and Technology 15(3): 305-311.

Braun, P. and J. Sutton (1988). "Infection cycles and population dynamics of Botrytis cinerea in strawberry leaves." Canadian Journal of Plant Pathology 10(2): 133-141.

Centers for Disease Control and Prevention. (2011). "CDC Estimates of Foodborne Illness in the United States." Retrieved 9 April, 2013, 2013, from http://www.cdc.gov/foodborneburden/PDFs/FACTSHEET_A_FINDINGS_updated4-13.pdf.

Centers for Disease Control and Prevention. (2013). "Surveillance for Foodborne Disease Outbreaks — United States, 2009–2010." Retrieved June 1st, 2013, from http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6203a1.htm?s_cid=mm6203a1_w.

Condie, L. W. (1986). "Toxicological Problems Associated With Chlorine Dioxide." Journal (American Water Works Association) 78(6): 73-78.

Costilow, R. N., M. A. Uebersax, et al. (1984). "Use of Chlorine Dioxide for Controlling Microorganisms During the Handling and Storage of Fresh Cucumbers." Journal of Food Science 49(2): 396-401.

Couri, D., M. S. Abdel-Rahman, et al. (1982). "Toxicological Effects of Chlorine Dioxide, Chlorite and Chlorate." Environmental Health Perspectives 46: 13-17.

Czarneski, M. A. and P. Lorcheim (2005). "Isolator decontamination using chlorine dioxide gas." Pharmaceutical technology: 124-133.

Dagnas, S. and J.-M. Membre (2013). "Predicting and Preventing Mold Spoilage of Food Products." Journal of Food Protection 76(3): 538-551.

Dantigny, P., M. Bensoussan, et al. (2006). "Standardisation of methods for assessing mould germination: A workshop report." International journal of food microbiology 108(2): 286-291.

Dantigny, P., A. Guilmart, et al. (2005). "Basis of predictive mycology." International journal of food microbiology 100(1-3): 187-196.

Dantigny, P., S. Marín, et al. (2007). "Mould germination: Data treatment and modelling." International journal of food microbiology 114(1): 17-24.

Daş, E., G. C. Gürakan, et al. (2006). "Effect of controlled atmosphere storage, modified atmosphere packaging and gaseous ozone treatment on the survival of Salmonella Enteritidis on cherry tomatoes." Food Microbiology 23(5): 430-438.

Declerck, S., D. D'or, et al. (2001). "Modelling the sporulation dynamics of arbuscular mycorrhizal fungi in monoxenic culture." Mycorrhiza 11(5): 225-230.

Du, J., Y. Han, et al. (2002). "Inactivation by chlorine dioxide gas (ClO2) of Listeria monocytogenes spotted onto different apple surfaces." Food Microbiology 19(5): 481-490.

Du, J., Y. Han, et al. (2003). "Efficacy of chlorine dioxide gas in reducing Escherichia coli O157:H7 on apple surfaces." Food Microbiology 20(5): 583-591.

El-Samahy, S., B. M. Youssef, et al. (2000). "Microbiological and chemical properties of irradiated mango." Journal of food safety 20(3): 139-156.

Ellis, M., K. Cooksey, et al. (2006). "Quality of Fresh Chicken Breasts Using a Combination of Modified Atmosphere Packaging and Chlorine Dioxide Sachets." Journal of Food Protection 69: 1991-1996.

Exama, A., J. Arul, et al. (1993). "Suitability of Plastic Films for Modified Atmosphere Packaging of Fruits and Vegetables." Journal of Food Science 58(6): 1365-1370.

Garcia, D., A. J. Ramos, et al. (2009). "Predicting mycotoxins in foods: A review." Food Microbiology 26(8): 757-769.

Gibson, A. M., N. Bratchell, et al. (1987). "The effect of sodium chloride and temperature on the rate and extent of growth of Clostridium botulinum type A in pasteurized pork slurry." Journal of Applied Microbiology 62(6): 479-490.

Gómez-López, V. M., A. Rajkovic, et al. (2009). "Chlorine dioxide for minimally processed produce preservation: a review." Trends in Food Science & Technology 20(1): 17-26.

Gougouli, M. and K. P. Koutsoumanis (2012). "Modeling germination of fungal spores at constant and fluctuating temperature conditions." International journal of food microbiology 152(3): 153-161.

Greenberg, A. E., L. S. Clesceri, et al. (1992). Standard methods for the examination of water and wastewater. Washington, D.C., American Public Health Association: 4-56 - 54.81.

Gustavsson, J., C. Cederberg, et al. (2011). Global Food Losses and Food Waste. International Congress SAVE FOOD!, Dusseldorf, Germany.

Han, Y., J. D. Floros, et al. (2001). "Response Surface Modeling for the Inactivation of Escherichia coli O157:H7 on Green Peppers (Capsicum annuum L.) by Chlorine Dioxide Gas Treatments." Journal of Food Protection 64: 1128-1133.

Han, Y., A. M. Guentert, et al. (1999). "Efficacy of chlorine dioxide gas as a sanitizer for tanks used for aseptic juice storage." Food Microbiology 16(1): 53-61.

Han, Y., R. H. Linton, et al. (2000). "Inactivation of Escherichia coli O157:H7 on surface-uninjured and -injured green pepper (Capsicum annuum L.) by chlorine dioxide

gas as demonstrated by confocal laser scanning microscopy." Food Microbiology 17(6): 643-655.

Han, Y., R. H. Linton, et al. (2001). "Reduction of Listeria monocytogenes on Green Peppers (Capsicum annuum L.) by Gaseous and Aqueous Chlorine Dioxide and Water Washing and Its Growth at 7C." Journal of Food Protection 64: 1730-1738.

Han, Y., T. L. Selby, et al. (2004). "Decontamination of Strawberries Using Batch and Continuous Chlorine Dioxide Gas Treatments." Journal of Food Protection 67: 2450-2455.

Han, Y., D. M. Sherman, et al. (2000). "The effects of washing and chlorine dioxide gas on survival and attachment of Escherichia coli O157: H7 to green pepper surfaces." Food Microbiology 17(5): 521-533.

Harris, L. J., J. N. Farber, et al. (2003). "Outbreaks Associated with Fresh Produce: Incidence, Growth, and Survival of Pathogens in Fresh and Fresh-Cut Produce." Comprehensive Reviews in Food Science and Food Safety 2: 78-141.

Hautman, D. P., D. J. Munch, et al. (1997). "US EPA Method 300.1, Determination of Inorganic Anions in Drinking Water by Ion Chromatography, EPA/600/R-98/118, NIST PB-98-169196INZ." (Revision 1.0).

Heffernan, W., C. Guion, et al. (1979). "Oxidative damage to the erythrocyte induced by sodium chlorite, in vivo." Journal of environmental pathology and toxicology 2(6): 1487.

Heller, M. C. and G. A. Keoleian (2000). Life cycle-based sustainability indicators for assessment of the US food system, Center for Sustainable Systems, University of Michigan.

Jarvis, W. R. (1962). "The infection of strawberry and raspberry fruits by Botrytis cinerea Fr." Annals of Applied Biology 50(3): 569-575.

Kaczur, J. J. and D. W. Cawlfield (2000). Chlorine Oxygen Acids and Salts, Chlorous Acid, Chlorites, and Chlorine Dioxide. Kirk-Othmer Encyclopedia of Chemical Technology. John Wiley and Son. New York. NY, John Wiley & Sons, Inc.: 1-26.

Kallio, H., M. Hakala, et al. (2000). "Sugars and acids of strawberry varieties." European Food Research and Technology 212(1): 81-85.

Kantor, L. S., K. Lipton, et al. (1997). "Estimating and addressing America’s food losses." Food Review 20(1): 2-12.

Keskinen, L. A. and B. A. Annous (2011). Chlorine Dioxide (Gas). Nonthermal Processing Technologies for Food. H. Q. Zhang, G. V. Barbosa-Cánovas, V. M. Balasubramaniamet al. Oxford, UK, Wiley-Blackwell: 359 - 365.

Keskinen, L. A., A. Burke, et al. (2009). "Efficacy of chlorine, acidic electrolyzed water and aqueous chlorine dioxide solutions to decontaminate Escherichia coli O157:H7 from lettuce leaves." International Journal of Food Microbiology 132(2-3): 134-140.

Kim, J., M. R. Marshall, et al. (1999). "Determination of Chlorate and Chlorite and Mutagenicity of Seafood Treated with Aqueous Chlorine Dioxide." Journal of Agricultural and Food Chemistry 47(9): 3586-3591.

Kim, J. Y., H. J. Kim, et al. (2010). "The effects of aqueous chlorine dioxide or fumaric acid treatment combined with UV-C on postharvest quality of ‘Maehyang’ strawberries." Postharvest Biology and Technology 56(3): 254-256.

Knapp, J. E. and D. L. Battisti (2001). Chlorine Dioxide. Disinfection, Sterilization, and Preservation. S. S. Block. Philadelphia, Lippincott Williams & Wilkins: 215-227.

Kromminga, H., J. Orphal, et al. (2003). "New measurements of OClO absorption cross-sections in the 325–435 nm region and their temperature dependence between 213 and 293 K." Journal of Photochemistry and Photobiology A: Chemistry 157(2–3): 149-160.

Lee, S.-Y., M. Costello, et al. (2004). "Efficacy of Chlorine Dioxide Gas as a Sanitizer of Lettuce Leaves." Journal of Food Protection 67: 1371-1376.

Linton, R., Y. Han, et al. (2006). "Gas-/vapor-phase sanitation (decontamination) treatments." Microbiology of fruits and vegetables: 401-435.

López, S., M. Prieto, et al. (2004). "Statistical evaluation of mathematical models for microbial growth." International journal of food microbiology 96(3): 289-300.

Lynch, M., R. Tauxe, et al. (2009). "The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities." Epidemiology and infection 137(3): 307.

Maas, J. (1995). Compendium of Strawberry Diseases. APS Press, St. Paul, MN.

Mahmoud, B. S. M., A. R. Bhagat, et al. (2007). "Inactivation kinetics of inoculated Escherichia coli O157:H7, Listeria monocytogenes and Salmonella enterica on strawberries by chlorine dioxide gas." Food Microbiology 24(7–8): 736-744.

Mahovic, M., J. A. Bartz, et al. "Chlorine Dioxide Gas from an Aqueous Solution: Reduction of Salmonella in Wounds on Tomato Fruit and Movement to Sinks in a Treatment Chamber." Journal of Food Protection® 72(5): 952-958.

Netramai, S. (2011). Utilization of chlorine dioxide gas in food packaging application. Doctor of Philosophy Ph.D., Michigan State University.

Nieuwenhuijsen, M. J., M. B. Toledano, et al. (2000). "Uptake of chlorination disinfection by-products; a review and a discussion of its implications for exposure assessment in epidemiological studies." Journal of exposure analysis and environmental epidemiology 10(6 Pt 1): 586-599.

O'Beirne, D. and G. Francis (2003). "Reducing the pathogen risk in MAP-prepared produce." Novel food packaging techniques 12: 231-275.

Parish, M. E., L. R. Beuchat, et al. (2003). "Methods to Reduce/Eliminate Pathogens from Fresh and Fresh-Cut Produce." Comprehensive Reviews in Food Science and Food Safety 2: 161-173.

Peano, C., V. Girgenti, et al. (2012). Strawberry Production Chain: Application of a LCA model Acta Horticulturae. (ISHS)

Pepich, B. V., T. A. Dattilio, et al. (2007). "An improved colorimetric method for chlorine dioxide and chlorite ion in drinking water using lissamine green B and horseradish peroxidase." Analytica Chimica Acta 596(1): 37-45.

Phillips, C. A. (1996). "Review: Modified Atmosphere Packaging and its effects on the microbiological quality and safety of produce." International Journal of Food Science & Technology 31(6): 463-479.

Plattner, K. and A. Perez. (2012). "United States Department of Agriculture (USDA) Report: Fruit and Tree Nuts Outlook." from http://www.ers.usda.gov/media/970458/fts354.pdf.

Powelson, R. (1960). "Initiation of strawberry fruit rot caused by Botrytis cinérea." Phytopathology 50: 491-494.

Qingdong, X., Z. Guangming, et al. (2006). "Study on Subchronic Toxicity of Chlorine Dioxide and By-Products in Water." Journal of Environmental Science and

Health, Part A: Toxic/Hazardous Substances and Environmental Engineering 41(7): 1347 - 1353.

Reina, L. D., H. P. Fleming, et al. "Microbiological Control of Cucumber Hydrocooling Water with Chlorine Dioxide." Journal of Food Protection 58(5): 541-546.

Rico, D., A. B. Martín-Diana, et al. (2007). "Extending and measuring the quality of fresh-cut fruit and vegetables: a review." Trends in Food Science & Technology 18(7): 373-386.

Roberts, R. G. and S. T. Reymond (1994). "Chlorine dioxide for reduction of postharvest pathogen inoculum during handling of tree fruits." Applied and environmental microbiology 60(8): 2864-2868.

Robinson, J. W., Skelly Frame, Frame II (2005). Undergraduate Instrumental Analysis. New York.

Rodgers, S. L., J. N. Cash, et al. (2004). "A Comparison of Different Chemical Sanitizers for Inactivating Escherichia coli O157:H7 and Listeria monocytogenes in Solution and on Apples, Lettuce, Strawberries, and Cantaloupe." Journal of Food Protection 67(4): 721-731.

Schilder, A. M. C., J. M. Gillett, et al. (2013). "Evaluation of fungicides for control of Botrytis fruit rot and leaf spot in strawberry, 2012." Plant Disease Management Reports.

Seo, K. H. and J. F. Frank (1999). "Attachment of Escherichia coli O157:H7 to Lettuce Leaf Surface and Bacterial Viability in Response to Chlorine Treatment as Demonstrated by Using Confocal Scanning Laser Microscopy." Journal of Food Protection 62: 3-9.

Seymour, I. J. and H. Appleton (2001). "Foodborne viruses and fresh produce." Journal of Applied Microbiology 91(5): 759-773.

Singh, N., R. K. Singh, et al. (2002). "Effect of inoculation and washing methods on the efficacy of different sanitizers against Escherichia coli O157:H7 on lettuce." Food Microbiology 19(2-3): 183-193.

Sloan, A. E. (2013). "Top 10 Food Trends." from http://sloantrend.com/attachments/article/9/2013-04IFTTop10Trends.pdf.

Spotts, R. and B. Peters (1980). "Chlorine and chlorine dioxide for control of d'Anjou pear decay." Plant Dis 64(1): 2.

Staschower, F. (2012). Residues from Chlorine Dioxide Gas Treatment, Generated by Different Delivery Systems, on Fresh Produce. Master of Science, Michigan State University.

Sumathi, S., R. F. Cindy, et al. (2004). "Fresh Produce: A Growing Cause of Outbreaks of Foodborne Illness in the United States, 1973 through 1997." Journal of Food Protection 67(10): 2342-2353.

Sy, K. V., K. H. McWatters, et al. (2005). "Efficacy of Gaseous Chlorine Dioxide as a Sanitizer for Killing Salmonella, Yeasts, and Molds on Blueberries, Strawberries, and Raspberries." Journal of Food Protection 68: 1165-1175.

Sy, K. V., M. B. Murray, et al. (2005). "Evaluation of Gaseous Chlorine Dioxide as a Sanitizer for Killing Salmonella, Escherichia coli O157:H7, Listeria monocytogenes, and Yeasts and Molds on Fresh and Fresh-Cut Produce." Journal of Food Protection 68: 1176-1187.

Trinetta, V., N. Vaidya, et al. (2011). "Evaluation of Chlorine Dioxide Gas Residues on Selected Food Produce." Journal of Food Science 76(1): T11-T15.

Tsai, L. S., C. C. Huxsoll, et al. (2001). "Prevention of Potato Spoilage During Storage by Chlorine Dioxide." Journal of Food Science 66(3): 472-477.

United States Department of Agriculture (USDA). (2003). "Pathogen modelling program, version 7.0." Retrieved June 1st, 2013, from http://ars.usda.gov/services/docs.htm?docid=6786.

United States Environmental Protection Agency (EPA) (1999). "Alternative Disinfectants and Oxidants Guidance Manual: Chapter 4. Chlorine Dioxide, EPA 815-R-99-014."

United States Environmental Protection Agency (EPA) (2006). Registration eligibility decision for Chlorine Dioxide and Sodium Chlorite (Case Number 4023). http://www.epa.gov/oppsrrd1/REDs/chlorine_dioxide_red.pdf.

United States Environmental Protection Agency (EPA). (2007). "Anthrax spore decontamination using chlorine dioxide." Retrieved June 1st, 2013, from http://www.epa.gov/pesticides/factsheets/chemicals/chlorinedioxidefactsheet.htm.

United States Food and Drug Administration (FDA). (2004). "Produce Safety From Production to Consumption: 2004 Action Plan to Minimize Foodborne Illness Associated with Fresh Produce Consumption." Retrieved June 1st, 2013, from

http://www.fda.gov/Food/FoodborneIllnessContaminants/BuyStoreServeSafeFood/ucm129487.htm.

United States Food and Drug Administration (FDA). (2011). "Agency Response Letter GRAS Notice No. GRN 000062." Retrieved June 1st, 2013, from http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm153971.htm.

United States Food and Drug Administration (FDA). (2012). "Secondary direct food additives permitted in food for human consumption. Chlorine dioxide. CFR Title 21, part 173.300." Retrieved 9 Apr 2013, 2013, from http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=173.300.

Vardar, C., K. Ilhan, et al. (2012). "The application of various disinfectants by fogging for decreasing postharvest diseases of strawberry." Postharvest Biology and Technology 66(0): 30-34.

Wahner, A., G. S. Tyndall, et al. (1987). "Absorption cross sections for symmetric chlorine dioxide as a function of temperature in the wavelength range 240-480nm." The Journal of Physical Chemistry 91(11): 2734-2738.

Wei, C. I., T. S. Huang, et al. (1995). "Growth and Survival of Salmonella montevideo on Tomatoes and Disinfection with Chlorinated Water." Journal of Food Protection 58(8): 829-836.

Williamson, B., B. Tudzynski, et al. (2007). "Botrytis cinerea: the cause of grey mould disease." Molecular Plant Pathology 8(5): 561-580.

Windholz, M., S. Budavari, et al. (1976). The Merck index. An encyclopedia of chemicals and drugs. Rahway, New Jersey, Merck & Co.

Wise, J. C., L. J. Gut, et al. (2012). Michigan Fruit Management Guide 2013. Extension Bulletin E-154. East Lansing, MI, Michigan State University.

Yuk, H.-G., J. A. Bartz, et al. (2006). "The Effectiveness of Sanitizer Treatments in Inactivation of Salmonella spp. from Bell Pepper, Cucumber, and Strawberry." Journal of Food Science 71(3): M95-M99.

5. EXPLORATORY – PRELIMINARY DATA OF EFFECT OF SURFACE AREA AND WEIGHT ON ClO_2 CONSUMPTION BY FRESH PRODUCE

This section shows the preliminary results found when attempting to determine the correlation between total absorption of ClO_2 (measured using new approach developed) with surface area and weight of the produce, as well as with the surface residues from the produce recovered through the amperometric titration described previously (Netramai 2011; Staschower 2012). Romaine lettuce leaves were selected because it was amenable to easy modification and measurement of surface area and mass. For this study, the leaves were cut into rectangles of various sizes: 1.27 cm x 2.54 cm, 2.54 cm x 2.54 cm, 5.08 cm x 5.08 cm, and 2.54 cm x 12.7 cm corresponding to 3.23 cm^2 (or 0.5 in^2), 6.45 cm^2 (or 1 in^2), 25.81 cm^2 (or 4 in^2) and 32.26 cm^2 (or 5 $inch^2$) surface area respectively. After weighting them, 18 pieces of each surface area type were placed inside the chamber using the three-legged stands and exposed to a treatment of 5 mg/L ClO_2 concentration for 15 min. Total ClO_2 absorption was quantified *in situ* using the new method described above. As soon as each treatment was carried out, the lettuce pieces were washed with 300 mL of distilled and deionized water for 15 min and kept in the dark, after which the washing solution was titrated for residual ClO_2 and chlorite (ClO_2 -) with phenylarsine oxide (C_6H_5AsO). The results from both methods were statistically compared.

Table 11 shows total ClO_2 absorption by the different romaine lettuce sizes exposed to a concentration of 5 mg/L during 15 min. Absorption of ClO_2 increases as surface area and weight increase. Although Figure 25 shows that there is a positive linear relationship between absorption with surface area and weight, the surface area seems to be more strongly correlated to ClO_2 absorption by the produce with a higher coefficient of determination (R^2=0.78 versus R^2=0.58, p≤0.001) and narrower confidence intervals. At the same time, the slope difference between the two factors implies that with small surface area increments, the total absorption of ClO_2 by the fresh produce increases faster than with smaller changes in weight. Surface area showed a greater impact on ClO_2 absorption than the overall mass of the fresh produce.

The results of this trial were compared using the amperometric titration method. The total mass of ClO_2 absorbed by the product upon treatment with the gas could either stay the form of ClO_2 or it can freely participate in oxidation reactions and rapidly break down to chlorate (ClO_3^-) and chlorite (ClO_2^-) ions, which could then be converted into to chloride (Cl^-). With the UV-vis method described in this document, only ClO_2 was measured as it becomes unavailable inside the chamber due to absorption by the fresh produce. With the amperometric titration method used in this trial, only ClO_2^- and ClO_2 species could be recovered from the measured surface, which in part explain the lower percent of mass recover as shown in Table 11. The

amperometric titration method does not measure directly the amount of ClO_2 absorbed, but it quantified products absorbed below the surface or bound to the strawberries since they cannot be recovered through the successive washes that are required by the method However, it can also be observed that there is an equivalent trend between both approaches where total ClO_2 absorption by produce is impacted by surface area rather than by weight.

Table 11. Comparison of total chlorine dioxide absorption versus chlorites and chlorine dioxide residues recovered after exposure

Surface Area [inch^2]	Weight [g]	Total ClO_2 absorption [mg]	Recovered ClO_2 & ClO_2- [mg]	%Recovery [mg]
0.5	3.6 ± 0.30	1.46 ± 0.54	2.58E-02 ± 1.50E-03	1.8%
1	4.0 ± 0.42	2.34 ± 0.63	2.24E-02 ± 1.84E-03	1.0%
4	22 ± 0.14	4.88 ± 0.19	2.16E-01 ± 1.20E-02	4.4%
5	30.82 ± 4.3	5.63 ± 0.01	2.96E-01 ± 2.88E-03	5.3%

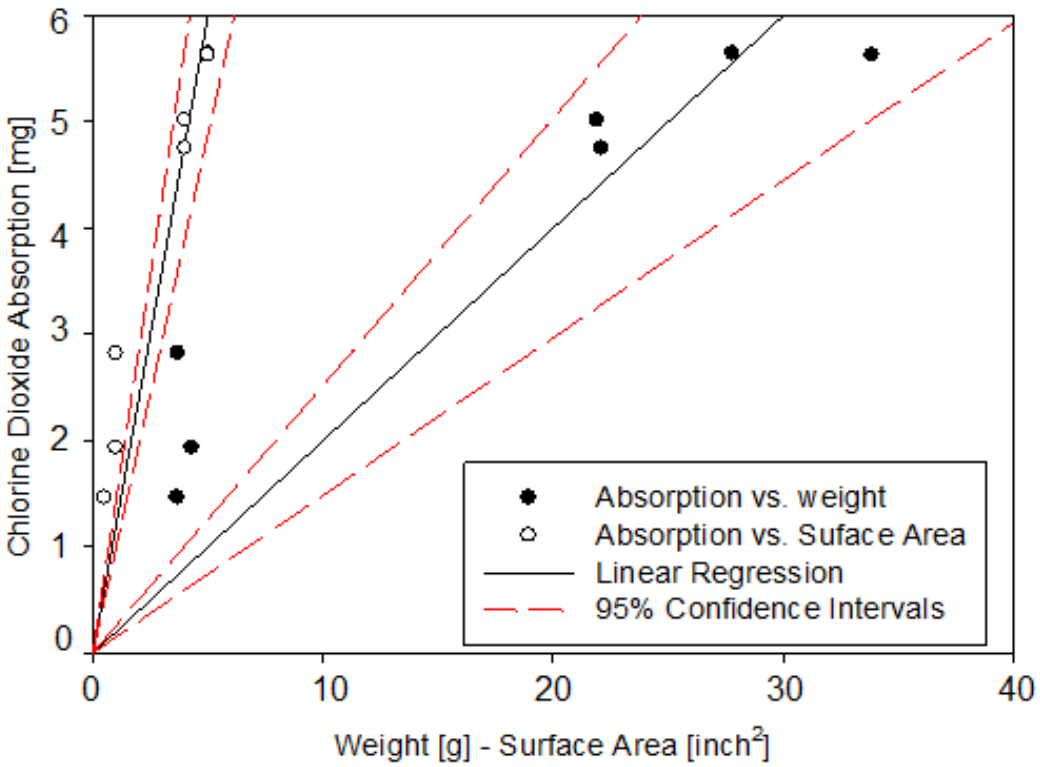

FIGURE 25. Chlorine dioxide absorption versus weight [g] and surface area [in^2]

6. CONCLUSIONS AND FUTURE WORK

To be able to consider and fully assess the potential of ClO_2 gas as an antimicrobial agent for fresh produce, it is necessary to be able to monitor and accurately measure both exposure of the ClO_2 gas to the food product and absorption or uptake of ClO_2 by the food. Quantifying and correlating exposure dose of ClO_2 to efficacy and absorption by the fresh produce is crucial for safety, sanitation and packaging design purposes among others.

From the safety perspective prior to human ingestion, it is important to assess and quantify surface by-product residuals as well as potentially ClO_2 or other chlorinated absorbed species by the fresh produce. Results from this assessment could be transformed into input for the sanitation process, where it would be defined in a case by case basis the maximum concentration levels and exposure times allowed in order to keep absorption of ClO_2 under safe limits. Finally, from the packaging design perspective, understanding how much and how fast fresh produce absorbs or uptakes ClO_2 s during ClO_2 gas treatments would provide a reference to determine gas release-speed and concentration required in the headspace.

Also, considering how effective ClO_2 gas has proven to be against pathogenic microorganisms, it makes sense to also investigate its potential against spoilage microorganisms.

This study provides a new approach to the quantification of ClO_2 gas absorption by fresh produce and the fungicidal potential of the gas against *Botrytis cinerea* on fresh

strawberries. The main goal of this work was to assess the potential use of ClO_2 as a sanitizer in strawberries considering ClO_2 consumption by the fruit as well. This work aimed to answer two specific questions stated in Chapter 1 and the key findings are described here:

Development of a new system and online method to quantify ClO_2 exposure concentration and consumption by fresh produce.

The system built is robust, easy to use and fast in determining in situ measurements of ClO_2 gas concentration exposure to produce and concentration of ClO_2 consumed by fresh produce. Even though results presented in this study are based on fresh strawberries, the equipment allows for study of any other kind of fresh produce provided that it fits in the current glass chamber.

The two routes on which the system could be operated provides a good resource for research looking for answers when it comes to understanding the mechanisms of inactivation by ClO_2 of different microorganism and the way fresh produce consume ClO_2.

Some of the key findings with route 2 are the fast consumption kinetics on strawberry fruit at all three tested concentrations (1, 3, and 5 mg/L).

Assessment on the application of the new equipment by evaluating ClO_2 gas efficacy against Botrytis cinerea on strawberries.

A total of three experiments were run to test the efficacy of the gas against *Botrytis cinerea* in strawberries as well as the impact of the treatments on visual quality. Despite the high variability found within treatments, it was found that the treatment with ClO_2 gas concentration set at 0.63 mg/L and exposure time of 7 minutes was effective.

Although the treatment with ClO_2 gas concentration set at 5 mg/L and exposure time of 7 minutes was effective as well, it damaged the fruit from the bleaching point of view.

From the assessment done to percent bleaching of strawberries as a function of total ClO_2 consumption by the fruit, it was found that the visual quality of strawberries is highly sensitive to treatments with ClO_2 gas, exposing very rapidly changes in color and texture on both the soft red tissue and the calyx.

As a complement to this work, and considering the handling and operating advantages of the new method developed, it would be interesting to balance a stoichiometric formula and run comparative tests in order to correlate the ClO_2 consumption results from this methodology with another current valid method used to quantify surface residues on fresh produce. This would allow not only for a validation of the method, but it would serve as a calibration to transform data from one method to another.

Also, the tests performed on this study provide some preliminary insight from where it seemed like surface area would be a more significant factor than weight

when correlation to total ClO_2 absorption, but more experiments are needed in order to establish this with confidence. Exploring deeper into the effects of surface area and weight on ClO_2 consumption by fresh produce would also help understand the mechanism of inactivation of ClO_2 gas: whether it acts only on the surface of the produce or if it actually penetrates inside the fruit. Additionally, performing these type of experiments in more than just one type of fresh produce could also provide additional information as to whether those mechanisms of inactivation would differ from produce to produce considering that some of them have softer, open cell tissues (e.g. strawberries) while others are protected by a thicker skin (e.g tomatoes).

APPENDICES

APPENDIX 1: Antimicrobial effectiveness of ClO_2 to inactivate microorganisms in fresh produce

Table 12. Summary of antimicrobial effectiveness of ClO_2 gas to inactivate microorganisms in fresh produce

MICROORGANISMS	PRODUCE	AUTHOR
Escherichia Coli	Green Peppers	Y. Han, D.M. Sherman
Escherichia Coli, Listeria monocytogenes, and Salmonella	Lettuce Leaves	Sun-Young Lee
Escherichia Coli	Green Peppers	Y. Han, R.H. Linton
Perservation Quality	Green Bell Peppers	Du Jin-Hua
Botrytis cinerea, Penicillium, alternaria	Grape	Ri ya Jin
Escherichia Coli, Listeria monocytogenes, and Salmonella	Blueberries	Iuliano Popa
Listeria monocytogenes, Pseudomas aeruginosa. Salmonella Typhimurim, Yersinia enterocolitica	Blueberries	Vivian C.H. Wu
Lactabacillus buchneri, Leuconostoc mesenteroides, Eurotium spp., Penicilluim spp, Candida spp, and Saccharomyces cerevisiae	Juice	Y Han, A.M. Guentert
Salmonella enterica spp	Roma tomatoes	V. Trinetta
Escherichia Coli and Listeria monocytogenes	Green Peppers	Y. Han, R.H. Linton
Escherichia Coli	Iceberg Lettuce	C Diaz
Escherichia Coli	Salad Vegetables	U.M. Abdul-Raouf
Listeria monocytogenes	Lettuce	Larry R. Beuchat
Salmonella baildon	Shredded lettuce and diced tomatoes	W.R. Weissinger
Escherichia Coli	Lettuce	Larry R. Beuchat
Escherichia Coli, Listeria monocytogenes, and Salmonella	Blueberries	Iuliano Popa
Perservation Quality	Strawberries	Mehment Seckin Aday

Table 12 (CONT'D)

Salmonella	Bell pepper, cucumber, and Strawberry	Hyun-Gyun Yuk
Listeria Monocytogenes	Apples, lettuce, Strawberries, and cantaloupe	Stephanie L. Rodgers
Escherichia Coli	Apples	G.M. Sapers
Salmonella enterica and Erwinia carotovora	Tomatoes	S. Pao
Escherichia Coli	Green Peppers	Y. Han, J.D. Floros
Escherichia Coli	Apples	J. Du
Listeria Monocytogenes	Apples	Jinhua Du
Escherichia Coli	Green Peppers	Y. Han, J.D. Floros
Listeria Monocytogenes	Green Peppers	Y. Han, R.H. Linton
Escherichia Coli, Listeria monocytogenes, and Salmonella	Lettuce Leaves	Sun-Young Lee
Escherichia Coli, Listeria monocytogenes, and Salmonella	Cabage, carrot, and iceberg lettuce	Kaye V. Sy
Escherichia Coli	Lettuce Leaf	K.H. Seo
Escherichia Coli, Listeria monocytogenes, and Salmonella	Raw Apples, Tomatoes, and Lettuce	L.R. Beuchat
Escherichia Coli	Lettuce Leaves	Lidsey A. Keskinen
Listeria Monocytogenes	Iceberg Lettuce and Romaine Lettuce	Larry R. Beuchat
Escherichia Coli	Tomato Fruits and Lettuce Leaves	Suwimon Keeratipibul
Escherichia Coli	Apples	G.M. Sapers
Salmonella, Yeast and Molds	Blueberries, Strawberries, and Raspberries	Kaye V. Sy
Escherichia Coli and Listeria monocytogenes	Strawberries	Y. Han

APPENDIX 2: Amperometric titration for determination of ClO_2 and ClO_2^- in solution

This procedure was outlined by Netramai (2010), it is a modification of the original amperometric titration method for water and wastewater, 4500- ClO_2 C (Greenberg, Clesceri et al. 1992). The method is used to determine the residual ClO_2 and ClO_2 in a solution by dividing the sample in three parts in order to determine each component separately.

Materials

- 60 mL sample cup for titrator
- pH meter
- Titrator equipped with electrode – Dual Ring PT (Mettler Tolledo, Columbus, OH)

Reagents

- Deionized water
- Phenylarsine oxide - PAO ((C6H5)AsO) – 0.00564N
- Phosphate buffer solution (pH 7)
- Potassium Iodide (KI) solution – 5% (weight)
- Sodium Hydroxide (NaOH) – 6N, 0.02N and 0.002N
- Sulfuric Acid (H2SO4) – 6N, 0.3N and 0.003N

Procedure

- Sample 3 portions, 50 mL, of the sample solution (washed water) and place in the titrator cup.
- Portion 1 - determination of free available chlorine and chloramines
    - Adjust the pH to ≥ 12 adding NaOH,
    - Leave in dark for 10 min
    - Correct the pH to 7 by adding H2SO4
    - Add 1 mL of KI solution
    - Titrate with PAO until the end point
    - Record results as A

- Portion 2 – determination of free available chlorine, chloramines, and 1/5 of chlorine dioxide

    - Adjust the pH to 7 by adding phosphate buffer solution
    - Add 1 mL of KI solution
    - Titrate with PAO until the end point
    - Record results as B

- Portion 3 – determination of free available chlorine, chloramines, chlorine dioxide and chlorite
    - Add 1 mL of KI solution
    - Adjust the pH to ≤ 2 with H2SO4
    - Leave in dark for 10 min
    - Correct pH to 7 by adding NaOH
    - Titrate with PAO until the end point
    - Record result as C

- Discard all solutions

Calculations

To calculate ClO_2 in mg ClO_2/L

ClO_2 (mg/L) = 1.9(B - A)

To calculate ClO_2- in mg Cl_2/L

ClO_2- (mgCl_2/L) = 4A - 5B + C

APPENDIX 3: Determination of ClO_2 solution using Thiosulfate Titration

Below is described the procedure to determine ClO_2 concentration in solution modified from ICA TriNova LLC (Staschower, 2012). The method described is for an automatic titration.

Materials

- 60 mL sample cup for titrator
- pH meter
- Titrator with electrode – DMI 140 (Mettler Tolledo, Columbus, OH)

Solutions

- Potassium iodide (KI) - 10% (weight)
- Sodium Thiosulfate ($Na_2S_2O_3$) – Certified Solution – 0.1N, 0.01N, and 0.001N
- Sulfuric Acid (H_2SO_4) – 6N

Procedure

- Sample a known amount of ClO_2 solution and place in the sample cup (record as Vs (mL))
- Add KI solution if the volume is low
- Titrate the solution (by hand) with $Na_2S_2O_3$ until it is colorless
- Correct the pH to 7 by adding H_2SO_4
- Leave the solution in dark for 10 min
- Titrate with $Na_2S_2O_3$ until the end point (automatic titration)
- Record result as Va
- Discard the solution

Calculation

$$ClO_2\left(mg/_L\right) = \frac{V_A \times N \times 67500}{4 \times V_s}$$

N= normality of Na_2S_2O

APPENDIX 4: SAS CODES

EXPERIMENT 1 – REPEATED MEASURES

```
** Importing dataset;
PROC IMPORT OUT= WORK.bot
        DATAFILE= "C:\Users\Nico\Desktop\Statistical Analysis\trial1.csv"
        DBMS=CSV REPLACE;
    GETNAMES=YES;
    DATAROW=2;
RUN;
data bot; set bot;
date=day; run;
proc print data=bot; run;

* checking samples;
proc freq data=bot;
tables day*trt;
run;
* extracting means per chamber;
proc means data=bot mean;
var rot;
class trt rep day;
ods output summary=bot2;
run;
proc print data=bot2; run;
data bot2; set bot2;
if trt=1 AND rep=4 AND day=7 then delete;
run;


** BASIC GLMM;
* cheking the basic structure in a LINEAR model;
proc mixed data=bot2;
class trt day rep;
model rot_Mean=trt|day/outp=jresiduals;
random rep*trt;
repeated day/subject=rep*trt type=cs;
run;

** Checking stat. assumptions;
*normality;
proc univariate data=jresiduals normal plot;
var resid ; histogram resid; qqplot resid; run;
* Variances;
proc sort data=jresiduals; by trt; run;
```

```
proc univariate data=jresiduals normal plot;
var resid; by trt; run;
proc sort data=jresiduals; by day; run;
proc univariate data=jresiduals normal plot;
var resid; by day; run;


** Adjusting a repeated structure;
* Cheking the structure in the VAR/COV matrix;
proc mixed data=bot2;
class trt day rep;
model rot_Mean=trt|day/ddfm=sat;
*random rep*trt;
repeated day/ subject=rep*trt type=cs;
run;
* -2 Res Log Likelihood 407.5
AICC (smaller is better) 411.7 ;


proc mixed data=bot2;
class trt day rep;
model rot_Mean=trt|day/ddfm=sat;
random rep*trt;
repeated day/subject=rep*trt type=ar(1);
run;
* -2 Res Log Likelihood 392.5
AICC (smaller is better) 396.7  ;

proc mixed data=bot2;
class trt day rep;
model rot_Mean=trt|day/ddfm=sat;
*random rep*trt;
repeated day/ subject=rep*trt type=un;
run;
* -2 Res Log Likelihood 381.6
AICC (smaller is better) 406.5;

proc mixed data=bot;
class trt day rep;
model rot_Mean=trt|day/ddfm=sat;
*random rep*temp*conc;
repeated day/subject=rep*trt type=sp(pow)(day);
run;
*-2 Res Log Likelihood 392.5
AICC (smaller is better) 396.7 ;
```

```
** Final model and reporting-listo;
proc mixed data=bot2;
class trt day rep;
model rot_Mean=trt|day/ddfm=sat;
random rep*trt;
repeated day/subject=rep*trt type=ar(1);
lsmeans trt*day/pdiff;
ods output lsmeans=jmeans diffs=jdiff;
run;
proc print data=jmeans; run;


*reporting:plotting;
symbol1 v=dot c='black' i=join;
symbol2 v=dot c='red' i=join;
symbol3 v=dot c='green' i=join;
symbol4 v=dot c='blue' i=join;
symbol5 v=dot c='orange' i=join;
symbol6 v=dot c='purple' i=join;
symbol7 v=dot c='brown' i=join;
symbol8 v=dot c='yellow' i=join;
symbol9 v=dot c='pink' i=join;
symbol10 v=dot c='ochre' i=join;
proc gplot data=jmeans;
plot estimate*day=trt;
run;

proc print data=jdiff;
where day=_day AND trt=trt AND probt<0.05; run;

proc glimmix data=bot2;
class trt rep day;
model rot_Mean=trt|day/ddfm=sat;
random day/subject=rep*trt type=ar(1);
lsmeans trt*day/slicediff=(trt day);
lsmeans trt*day/plot=meanplot(sliceby=trt join cl);;
run;
```

```
EXPERIMENT 1 - GOMPERTZ MODEL
** 1. Importing dataset;
PROC IMPORT OUT= WORK.bot
            DATAFILE= "C:\Users\Nico\Desktop\Statistical Analysis\trial1.csv"
            DBMS=CSV REPLACE;
     GETNAMES=YES;
     DATAROW=2;
RUN;
data bot; set bot;
date=day; run;
proc print data=bot; run;

** Plotting;
symbol1 v=dot c='black'; symbol2 v=dot c='red';
proc gplot data=bot;
where conc=0; plot rot*day=temp;
run;
proc gplot data=bot;
where conc=0.63; plot rot*day=temp;
run;

** 2. Fitting a Non-Linear function using proc nlin;
************************************************;
* 2.1. Using Gompertz function;
proc sort data=bot; by trt; run;
proc nlin data=bot best=10 MAXITER=500 converge=0.05;
*where temp=4;
by trt;
parms alpha=60 to 100 by 0.1 beta=0 to 1 by 0.1 theta=0 to 5 by 0.1;
pa=alpha; pb=beta; pc=theta;
model rot = pa*2.71828**(-2.71828**(-pb*(day-pc)));
ods output ParameterEstimates=estimate1 ANOVA=ANOVA1 corrb=corrb1;
output out=mypred1 predicted=pred;
run;
proc print data=mypred1; run;
proc print data=estimate1; run;
proc print data=ANOVA1; run;
proc print data=corrb1; run;

* 2.2. Extracting estimated parameters;
proc sql;
select std(estimate) as sta into :sta from estimate1 where parameter='alpha';
select std(estimate) as stb into :stb from estimate1 where parameter='beta';
select std(estimate) as stc into :stc from estimate1 where parameter='theta';
select mean(alpha) as corrab into :corrab from corrb1 where parameter='beta';
select mean(alpha) as corrac into :corrac from corrb1 where parameter='theta';
```

```
select mean(beta) as corrbc into :corrbc from corrb1 where parameter='theta';
select mean(ms) as sse into :sse from anova1 where source='Error';
quit;

* 2.3. Extracting predicted values - Gompertz function;
proc nlmixed data=bot MAXITER=500;
where temp=4;
by conc;
bounds 0.5 < alpha < 1.5; bounds beta < 0; bounds theta < 0;
parms alpha=0.80 beta=-600 theta=-0.35;
pa=alpha; pb=beta; pc=theta;
pred= pa*2.71828**(-2.71828**(-pb*(day-pc)));
model rot~normal(pred,s2);
predict pred out=pred1;
run;
proc nlmixed data=bot MAXITER=500;
where temp=23;
by conc;
bounds 0.5 < alpha < 1.5; bounds beta < 0; bounds theta < 0;
parms alpha=0.80 beta=-20 theta=-0.35;
pa=alpha; pb=beta; pc=theta;
pred= pa*2.71828**(-2.71828**(-pb*(day-pc)));
model rot~normal(pred,s2);
predict pred out=pred2;
run;

* 2.4. plotting an overlayed graph;
*extracting data means;
data preds; set pred1 pred2;
if temp=4 AND conc=0 then trt='Tem4-Conc0';
if temp=4 AND conc=0.63 then trt='Tem4-C0.63';
if temp=23 AND conc=0 then trt='T23-Conc0';
if temp=23 AND conc=0.63 then trt='T23-C0.63';
run;
proc print data=preds; run;
proc sort data=preds; by trt; by day; run;
proc means data=preds nway chartype;
class trt day; var rot pred;
output out=mms1; run;
proc print data=mms1; run;
data means1; set mms1;
where _stat_='MEAN';
drop _TYPE_ _FREQ_ _STAT_;
run;
proc print data=means1; run;
*plotting;
```

```
symbol1 color=red interpol=none value=dot;
symbol2 color=black interpol=none value=dot;
symbol3 color=blue interpol=none value=dot;
symbol4 color=gray interpol=none value=dot;
symbol5 color=red width=4 interpol=spline2s value=none;
symbol6 color=black width=4 interpol=spline2s value=none;
symbol7 color=blue width=4 interpol=spline2s value=none;
symbol8 color=gray width=4 interpol=spline2s value=none;
axis1 label=('Percentage') order=(0 to 1 by 0.1) width=3;
axis2 label=('Model') order=(0 to 1 by 0.1) width=3;
legend1 label=('Mean Percentage');
legend2 label=('Model fitted');
proc sort data=means1; by trt; run;
proc gplot data=means1;
plot rot*day=trt/ vaxis=axis1 legend=legend1;
plot2 pred*day=trt/ vaxis=axis2 legend=legend2;
run;
```

```
EXPERIMENT 2 – REPEATED MEASURES
** Importing dataset;
PROC IMPORT OUT= WORK.bot
        DATAFILE= "C:\SCC\13-03-27-Juliana Arango\trial2.csv"
        DBMS=CSV REPLACE;
    GETNAMES=YES;
    DATAROW=2;
RUN;
data bot; set bot;
date=day; run;
proc print data=bot; run;

* checking samples;
proc freq data=bot;
tables day*trt;
run;
* extracting means per chamber;
proc means data=bot mean;
var rot;
class trt rep day;
ods output summary=bot2;
run;
proc print data=bot2; run;
data bot2; set bot2;
if trt=1 AND rep=4 AND day=7 then delete;
run;


** BASIC GLMM;
* cheking the basic structure in a LINEAR model;
proc mixed data=bot2;
class trt day rep;
model rot_Mean=trt|day/outp=jresiduals;
random rep*trt;
repeated day/subject=rep*trt type=cs;
run;

** Checking stat. assumptions;
*normality;
proc univariate data=jresiduals normal plot;
var resid ; histogram resid; qqplot resid; run;
* Variances;
proc sort data=jresiduals; by trt; run;
proc univariate data=jresiduals normal plot;
var resid; by trt; run;
proc sort data=jresiduals; by day; run;
```

```
proc univariate data=jresiduals normal plot;
var resid; by day; run;


** Adjusting a repeated structure;
* Cheking the structure in the VAR/COV matrix;
proc mixed data=bot2;
class trt day rep;
model rot_Mean=trt|day/ddfm=sat;
*random rep*trt;
repeated day/ subject=rep*trt type=cs;
run;
* -2 Res Log Likelihood          895.1
* BIC (smaller is better)        901.4;

proc mixed data=bot2;
class trt day rep;
model rot_Mean=trt|day/ddfm=sat;
random rep*trt;
repeated day/subject=rep*trt type=ar(1);
run;
* AIC (smaller is better)        844.6
* BIC (smaller is better)        847.0 ;

*tarea;
proc mixed data=bot2;
class trt day rep;
model rot_Mean=trt|day/ddfm=sat;
*random rep*trt;
repeated day/ subject=rep*trt type=un;
run;

proc mixed data=bot;
class temp conc day rep date;
model rot=temp|conc|day/ddfm=sat;
*random rep*temp*conc;
repeated day/subject=rep*temp*conc type=sp(pow) (date) group=conc*temp;
run;


** Final model and reporting-listo;
proc mixed data=bot2;
class trt day rep;
model rot_Mean=trt|day/ddfm=sat;
random rep*trt;
repeated day/subject=rep*trt type=ar(1);
```

```
lsmeans trt*day/pdiff;
ods output lsmeans=jmeans diffs=jdiff;
run;
proc print data=jmeans; run;


*reporting:plotting;
symbol1 v=dot c='black' i=join;
symbol2 v=dot c='red' i=join;
symbol3 v=dot c='red' i=join;
symbol4 v=dot c='red' i=join;
symbol5 v=dot c='red' i=join;
symbol6 v=dot c='red' i=join;
proc gplot data=jmeans;
plot estimate*day=trt;
run;

proc print data=jdiff;
where day=_day AND trt=trt AND probt<0.05; run;
```

EXPERIMENT 2 - GOMPERTZ MODEL

```
** 1. Importing dataset;
PROC IMPORT OUT= WORK.bot
        DATAFILE= "C:\Users\Nico\Desktop\Statistical Analysis\jdlast\trial2.csv"
        DBMS=CSV REPLACE;
    GETNAMES=YES;
    DATAROW=2;
RUN;
data bot; set bot;
date=day; run;
proc print data=bot; run;

** Plotting;
symbol1 v=dot c='black'; symbol2 v=dot c='red';
proc gplot data=bot;
where conc=0; plot rot*day=temp;
run;
proc gplot data=bot;
where conc=0.63; plot rot*day=temp;
run;

** 2. Fitting a Non-Linear function using proc nlin;
**************************************************;
* 2.1. Using Gompertz function;
proc sort data=bot; by conc; run;
proc nlin data=bot best=10 MAXITER=500 converge=0.05;
*where temp=4;
by conc;
parms alpha=70 to 150 by 0.1 beta=0 to 1 by 0.1 theta=0 to 7 by 0.1;
pa=alpha; pb=beta; pc=theta;
model rot = pa*2.71828**(-2.71828**(-pb*(day-pc)));
ods output ParameterEstimates=estimate1 ANOVA=ANOVA1 corrb=corrb1;
output out=mypred1 predicted=pred;
run;
proc print data=mypred1; run;
proc print data=estimate1; run;
proc print data=ANOVA1; run;
proc print data=corrb1; run;

* 2.2. Extracting estimated parameters;
proc sql;
select std(estimate) as sta into :sta from estimate1 where parameter='alpha';
select std(estimate) as stb into :stb from estimate1 where parameter='beta';
select std(estimate) as stc into :stc from estimate1 where parameter='theta';
select mean(alpha) as corrab into :corrab from corrb1 where parameter='beta';
```

```
select mean(alpha) as corrac into :corrac from corrb1 where parameter='theta';
select mean(beta) as corrbc into :corrbc from corrb1 where parameter='theta';
select mean(ms) as sse into :sse from anova1 where source='Error';
quit;

* 2.3. Extracting predicted values - Gompertz function;
proc nlmixed data=bot MAXITER=500;
where temp=4;
by conc;
bounds 0.5 < alpha < 1.5; bounds beta < 0; bounds theta < 0;
parms alpha=0.80 beta=-600 theta=-0.35;
pa=alpha; pb=beta; pc=theta;
pred= pa*2.71828**(pb*2.71828**(pc*day));
model rot~normal(pred,s2);
predict pred out=pred1;
run;
proc nlmixed data=bot MAXITER=500;
where temp=23;
by conc;
bounds 0.5 < alpha < 1.5; bounds beta < 0; bounds theta < 0;
parms alpha=0.80 beta=-20 theta=-0.35;
pa=alpha; pb=beta; pc=theta;
pred= pa*2.71828**(pb*2.71828**(pc*day));
model rot~normal(pred,s2);
predict pred out=pred2;
run;

* 2.4. plotting an overlayed graph;
*extracting data means;
data preds; set pred1 pred2;
if temp=4 AND conc=0 then trt='Tem4-Conc0';
if temp=4 AND conc=0.63 then trt='Tem4-C0.63';
if temp=23 AND conc=0 then trt='T23-Conc0';
if temp=23 AND conc=0.63 then trt='T23-C0.63';
run;
proc print data=preds; run;
proc sort data=preds; by trt; by day; run;
proc means data=preds nway chartype;
class trt day; var rot pred;
output out=mms1; run;
proc print data=mms1; run;
data means1; set mms1;
where _stat_='MEAN';
drop _TYPE_ _FREQ_ _STAT_;
run;
proc print data=means1; run;
```

```
*plotting;
symbol1 color=red interpol=none value=dot;
symbol2 color=black interpol=none value=dot;
symbol3 color=blue interpol=none value=dot;
symbol4 color=gray interpol=none value=dot;
symbol5 color=red width=4 interpol=spline2s value=none;
symbol6 color=black width=4 interpol=spline2s value=none;
symbol7 color=blue width=4 interpol=spline2s value=none;
symbol8 color=gray width=4 interpol=spline2s value=none;
axis1 label=('Percentage') order=(0 to 1 by 0.1) width=3;
axis2 label=('Model') order=(0 to 1 by 0.1) width=3;
legend1 label=('Mean Percentage');
legend2 label=('Model fitted');
proc sort data=means1; by trt; run;
proc gplot data=means1;
plot rot*day=trt/ vaxis=axis1 legend=legend1;
plot2 pred*day=trt/ vaxis=axis2 legend=legend2;
run;
```

EXPERIMENT 3 – REPEATED MEASURES

```
** Importing dataset;
PROC IMPORT OUT= WORK.bot
        DATAFILE= "C:\Users\Nico\Desktop\Statistical Analysis\julianadata.csv"
        DBMS=CSV REPLACE;
    GETNAMES=YES;
    DATAROW=2;
RUN;
data bot; set bot;
date=day; run;
proc print data=bot; run;

************OLD COMPUTATION**************************;
** Organizing values;
data bot2; set bot;
if s1=2 then s1=1; if s2=2 then s2=1;
if s3=2 then s3=1; if s4=2 then s4=1;
if s5=2 then s5=1; if s6=2 then s6=1;
if s7=2 then s7=1; if s8=2 then s8=1;
if s9=2 then s9=1;
sum=s1+s2+s3+s4+s5+s6+s7+s8+s9;
mean=total/9;
event=0;
if total>0 then event=1;
drop s1 s2 s3 s4 s5 s6 s7 s8 s9;
total=9;
run;
proc print data=bot; run;
******************END*********************************;

** Plotting;
symbol1 v=dot c='black';
symbol2 v=dot c='red';
proc gplot data=bot;
where conc=0;
plot rot*day=temp;
run;
proc gplot data=bot;
where conc=0.63;
plot rot*day=temp;
run;


** BASIC GLMM;
```

```
* cheking the basic structure in a LINEAR model;
proc mixed data=bot;
class temp conc day rep;
model rot=temp|conc|day/outp=jresiduals;
random rep*temp*conc;
repeated day/ subject=rep*temp*conc type=cs;
run;

** Checking stat. assumptions;
*normality;
proc univariate data=jresiduals normal plot;
var resid ; histogram resid; qqplot resid; run;
* Variances;
proc sort data=jresiduals; by temp; run;
proc univariate data=jresiduals normal plot;
var resid; by temp; run;
proc sort data=jresiduals; by conc; run;
proc univariate data=jresiduals normal plot;
var resid; by conc; run;
proc sort data=jresiduals; by day; run;
proc univariate data=jresiduals normal plot;
var resid; by day; run;

* Checking unequal variances acros treatments;
proc mixed data=bot;
class temp conc day rep;
model rot=temp|conc|day/ddfm=sat;
*random rep*temp*conc;
repeated day/subject=rep*temp*conc type=cs;
run;
*AIC (smaller is better) -1298.8
*BIC (smaller is better) -1294.5;

proc mixed data=bot;
class temp conc day rep;
model rot=temp|conc|day/ddfm=sat;
*random rep*temp*conc;
repeated day/subject=rep*temp*conc type=cs group=temp;
run;
*AIC (smaller is better) -1310.6
*BIC (smaller is better) -1302.0 ;

proc mixed data=bot;
class temp conc day rep;
model rot=temp|conc|day/ddfm=sat;
*random rep*temp*conc;
```

```
repeated day/subject=rep*temp*conc type=cs group=conc;
run;
*AIC (smaller is better) -1301.9
*BIC (smaller is better) -1293.2 ;

proc mixed data=bot;
class temp conc day rep;
model rot=temp|conc|day/ddfm=sat;
*random rep*temp*conc;
repeated day/subject=rep*temp*conc type=cs group=conc*temp;
run;
*AIC (smaller is better) -1312.3
*BIC (smaller is better) -1295.0 ;


** Adjusting a repeated structure;
* Cheking the structure in the VAR/COV matrix;
proc mixed data=bot;
class temp conc day rep;
model rot=temp|conc|day/ddfm=sat;
*random rep*temp*conc;
repeated day/subject=rep*temp*conc type=cs group=conc*temp;
run;
*AIC (smaller is better) -1312.3
*BIC (smaller is better) -1295.0 ;

proc mixed data=bot;
class temp conc day rep;
model rot=temp|conc|day/ddfm=sat;
random rep*temp*conc;
repeated day/subject=rep*temp*conc type=ar(1) group=conc*temp;
run;
*AIC (smaller is better) -2568.2
*BIC (smaller is better) -2550.9 ;

proc mixed data=bot;
class temp conc day rep date;
model rot=temp|conc|day/ddfm=sat;
*random rep*temp*conc;
repeated day/subject=rep*temp*conc type=sp(pow) (date) group=conc*temp;
run;
*AIC (smaller is better) -2568.2
*BIC (smaller is better) -2550.9;
** Final model and reporting;
proc mixed data=bot;
class temp conc day rep;
```

```
model rot=temp|conc|day/ddfm=sat;
random rep*temp*conc;
repeated day/subject=rep*temp*conc type=ar(1) group=conc*temp;
lsmeans conc*day*temp/pdiff; * alpha=0.1;
ods output lsmeans=jmeans diffs=jdiff;
run;
proc print data=jmeans; run;


*reporting:plotting;
proc sort data=jmeans; by temp; run;
symbol1 v=dot c='black' i=join;
symbol2 v=dot c='red' i=join;
proc gplot data=jmeans;
by temp;
plot estimate*day=conc;
run;

proc sort data=jdiff; by temp; run;
proc print data=jdiff;
where day=_day AND temp=_temp AND probt<0.1; run;

** Final model and reporting;
proc mixed data=bot;
class temp conc day rep;
model rot=temp|conc|day/ddfm=sat;
random rep*temp*conc;
*repeated day/subject=rep*temp*conc type=ar(1) group=conc*temp;
estimate 'con vs conc 17-20' conc 1 -1 temp*conc 1 -1 0 0 conc*day 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0.25 0.25 0.25 0.25 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -0.25 -0.25 -0.25 -0.25 0 0 0 0
temp*conc*day 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0.25 0.25 0.25 0.25 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -0.25 -0.25 -0.25 -0.25 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0/e;
estimate 'con vs conc 17-21' conc 1 -1 temp*conc 1 -1 0 0 conc*day 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0.2 0.2 0.2 0.2 0.2 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -0.2 -0.2 -0.2 -0.2 -0.2 0 0 0
temp*conc*day 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0.2 0.2 0.2 0.2 0.2 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 -0.2 -0.2 -0.2 -0.2 -0.2 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0/e;
run;
```

EXPERIMENT 3 - GOMPERTZ MODEL

```
** 1. Importing dataset;
PROC IMPORT OUT= WORK.bot
        DATAFILE= "C:\Users\Nico\Desktop\Statistical
Analysis\jdlast\julianadata.csv"
        DBMS=CSV REPLACE;
     GETNAMES=YES;
     DATAROW=2;
RUN;
data bot; set bot;
date=day; run;
proc print data=bot; run;

** Plotting;
symbol1 v=dot c='black'; symbol2 v=dot c='red';
proc gplot data=bot;
where conc=0; plot rot*day=temp;
run;
proc gplot data=bot;
where conc=0.63; plot rot*day=temp;
run;

** 2. Fitting a Non-Linear function using proc nlin;
**************************************************;
* 2.1. Using Gompertz function;
proc sort data=bot; by conc; run;
proc nlin data=bot best=10 MAXITER=500 converge=0.05;
where temp=4;
by conc;
parms alpha=0.5 to 1.5 by 0.05 beta=0 to 1 by 0.1 theta=0 to 13 by 0.1;
pa=alpha; pb=beta; pc=theta;
model rot = pa*2.71828**(-2.71828**(-pb*(day-pc)));
ods output ParameterEstimates=estimate1 ANOVA=ANOVA1 corrb=corrb1;
output out=mypred1 predicted=pred;
run;
proc print data=mypred1; run;
proc print data=estimate1; run;
proc print data=ANOVA1; run;
proc print data=corrb1; run;

* 2.2. Extracting estimated parameters;
proc sql;
select std(estimate) as sta into :sta from estimate1 where parameter='alpha';
select std(estimate) as stb into :stb from estimate1 where parameter='beta';
select std(estimate) as stc into :stc from estimate1 where parameter='theta';
```

```
select mean(alpha) as corrab into :corrab from corrb1 where parameter='beta';
select mean(alpha) as corrac into :corrac from corrb1 where parameter='theta';
select mean(beta) as corrbc into :corrbc from corrb1 where parameter='theta';
select mean(ms) as sse into :sse from anova1 where source='Error';
quit;

* 2.3. Extracting predicted values - Gompertz function;
proc nlmixed data=bot MAXITER=500;
where temp=4;
by conc;
bounds 0.5 < alpha < 1.5; bounds beta < 0; bounds theta < 0;
parms alpha=0.80 beta=-600 theta=-0.35;
pa=alpha; pb=beta; pc=theta;
pred= pa*2.71828**(-2.71828**(-pb*(day-pc)));
model rot~normal(pred,s2);
predict pred out=pred1;
run;
proc nlmixed data=bot MAXITER=500;
where temp=23;
by conc;
bounds 0.5 < alpha < 1.5; bounds beta < 0; bounds theta < 0;
parms alpha=0.80 beta=-20 theta=-0.35;
pa=alpha; pb=beta; pc=theta;
pred= pa*2.71828**(-2.71828**(-pb*(day-pc)));
model rot~normal(pred,s2);
predict pred out=pred2;
run;

* 2.4. plotting an overlayed graph;
*extracting data means;
data preds; set pred1 pred2;
if temp=4 AND conc=0 then trt='Tem4-Conc0';
if temp=4 AND conc=0.63 then trt='Tem4-C0.63';
if temp=23 AND conc=0 then trt='T23-Conc0';
if temp=23 AND conc=0.63 then trt='T23-C0.63';
run;
proc print data=preds; run;
proc sort data=preds; by trt; by day; run;
proc means data=preds nway chartype;
class trt day; var rot pred;
output out=mms1; run;
proc print data=mms1; run;
data means1; set mms1;
where _stat_='MEAN';
drop _TYPE_ _FREQ_ _STAT_;
run;
```

```
proc print data=means1; run;
*plotting;
symbol1 color=red interpol=none value=dot;
symbol2 color=black interpol=none value=dot;
symbol3 color=blue interpol=none value=dot;
symbol4 color=gray interpol=none value=dot;
symbol5 color=red width=4 interpol=spline2s value=none;
symbol6 color=black width=4 interpol=spline2s value=none;
symbol7 color=blue width=4 interpol=spline2s value=none;
symbol8 color=gray width=4 interpol=spline2s value=none;
axis1 label=('Percentage') order=(0 to 1 by 0.1) width=3;
axis2 label=('Model') order=(0 to 1 by 0.1) width=3;
legend1 label=('Mean Percentage');
legend2 label=('Model fitted');
proc sort data=means1; by trt; run;
proc gplot data=means1;
plot rot*day=trt/ vaxis=axis1 legend=legend1;
plot2 pred*day=trt/ vaxis=axis2 legend=legend2;
run;
```

BIBLIOGRAPHY

BIBLIOGRAPHY

Abdel-Rahman, M. S., D. Couri, et al. (1979). "Kinetics of Cl02 and effects of Cl02, Cl02-, and Cl03- in drinking water on blood glutathione and hemolysis in rat and chicken." Journal of environmental pathology and toxicology 3(1-2): 431-449.

Abdul-Raouf, U. M., L. R. Beuchat, et al. (1993). "Survival and growth of Escherichia coli O157:H7 on salad vegetables." Appl. Environ. Microbiol. 59(7): 1999-2006.

Aday, M. S., M. B. Buyukcan, et al. (2012). "MAINTAINING THE QUALITY OF STRAWBERRIES BY COMBINED EFFECT OF AQUEOUS CHLORINE DIOXIDE WITH MODIFIED ATMOSPHERE PACKAGING." Journal of Food Processing and Preservation: no-no.

Agency for Toxic Substances and Disease Registry Division of Toxicology and Envirommental Medicine. (2004). "Toxicological Profile for Chlorine Dioxide and Chlorite." Retrieved June 1st, 2013, from http://www.atsdr.cdc.gov/toxprofiles/tp160.pdf.

Aieta, E. M. and J. M. Berg (1986). "A Review of Chlorine Dioxide in Drinking Water Treatment." J. Am. Water Works Assoc. 78 (6): 62-72.

Aieta, E. M., P. V. Roberts, et al. (1984). "Determination of Chlorine Dioxide, Chlorine, Chlorite, and Chlorate in Water." J. Am. Water Works Assoc. 76: 64-70.

Akin, E. W., J. C. Hoff, et al. (1982). "Waterborne outbreak control: which disinfectant?" Environmental Health Perspectives 46: 7-12.

Allende, A., F. A. Tomás-Barberán, et al. (2006). "Minimal processing for healthy traditional foods." Trends in Food Science & Technology 17(9): 513-519.

Appendini, P. and J. H. Hotchkiss (2002). "Review of antimicrobial food packaging." Innovative Food Science & Emerging Technologies 3(2): 113-126.

Arango, J., M. Rubino, et al. (2013). Quantification of Total Chlorine Dioxide Absorption by UV-visible Spectroscopy. Michigan, US, Michigan State University.

Balla, C. S. and J. Farkas (2007). Minimally Processed Fruits and Fruit Products and their Microbiological Safety. Handbook of Fruits and Fruit Processing, Blackwell Publishing: 115-128.

Bean, N. H. and P. M. Griffin (1990). "Foodborne disease outbreaks in the United States, 1973-1987: pathogens, vehicles, and trends." Journal of Food Protection 53.

Benarde, M. A., B. M. Israel, et al. (1965). "Efficiency of chlorine dioxide as a bactericide." Applied Microbiology 13(5): 776-780.

Benarde, M. A., W. B. Snow, et al. (1967). "Chlorine Dioxide Disinfection Temperature Effects." Journal of Applied Microbiology 30(1): 159-167.

Beuchat, L. (1998). "Surface decontamination of fruits and vegetables eaten raw: a review."

Beuchat, L. R. (1996). "Pathogenic Microorganisms Associated with Fresh Produce." Journal of Food Protection 59(2): 204-216.

Beuchat, L. R. (1999). "Survival of Enterohemorrhagic Escherichia coli O157:H7 in Bovine Feces Applied to Lettuce and the Effectiveness of Chlorinated Water as a Disinfectant." Journal of Food Protection 62: 845-849.

Beuchat, L. R. (2002). "Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables." Microbes and Infection 4(4): 413-423.

Bhagat, A. R. (2010). Modeling Critical Factors to Optimize the Treatment of Selected Fruits and Vegetables with Chlorine Dioxide Gas Using a Miniaturized Insustrial-Size Tunnel System. Doctor of Philosophy, Purdue University.

Bhaskara Reddy, M. V., K. Belkacemi, et al. (2000). "Effect of pre-harvest chitosan sprays on post-harvest infection by Botrytis cinerea and quality of strawberry fruit." Postharvest Biology and Technology 20(1): 39-51.

Brackett, R. E. (1999). "Incidence, contributing factors, and control of bacterial pathogens in produce." Postharvest Biology and Technology 15(3): 305-311.

Braun, P. and J. Sutton (1988). "Infection cycles and population dynamics of Botrytis cinerea in strawberry leaves." Canadian Journal of Plant Pathology 10(2): 133-141.

Centers for Disease Control and Prevention. (2011). "CDC Estimates of Foodborne Illness in the United States." Retrieved 9 April, 2013, 2013, from http://www.cdc.gov/foodborneburden/PDFs/FACTSHEET_A_FINDINGS_updated4-13.pdf.

Centers for Disease Control and Prevention. (2013). "Surveillance for Foodborne Disease Outbreaks — United States, 2009–2010." Retrieved June 1st, 2013, from http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6203a1.htm?s_cid=mm6203a1_w.

Condie, L. W. (1986). "Toxicological Problems Associated With Chlorine Dioxide." Journal (American Water Works Association) 78(6): 73-78.

Costilow, R. N., M. A. Uebersax, et al. (1984). "Use of Chlorine Dioxide for Controlling Microorganisms During the Handling and Storage of Fresh Cucumbers." Journal of Food Science 49(2): 396-401.

Couri, D., M. S. Abdel-Rahman, et al. (1982). "Toxicological Effects of Chlorine Dioxide, Chlorite and Chlorate." Environmental Health Perspectives 46: 13-17.

Czarneski, M. A. and P. Lorcheim (2005). "Isolator decontamination using chlorine dioxide gas." Pharmaceutical technology: 124-133.

Dagnas, S. and J.-M. Membre (2013). "Predicting and Preventing Mold Spoilage of Food Products." Journal of Food Protection 76(3): 538-551.

Dantigny, P., M. Bensoussan, et al. (2006). "Standardisation of methods for assessing mould germination: A workshop report." International journal of food microbiology 108(2): 286-291.

Dantigny, P., A. Guilmart, et al. (2005). "Basis of predictive mycology." International journal of food microbiology 100(1-3): 187-196.

Dantigny, P., S. Marín, et al. (2007). "Mould germination: Data treatment and modelling." International journal of food microbiology 114(1): 17-24.

Daş, E., G. C. Gürakan, et al. (2006). "Effect of controlled atmosphere storage, modified atmosphere packaging and gaseous ozone treatment on the survival of Salmonella Enteritidis on cherry tomatoes." Food Microbiology 23(5): 430-438.

Declerck, S., D. D'or, et al. (2001). "Modelling the sporulation dynamics of arbuscular mycorrhizal fungi in monoxenic culture." Mycorrhiza 11(5): 225-230.

Du, J., Y. Han, et al. (2002). "Inactivation by chlorine dioxide gas (ClO2) of Listeria monocytogenes spotted onto different apple surfaces." Food Microbiology 19(5): 481-490.

Du, J., Y. Han, et al. (2003). "Efficacy of chlorine dioxide gas in reducing Escherichia coli O157:H7 on apple surfaces." Food Microbiology 20(5): 583-591.

El-Samahy, S., B. M. Youssef, et al. (2000). "Microbiological and chemical properties of irradiated mango." Journal of food safety 20(3): 139-156.

Ellis, M., K. Cooksey, et al. (2006). "Quality of Fresh Chicken Breasts Using a Combination of Modified Atmosphere Packaging and Chlorine Dioxide Sachets." Journal of Food Protection 69: 1991-1996.

Exama, A., J. Arul, et al. (1993). "Suitability of Plastic Films for Modified Atmosphere Packaging of Fruits and Vegetables." Journal of Food Science 58(6): 1365-1370.

Garcia, D., A. J. Ramos, et al. (2009). "Predicting mycotoxins in foods: A review." Food Microbiology 26(8): 757-769.

Gibson, A. M., N. Bratchell, et al. (1987). "The effect of sodium chloride and temperature on the rate and extent of growth of Clostridium botulinum type A in pasteurized pork slurry." Journal of Applied Microbiology 62(6): 479-490.

Gómez-López, V. M., A. Rajkovic, et al. (2009). "Chlorine dioxide for minimally processed produce preservation: a review." Trends in Food Science & Technology 20(1): 17-26.

Gougouli, M. and K. P. Koutsoumanis (2012). "Modeling germination of fungal spores at constant and fluctuating temperature conditions." International journal of food microbiology 152(3): 153-161.

Greenberg, A. E., L. S. Clesceri, et al. (1992). Standard methods for the examination of water and wastewater. Washington, D.C., American Public Health Association: 4-56 - 54.81.

Gustavsson, J., C. Cederberg, et al. (2011). Global Food Losses and Food Waste. International Congress SAVE FOOD!, Dusseldorf, Germany.

Han, Y., J. D. Floros, et al. (2001). "Response Surface Modeling for the Inactivation of Escherichia coli O157:H7 on Green Peppers (Capsicum annuum L.) by Chlorine Dioxide Gas Treatments." Journal of Food Protection 64: 1128-1133.

Han, Y., A. M. Guentert, et al. (1999). "Efficacy of chlorine dioxide gas as a sanitizer for tanks used for aseptic juice storage." Food Microbiology 16(1): 53-61.

Han, Y., R. H. Linton, et al. (2000). "Inactivation of Escherichia coli O157:H7 on surface-uninjured and -injured green pepper (Capsicum annuum L.) by chlorine dioxide

gas as demonstrated by confocal laser scanning microscopy." Food Microbiology 17(6): 643-655.

Han, Y., R. H. Linton, et al. (2001). "Reduction of Listeria monocytogenes on Green Peppers (Capsicum annuum L.) by Gaseous and Aqueous Chlorine Dioxide and Water Washing and Its Growth at 7C." Journal of Food Protection 64: 1730-1738.

Han, Y., T. L. Selby, et al. (2004). "Decontamination of Strawberries Using Batch and Continuous Chlorine Dioxide Gas Treatments." Journal of Food Protection 67: 2450-2455.

Han, Y., D. M. Sherman, et al. (2000). "The effects of washing and chlorine dioxide gas on survival and attachment of Escherichia coli O157: H7 to green pepper surfaces." Food Microbiology 17(5): 521-533.

Harris, L. J., J. N. Farber, et al. (2003). "Outbreaks Associated with Fresh Produce: Incidence, Growth, and Survival of Pathogens in Fresh and Fresh-Cut Produce." Comprehensive Reviews in Food Science and Food Safety 2: 78-141.

Hautman, D. P., D. J. Munch, et al. (1997). "US EPA Method 300.1, Determination of Inorganic Anions in Drinking Water by Ion Chromatography, EPA/600/R-98/118, NIST PB-98-169196INZ." (Revision 1.0).

Heffernan, W., C. Guion, et al. (1979). "Oxidative damage to the erythrocyte induced by sodium chlorite, in vivo." Journal of environmental pathology and toxicology 2(6): 1487.

Heller, M. C. and G. A. Keoleian (2000). Life cycle-based sustainability indicators for assessment of the US food system, Center for Sustainable Systems, University of Michigan.

Jarvis, W. R. (1962). "The infection of strawberry and raspberry fruits by Botrytis cinerea Fr." Annals of Applied Biology 50(3): 569-575.

Kaczur, J. J. and D. W. Cawlfield (2000). Chlorine Oxygen Acids and Salts, Chlorous Acid, Chlorites, and Chlorine Dioxide. Kirk-Othmer Encyclopedia of Chemical Technology. John Wiley and Son. New York. NY, John Wiley & Sons, Inc.: 1-26.

Kallio, H., M. Hakala, et al. (2000). "Sugars and acids of strawberry varieties." European Food Research and Technology 212(1): 81-85.

Kantor, L. S., K. Lipton, et al. (1997). "Estimating and addressing America’s food losses." Food Review 20(1): 2-12.

Keskinen, L. A. and B. A. Annous (2011). Chlorine Dioxide (Gas). Nonthermal Processing Technologies for Food. H. Q. Zhang, G. V. Barbosa-Cánovas, V. M. Balasubramaniamet al. Oxford, UK, Wiley-Blackwell: 359 - 365.

Keskinen, L. A., A. Burke, et al. (2009). "Efficacy of chlorine, acidic electrolyzed water and aqueous chlorine dioxide solutions to decontaminate Escherichia coli O157:H7 from lettuce leaves." International Journal of Food Microbiology 132(2-3): 134-140.

Kim, J., M. R. Marshall, et al. (1999). "Determination of Chlorate and Chlorite and Mutagenicity of Seafood Treated with Aqueous Chlorine Dioxide." Journal of Agricultural and Food Chemistry 47(9): 3586-3591.

Kim, J. Y., H. J. Kim, et al. (2010). "The effects of aqueous chlorine dioxide or fumaric acid treatment combined with UV-C on postharvest quality of ‘Maehyang’ strawberries." Postharvest Biology and Technology 56(3): 254-256.

Knapp, J. E. and D. L. Battisti (2001). Chlorine Dioxide. Disinfection, Sterilization, and Preservation. S. S. Block. Philadelphia, Lippincott Williams & Wilkins: 215-227.

Kromminga, H., J. Orphal, et al. (2003). "New measurements of OClO absorption cross-sections in the 325–435 nm region and their temperature dependence between 213 and 293 K." Journal of Photochemistry and Photobiology A: Chemistry 157(2–3): 149-160.

Lee, S.-Y., M. Costello, et al. (2004). "Efficacy of Chlorine Dioxide Gas as a Sanitizer of Lettuce Leaves." Journal of Food Protection 67: 1371-1376.

Linton, R., Y. Han, et al. (2006). "Gas-/vapor-phase sanitation (decontamination) treatments." Microbiology of fruits and vegetables: 401-435.

López, S., M. Prieto, et al. (2004). "Statistical evaluation of mathematical models for microbial growth." International journal of food microbiology 96(3): 289-300.

Lynch, M., R. Tauxe, et al. (2009). "The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities." Epidemiology and infection 137(3): 307.

Maas, J. (1995). Compendium of Strawberry Diseases. APS Press, St. Paul, MN.

Mahmoud, B. S. M., A. R. Bhagat, et al. (2007). "Inactivation kinetics of inoculated Escherichia coli O157:H7, Listeria monocytogenes and Salmonella enterica on strawberries by chlorine dioxide gas." Food Microbiology 24(7–8): 736-744.

Mahovic, M., J. A. Bartz, et al. "Chlorine Dioxide Gas from an Aqueous Solution: Reduction of Salmonella in Wounds on Tomato Fruit and Movement to Sinks in a Treatment Chamber." Journal of Food Protection® 72(5): 952-958.

Netramai, S. (2011). Utilization of chlorine dioxide gas in food packaging application. Doctor of Philosophy Ph.D., Michigan State University.

Nieuwenhuijsen, M. J., M. B. Toledano, et al. (2000). "Uptake of chlorination disinfection by-products; a review and a discussion of its implications for exposure assessment in epidemiological studies." Journal of exposure analysis and environmental epidemiology 10(6 Pt 1): 586-599.

O'Beirne, D. and G. Francis (2003). "Reducing the pathogen risk in MAP-prepared produce." Novel food packaging techniques 12: 231-275.

Parish, M. E., L. R. Beuchat, et al. (2003). "Methods to Reduce/Eliminate Pathogens from Fresh and Fresh-Cut Produce." Comprehensive Reviews in Food Science and Food Safety 2: 161-173.

Peano, C., V. Girgenti, et al. (2012). Strawberry Production Chain: Application of a LCA model Acta Horticulturae. (ISHS)

Pepich, B. V., T. A. Dattilio, et al. (2007). "An improved colorimetric method for chlorine dioxide and chlorite ion in drinking water using lissamine green B and horseradish peroxidase." Analytica Chimica Acta 596(1): 37-45.

Phillips, C. A. (1996). "Review: Modified Atmosphere Packaging and its effects on the microbiological quality and safety of produce." International Journal of Food Science & Technology 31(6): 463-479.

Plattner, K. and A. Perez. (2012). "United States Department of Agriculture (USDA) Report: Fruit and Tree Nuts Outlook." from http://www.ers.usda.gov/media/970458/fts354.pdf.

Powelson, R. (1960). "Initiation of strawberry fruit rot caused by Botrytis cinérea." Phytopathology 50: 491-494.

Qingdong, X., Z. Guangming, et al. (2006). "Study on Subchronic Toxicity of Chlorine Dioxide and By-Products in Water." Journal of Environmental Science and

Health, Part A: Toxic/Hazardous Substances and Environmental Engineering 41(7): 1347 - 1353.

Reina, L. D., H. P. Fleming, et al. "Microbiological Control of Cucumber Hydrocooling Water with Chlorine Dioxide." Journal of Food Protection 58(5): 541-546.

Rico, D., A. B. Martín-Diana, et al. (2007). "Extending and measuring the quality of fresh-cut fruit and vegetables: a review." Trends in Food Science & Technology 18(7): 373-386.

Roberts, R. G. and S. T. Reymond (1994). "Chlorine dioxide for reduction of postharvest pathogen inoculum during handling of tree fruits." Applied and environmental microbiology 60(8): 2864-2868.

Robinson, J. W., Skelly Frame, Frame II (2005). Undergraduate Instrumental Analysis. New York.

Rodgers, S. L., J. N. Cash, et al. (2004). "A Comparison of Different Chemical Sanitizers for Inactivating Escherichia coli O157:H7 and Listeria monocytogenes in Solution and on Apples, Lettuce, Strawberries, and Cantaloupe." Journal of Food Protection 67(4): 721-731.

Schilder, A. M. C., J. M. Gillett, et al. (2013). "Evaluation of fungicides for control of Botrytis fruit rot and leaf spot in strawberry, 2012." Plant Disease Management Reports.

Seo, K. H. and J. F. Frank (1999). "Attachment of Escherichia coli O157:H7 to Lettuce Leaf Surface and Bacterial Viability in Response to Chlorine Treatment as Demonstrated by Using Confocal Scanning Laser Microscopy." Journal of Food Protection 62: 3-9.

Seymour, I. J. and H. Appleton (2001). "Foodborne viruses and fresh produce." Journal of Applied Microbiology 91(5): 759-773.

Singh, N., R. K. Singh, et al. (2002). "Effect of inoculation and washing methods on the efficacy of different sanitizers against Escherichia coli O157:H7 on lettuce." Food Microbiology 19(2-3): 183-193.

Sloan, A. E. (2013). "Top 10 Food Trends." from http://sloantrend.com/attachments/article/9/2013-04IFTTop10Trends.pdf.

Spotts, R. and B. Peters (1980). "Chlorine and chlorine dioxide for control of d'Anjou pear decay." Plant Dis 64(1): 2.

Staschower, F. (2012). Residues from Chlorine Dioxide Gas Treatment, Generated by Different Delivery Systems, on Fresh Produce. Master of Science, Michigan State University.

Sumathi, S., R. F. Cindy, et al. (2004). "Fresh Produce: A Growing Cause of Outbreaks of Foodborne Illness in the United States, 1973 through 1997." Journal of Food Protection 67(10): 2342-2353.

Sy, K. V., K. H. McWatters, et al. (2005). "Efficacy of Gaseous Chlorine Dioxide as a Sanitizer for Killing Salmonella, Yeasts, and Molds on Blueberries, Strawberries, and Raspberries." Journal of Food Protection 68: 1165-1175.

Sy, K. V., M. B. Murray, et al. (2005). "Evaluation of Gaseous Chlorine Dioxide as a Sanitizer for Killing Salmonella, Escherichia coli O157:H7, Listeria monocytogenes, and Yeasts and Molds on Fresh and Fresh-Cut Produce." Journal of Food Protection 68: 1176-1187.

Trinetta, V., N. Vaidya, et al. (2011). "Evaluation of Chlorine Dioxide Gas Residues on Selected Food Produce." Journal of Food Science 76(1): T11-T15.

Tsai, L. S., C. C. Huxsoll, et al. (2001). "Prevention of Potato Spoilage During Storage by Chlorine Dioxide." Journal of Food Science 66(3): 472-477.

United States Department of Agriculture (USDA). (2003). "Pathogen modelling program, version 7.0." Retrieved June 1st, 2013, from http://ars.usda.gov/services/docs.htm?docid=6786.

United States Environmental Protection Agency (EPA) (1999). "Alternative Disinfectants and Oxidants Guidance Manual: Chapter 4. Chlorine Dioxide, EPA 815-R-99-014."

United States Environmental Protection Agency (EPA) (2006). Registration eligibility decision for Chlorine Dioxide and Sodium Chlorite (Case Number 4023). http://www.epa.gov/oppsrrd1/REDs/chlorine_dioxide_red.pdf.

United States Environmental Protection Agency (EPA). (2007). "Anthrax spore decontamination using chlorine dioxide." Retrieved June 1st, 2013, from http://www.epa.gov/pesticides/factsheets/chemicals/chlorinedioxidefactsheet.htm.

United States Food and Drug Administration (FDA). (2004). "Produce Safety From Production to Consumption: 2004 Action Plan to Minimize Foodborne Illness Associated with Fresh Produce Consumption." Retrieved June 1st, 2013, from

http://www.fda.gov/Food/FoodborneIllnessContaminants/BuyStoreServeSafeFood/ucm129487.htm.

United States Food and Drug Administration (FDA). (2011). "Agency Response Letter GRAS Notice No. GRN 000062." Retrieved June 1st, 2013, from http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm153971.htm.

United States Food and Drug Administration (FDA). (2012). "Secondary direct food additives permitted in food for human consumption. Chlorine dioxide. CFR Title 21, part 173.300." Retrieved 9 Apr 2013, 2013, from http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=173.300.

Vardar, C., K. Ilhan, et al. (2012). "The application of various disinfectants by fogging for decreasing postharvest diseases of strawberry." Postharvest Biology and Technology 66(0): 30-34.

Wahner, A., G. S. Tyndall, et al. (1987). "Absorption cross sections for symmetric chlorine dioxide as a function of temperature in the wavelength range 240-480nm." The Journal of Physical Chemistry 91(11): 2734-2738.

Wei, C. I., T. S. Huang, et al. (1995). "Growth and Survival of Salmonella montevideo on Tomatoes and Disinfection with Chlorinated Water." Journal of Food Protection 58(8): 829-836.

Williamson, B., B. Tudzynski, et al. (2007). "Botrytis cinerea: the cause of grey mould disease." Molecular Plant Pathology 8(5): 561-580.

Windholz, M., S. Budavari, et al. (1976). The Merck index. An encyclopedia of chemicals and drugs. Rahway, New Jersey, Merck & Co.

Wise, J. C., L. J. Gut, et al. (2012). Michigan Fruit Management Guide 2013. Extension Bulletin E-154. East Lansing, MI, Michigan State University.

Yuk, H.-G., J. A. Bartz, et al. (2006). "The Effectiveness of Sanitizer Treatments in Inactivation of Salmonella spp. from Bell Pepper, Cucumber, and Strawberry." Journal of Food Science 71(3): M95-M99.