RESIDUES FROM CHLORINE DIOXIDE GAS TREATMENT, GENERATED BY DIFFERENT DELIVERY SYSTEMS, ON FRESH PRODUCE

By

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ABSTRACT

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Chlorine dioxide (ClO_2) gas, a promising sanitizing agent, could be deliver in the package directly as a gas or could be generated in situ by using a solid or a ClO_2 solution. The goal of this research was to quantify residues that remain in the produce surface after treatment with the different ClO_2 delivery systems.

After treating cherry tomatoes and fresh cut romaine lettuce with the different ClO_2 delivery system the residues (ClO_2 and Chlorite) on the produce surface were recovered and quantified with an amperometric titration method. The produce were treated at different: ClO_2 concentration, exposure time and temperature. Also the ClO_2 gas concentration profile for the different delivery system was created with an ultraviolet spectrophotometer.

It was found that when the ClO_2 is generated in situ and lower temperature the residues are lower. A model was developed for the prediction of residue in function of delivery system, concentration, time and temperature.

To those I love…

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CHAPTER 1 – INTRODUCTION

Since 1976, consumption of fresh produce in US has increased by 55% (Center for Disease Control and Prevention 2011). Centers for Disease Control and Prevention (CDC) that monitors foodborne cases of illness in the country has correlate 50% of them with the consumption of raw or minimally processed foods. In 2008, the CDC reported 1,034 cases of contaminated produce in United States, resulting in 23,152 incidents of reported illness with 22 deaths (Center for Disease Control and Prevention 2011) . In order to prevent future outbreaks, sanitizing steps were added to the food production line. Washing the produce with sanitizing solutions is the most common method used (Harris *et al.* 2003; Parish *et al.* 2003). Another method is the use of packaging systems that contain antimicrobial agents in order to sanitize the produce and prolong its shelf life. This can be done by coating the package material, inserting a sachet, or even changing the composition of the headspace by adding a sanitizing agents inside the produce package (Appendini and Hotchkiss 2002).

Different chemical compounds are being used, specially Chlorine Dioxide (ClO_2) that has been identified as a promising sanitizing agent for fruit and vegetables (Han *et al.* 1999; Sapers *et al.* 2003; Sy *et al.* 2005a; Yuk *et al.* 2006; Gómez-López *et al.* 2009; Netramai 2010).

Chlorine dioxide is effective due to its high oxidation potential that can inactivate a wide variety of microorganisms present in fresh produce. It was reported to be

effective against many pathogenic microorganisms, such as *Escherichia coli O157:H7, Listeria monocytogenes, and Salmonella* spp. (Rodgers *et al.* 2004; Ellis *et al.* 2006; Gómez-López *et al.* 2009). Unlike chlorine, widely used antimicrobial agent, chlorine dioxide produce less chlorinated by-products and does not produce carcinogenic compounds being an advantage in the sanitizing process (Gómez-López *et al.* 2009).

Chlorine dioxide 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. As a gas, ClO_2 can penetrate produce irregularities and areas that a solution cannot reach, making it a more effective sanitizer, inactivating more quantity of microorganisms (Han *et al.* 2001b; Du *et al.* 2002; Lee *et al.* 2004). Also, the moisture from the solution, left on the produce surface, increased the risk of growing molds (Trinetta *et al.* 2011b).

Gas ClO_2 can be introduced into a food packaging system throughout sachet, containing dry chemicals that will react and produce gas (Ellis *et al.* 2006; Shin 2007) or by inserting the gas directly using the reaction of chlorine gas and sodium chlorite. Those methods will generate different concentrations of gas. The sachet will deliver the gas that will permeate its wall, increasing the concentration over time until a maximum concentration is achieved. On the other hand, the direct injection gas, can be done with a commercial generator of ClO_2 that will deliver a constant concentration of gas throughout the treatment time, controlling the parameters automatically.

Another way that ClO_2 gas can be generated is by being released from a solution of ClO_2 and water in a closed chamber concentrating in the headspace, creating a ClO_2 environment. The microorganism inactivation with ClO_2 solution was studied by Netramai (2010) and she determined the interaction between lettuce and the ClO_2 gas.

Applying ClO_2 to a produce package raises some concerns that need to be evaluated. The first concern is related to how the gas will interact with the packaging materials, how much of the gas is being permeated through the package walls, and how it is affecting the materials. According to Netramai (2010) PET, nylon, BOPP, PLA and multilayer EVA/EVOH/EVA reported to be the most effective barriers to ClO_2. EVA/EVOH/EVA was the material with the highest barrier properties and its mechanical performance was not changed after ClO_2 exposure (Netramai 2010).

The second concern is related to how the gas circulates throughout the package in order to assure that the whole produce surface will be expose to the gas. With a good distribution the produce could accomplish an effective reduction of microorganisms. Various authors have studied different fruits and vegetables contaminated with different microorganisms using ClO_2 gas as a sanitizer. For example, a 5 log reduction of *Salmonella* with 0.5 mg/L for 10 min was achieved for tomatoes when used gas ClO_2 (Bhagat 2010) and lettuce contaminated with *E. coli* treated with 5 mg/L for 10 min showed a log reduction of 3.9 (Mahmoud and Linton 2008).

The interaction of the produce with ClO_2, specifically the amount of gas absorbed by the produce is the third concern. ClO_2 rapidly oxidizes forming chlorite ions (by-products) that will interact with the plant tissue of treated produce, possibly changing its nutrients, and physiology. (Trinetta *et al.* 2011b). Toxicological studies have shown that exposure by large amounts of ClO_2 can cause mouth, esophagus, or stomach irritation and problems with oxygen in blood, but it has not proved to be carcinogenic (Agency for Toxic Substances and Disease Registry Division of Toxicology and Envirommental Medicine 2004). The US Enviromental Protection Agency (EPA) has set 1mg/L as the maximum contaminant level chlorite and 0.8 mg/l for ClO_2 in drinking water treated with ClO_2.

Some of the studies focusing on ClO_2 absorption used a continuous concentration delivery system, which resulted in high absorption levels on lettuce and alfalfa sprouts (Trinetta *et al.* 2011b). However as mentioned before, a non-continuous ClO_2 generation system may show a different concentration profile, and the amount of gas in contact with the produce surface is different. Also a non-continuous system is a viable way to introduce the gas in the package system facilitating transport and logistics. The insertion of ClO_2 gas into a package is not yet approved by the US Food and Drug Administration (FDA) or US Department of Agriculture (USDA) due to high absorptions values and unknown effects on fruit and vegetable physiology and nutrient stability.

The goal of this study was to determine the absorption of ClO_2 and its by-products in fresh produce using different gas delivery systems and build a model that describe this behavior. The delivery systems consist of sachet, ClO_2 solution, and Minidox. Environmental conditions such, as temperature, exposure time and concentration were also taken into account. For this study two different produces types were chosen to evaluate the effect of produce surface on the absorption of ClO_2 gas and by-products.

The resulting data may be considered for the development of new regulations since most of the research was done with different ClO_2 delivery systems but the actual ClO_2 concentration profile and comparisons between delivery systems has not been discussed in any of the reported research.

CHAPTER 2 – LITERATURE REVIEW

2.1. Background

From 1976 to 2010 the consumption of fresh produce increased 26.7% while a 3.6% reduction on the consumption of processed fruits and vegetables (Cook 2011). This trend is due to the promotion and availability of fruits and vegetables took place in the same period, allowing the population to have easy access to those products (Harris *et al.* 2003). Is also related to the adoption of a healthy diet, rich in fresh or minimally processed produce, that according to scientific studies improves quality of life and prevents chronic and cardiovascular diseases (Bhagat 2010).

Produce is defined as minimally processed if the fruits or vegetables are processed (cut, clean or packed) by non-thermal methods in order to delivery a convenient and ready to eat food for consumers maintaining high quality, extending its freshness during storage and distribution (Allende *et al.* 2006; Balla and Farkas 2007; Bhagat 2010). Minimally processed fruits and vegetables normally do not includes preservatives (Seymour and Appleton 2001). The processing of fresh produce can expose produce cells and accelerates their degradation, changing color, texture and flavor and also letting the produce more exposed to possible microbial contamination (Allende *et al.* 2006).

During harvesting and sanitizing of fresh produce, once contaminated with pathogens is a risk as it can contaminate other produce throughout the packing line

causing a cross-contamination. This situation could be source of foodborne disease outbreak (FBDO) that can affect many consumers (Olsen *et al.* 2000).

2.2. Food safety

FBDO is a concern because raw fruits and vegetables can carry many kinds of microorganisms as they can easily attach to fresh products surface growing and reproducing (Bhagat 2010). The produce contamination may occur during harvest, processing and distribution; up to consumption. Contamination in any of the process step is critical as it can easily propagate with the potential to develop into an outbreak.

In the pre-harvest stage the soil can be contaminated by animal waste or flooding, that brings pollution from municipal and industrial wastes. During planting and harvesting the contamination can come from workers without a correct hygiene and from irrigation with contaminated water. Contamination can also take place during the processing step as it involves human contact, handling the produce to wash, cut, slice and package. During distribution the produce could be to contaminants and higher temperatures that promote the growth of microorganisms, therefore using appropriate shipping protocols to avoid excessive heat and allow circulation of air is critical (Brackett 1999; Gorny *et al.* 2006). A food production chain, with all possible contamination steps, is shown in Figure 1.

Also there are microorganisms that are responsible for affecting the quality of the fresh produce and reduce its shelf life and do not cause human health. Some of the critical pathogenic microorganism are: *Shigella* spp., *Salmonella*, *Escherichia coli*,

Campylobacter spp., *Listeria monocytogenes*, *Yersinia enterocolitica*, *Bacillus cereus*, *Clostridium botulinum*, viruses, and parasites such as *Giardia lamblia*, *Cyclospora cayetanensis*, and *Cryptosporidium parvum* (Beuchat 2002).

2.3. Strategies to assure safety and quality of fresh produce

Different preventive actions can be considered in order to mitigate contamination of fresh produce throughout the supply chain. Some of these actions may include a pre-planting step, it is critical to ensure that the area has not been used for life stock production and was not subjected to flooding as this could seriously compromise the soil quality due to presence of feces (Brackett 1999).

It is important to educate produce growers and handlers about good hygiene habits and manufacture practices; this will prevent contamination during harvesting and throughout packing line, specially washing, cutting and packaging, stages that may require human intervention. Also it is crucial to ship the produce using adequate distributions systems that are able to control temperature, humidity and hygiene conditions (Brackett 1999).

Specially for the distribution of safer produce to consumer new strategies were implemented during processing and packaging line. The strategy consists of treating the produce with sanitizing agents that are able kill microorganisms without damaging the produce. Many sanitizing agents are available, but their efficacy will depend on the characteristics of the produce surface, the type of pathogen, the application method and other variables such as temperature and pH (Beuchat 1998). Some of the disinfection

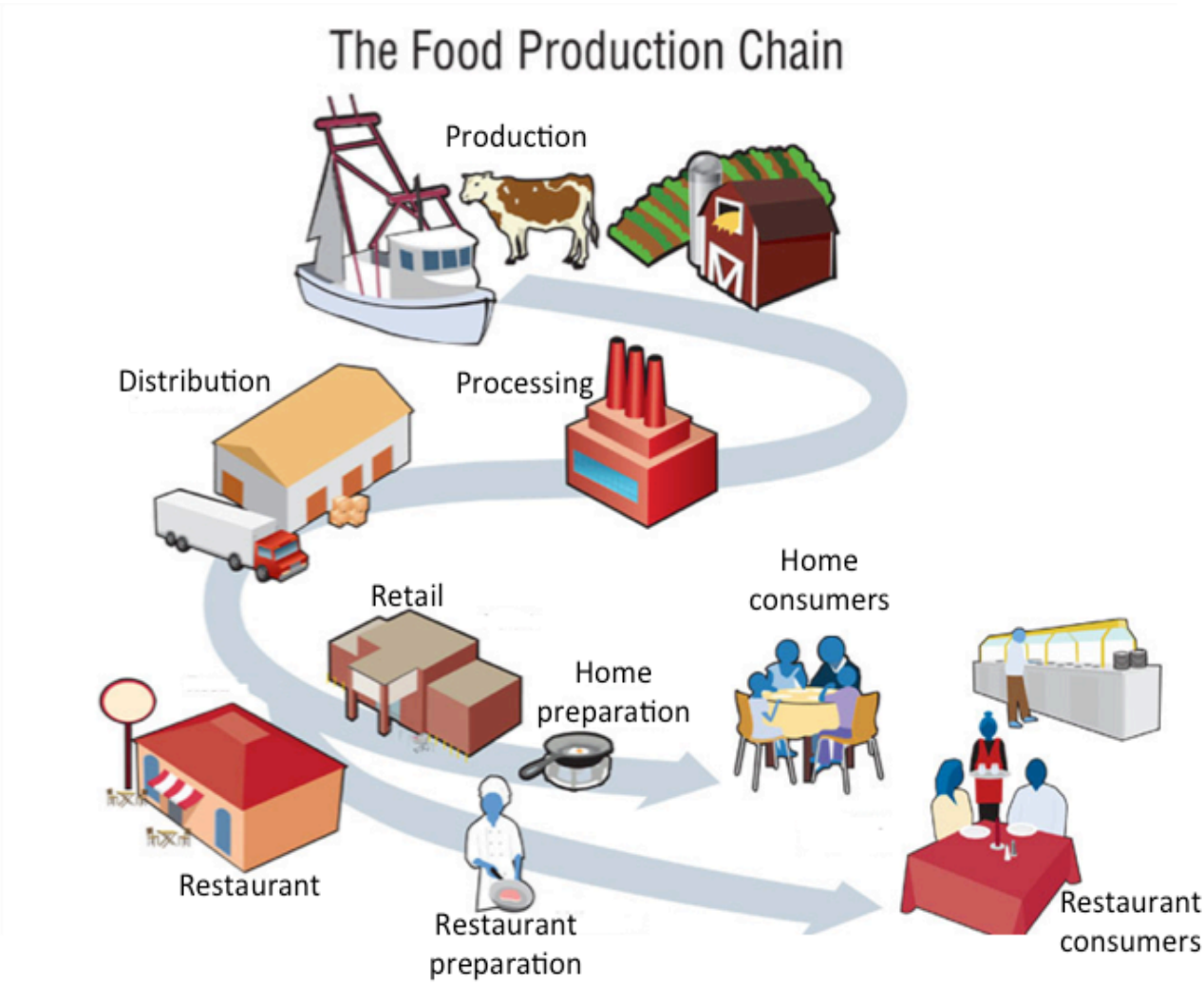

Figure 2.1 - Food production chain, stages where is possible an pathogen contamination (Centers for Disease Control and Prevention 2010)

For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

treatments are discussed below. They are known not to be 100% effective but to kill part of the population(Beuchat 1998).

2.3.1 Sanitization agents available

Different sanitizing agents are currently being used. Some of the advantages and disadvantages are outlined below.

Water: the produce is washed with tap water and packed. This process is not efficient in removing microorganism attached to irregular surface (Gorny *et al.* 2006) but with a vigorous washing it is possible to remove 10-100 fold of microorganism population (Beuchat 1998).

Chlorine can be used with water as a washing solution or as a spray to sanitize produce surfaces or the package operation (Parish *et al.* 2003; Bhagat 2010). It inactivates microorganism with the action of hypochlorous acid (HOCl), the free form of chlorine, it can form chlorinated compounds as trihaloethanes, considered carcinogenic (Parish *et al.* 2003; Bhagat 2010). Chorine is generally used in concentration between 50-200ppm for treatments of 1-2 minutes (Beuchat 1998).It is reported to reduce less than 2 log CFU g^{-1} on vegetables and fruits.

Peroxyacetic acid is a strong oxidizing agent, non-corrosive, that is added to the washing water with a maximum concentration of 80 ppm (Bhagat 2010). Its action is not affected by temperature and presence of organic compounds (Rodgers *et al.* 2004). It was reported to significantly reduce *Salmonella* and *E. coli* O157:H7 on the surface of cantaloupe and honeydew melon (Park and Beuchat 1999). But it was considered the

worse sanitizer agent by Rodgers (2004) when compared with solutions of sodium hypochlorite, chlorine dioxide and ozone but in the same comparison it was considered the best agent in decreasing mold and yeast.

Hydrogen peroxide acts as an oxidizing agent and its rapid breakdown makes it a good sanitizing agent for food surfaces (Rico *et al.* 2007). Its effectiveness depends on temperature and pH, which need to be controlled during treatment. It is more effective as vapor, but this treatment caused significant changes on quality characteristics of the treated fresh produce (Beuchat 1998). For example, shredded lettuce after treatment with H_2O_2 solution was brown (Rico *et al.* 2007).

Irradiation with gamma radiation is the most effective sanitizing treatment (Beuchat 1998), consist of beams that accelerate the ions to interact with microorganisms and inactivate them. This technique can inactivate spoilage microorganisms and pathogens present in a produce surface (El-Samahy *et al.* 2000). It is observed the formation of off-flavors in some fruits treatments, but is still necessary to test the dose tolerance for most produce disinfection and effects in sensory aspects (Beuchat 1998; Parish *et al.* 2003). The maximum dose permitted by FDA is 1 kGy that was not efficient in killing microbial population in some produces so it is necessary to combine this technique with other sanitizing strategies (Hagenmaier and Baker 1998). Mangoes has their shelf life doubled after combination of irradiation (1.0 kGy) and hot water without affecting its sensory characteristics (El-Samahy *et al.* 2000).

Ozone is used in gaseous form or dissolved in water, it is a strong oxidizing agent able to destroy microbial cells (Rico *et al.* 2007). It has strong penetrability in produce surface, and it will decompose into non-toxic product, and its performance is not affected by pH (Rodgers *et al.* 2004; Rico *et al.* 2007). Treatment with ozonated water showed an increase in shelf life of apples, grapes, oranges, pears, raspberries, and strawberries (Beuchat 1998), but it is known to cause some changes in fruit quality, for example cherry tomatoes were treated with ozone and turned yellow (Daş *et al.* 2006). Another concern related to this disinfectant is that ozone cannot be transported, due to its high corrosiveness, and has to be generated onsite (Beuchat 1998).

An approach to sanitize fresh produce is to use the Hurdle technology. This technique combines a series of sanitizing strategies and intervention methods in order to achieve a better disinfecting outcome without affecting the produce quality (Rico *et al.* 2007). It involves washing with different sanitizing agents, thermal and photochemical treatments, storage conditions, and the use of active packaging all combined with a synergistic action (Allende *et al.* 2006; Netramai 2010). This technology is reported to be effective in extending the produce shelf life and decreasing the pathogens on their surface (Allende *et al.* 2006).

All these sanitizer's agents are still in use but studies continue in order to identify the best approach for each specific applications. New sanitizers are being implemented, is the case of Chlorine Dioxide (ClO_2) it is a promising option as a sanitizer agent to disinfect fresh produce surface in efficient way.

2.4. Chlorine Dioxide

In 1950 Chlorine Dioxide (ClO_2) started to be used as a sanitizing agent in the treatment of drinking water in order to substitute chlorine, as it does not produce chlorinated by-products and can control the odor and taste of the final disinfected product (USEPA 1999; Keskinen and Annous 2011). Currently ClO_2 is also used in the pharmaceutical industry to sterilize equipment and as bleaching agent for the paper and pulp production (Keskinen and Annous 2011). Recent studies have been carried out in order to expand the application of ClO_2 as a sanitizer for fresh produce since it has a broad microorganism spectrum of inactivation.

2.4.1. Physical and chemical properties

Chlorine dioxide is a small, volatile and highly oxidant molecule (USEPA 1999). In the gas phase has a greenish yellow color and a strong characteristic odor. It exists most exclusively as free radicals as shown in Figure 2.2 (Knapp and Battisti 2001).

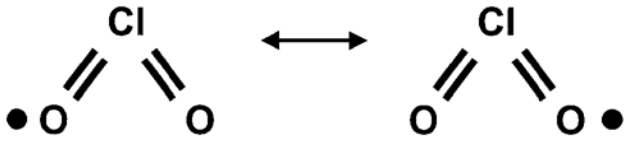

Figure 2.2 - Free radical structure of chlorine dioxide (Knapp and Battisti 2001)

Chlorine dioxide is stable in the dark and at room temperature. It is highly soluble in water, especially cold water (Mueller and Willner 1993; USEPA 1999; Kaczur and Cawlfield 2000; Gómez-López *et al.* 2009; Netramai 2010). Its solubility in water at different temperatures is shown in Figure 3. At 25°C the concentration of ClO_2 in solution is 23 times higher than the concentration of the gas phase in which it is in equilibrium (Gordon *et al.* 2007).

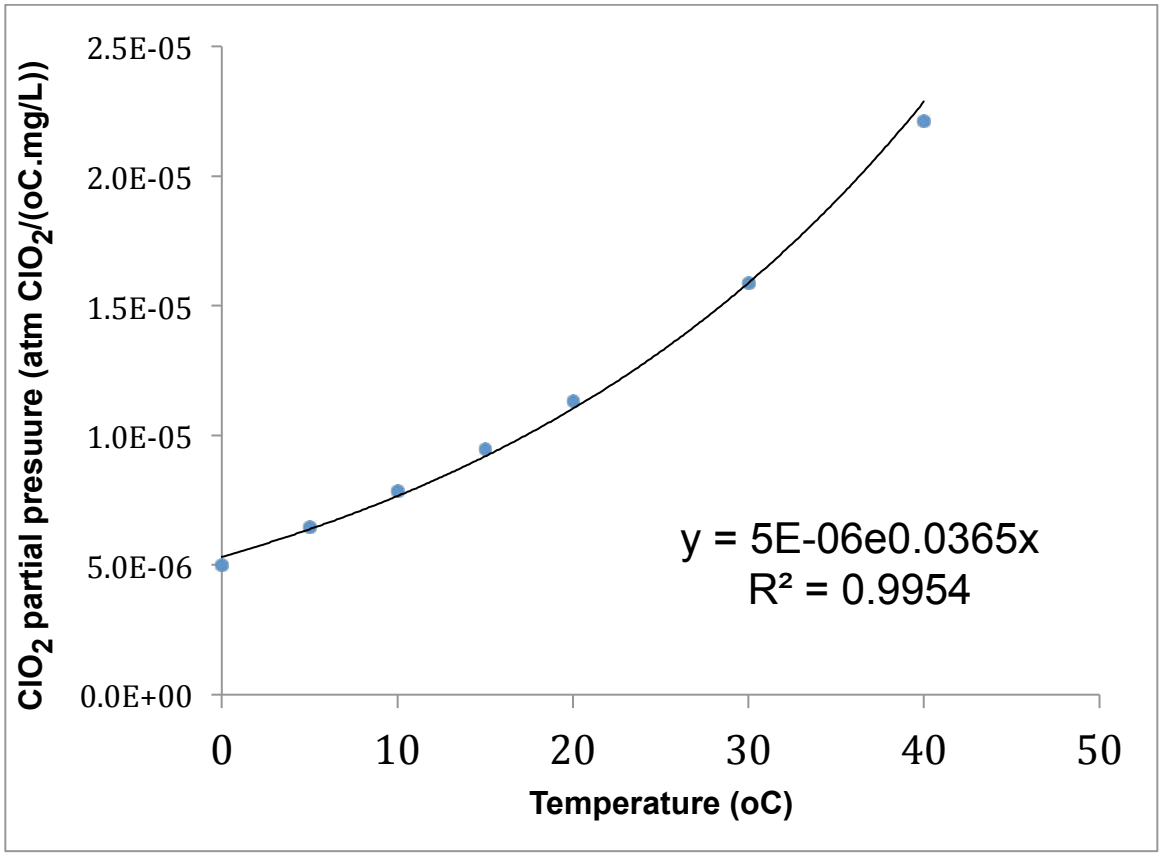

Figure 2.3 - Solubility of ClO_2 in water as a function of temperature (Ishi 1958; Netramai 2010)

UV or fluorescent light will degrade ClO_2, breaking the chlorine oxygen bond, forming O^- and ClO^- which are considered reactive forms as they can react with ozone and contribute to destroying the ozone layer (Kaczur and Cawlfield 2000; Netramai 2010).

Oxidative reactions of ClO_2 involve one electron transfer mechanism, forming chloride (Cl^-), chlorite (ClO_2^-) and chlorate (ClO_3^-). Their concentration will depend on the reaction pH and light conditions. At pH>10, ClO_2 will form chlorate and chlorite and under neutral conditions (pH 4 to10) the ClO_2 will be maintained as a free radical (Qingdong *et al.* 2006; USEPA 2006). Possible reactions are shown below (USEPA 1999):

$$ClO_2 + e^- = ClO_2^-$$

$$ClO_2^- + 2H_2O + 4e^- = Cl^- + 4OH^-$$

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

$$ClO_2 + H_2O = ClO_3^- + 2H^+ + e^-$$

In the oxidation process 50 to 70% of ClO_2 is immediately converted to chlorite and the rest to ClO_3^- and Cl^- (USEPA 1999). Table 2 provides a list of other ClO_2 properties

Table 2.1 - Characteristics of chlorine dioxide (Kaczur and Cawlfield 2000; Knapp and Battisti 2001; Keskinen and Annous 2011)

Property	Value
CAS Registry Number	10049-04-4
Molecular formula	ClO_2
Molecular Weight	67.5 mg/mol
Melting point	- 59^oC
Boiling point	11^oC
Density	1.6 g/mL at 0^oC (liquid) 3.1 g/L (gas)

2.4.2. Sanitizing properties of chlorine dioxide

Chlorine dioxide is a strong sanitizing agent and oxidation is the main mechanism of its bactericidal effect. The disinfection process will vary with produce surface, target microorganism, relative humidity (USEPA 1999), and temperature (Benarde *et al.* 1967), but ClO_2 is not affected by pH, as it does not ionize in water and

ClO_2 maintains its oxidizing efficacy, unlike hypochlorite and chlorite (Benarde *et al.* 1967).

Chlorine dioxide is reported to have a disinfection efficacy equal to or higher than Cl_2 on a mass-dose basis, but lower than ozone (USEPA 1999). Research has shown that ClO_2 kills different kinds of microorganism on different produce surfaces (Appendix 1). Chlorine dioxide is also capable of sanitizing seed without affecting its germination. A study comparing the inactivation of pathogens on seeds of tomatoes showed that ClO_2 resulted in the best log reduction compared to treatments with ozone gas and e-beam irradiation (Trinetta *et al.* 2011a).

2.4.3. Aqueous versus gaseous ClO_2

Most aqueous sanitizers are ineffective in disinfecting raw produce as they cannot eliminate cells located in morphological structures of the produce surface, while gases have the ability to penetrate small and complex spaces (Han *et al.* 2001b). Liquid solution also cannot inactivate microorganisms attached at the produce broken trichomes, cracks, stomata and cut edges. Some microorganisms can also penetrate the produce, entering through its cut edges and stomata; E. coli O157:H7 was found to be 20 μm of the surface inside the stomata (Seo and Frank 1999), making effective produce sanitization difficult, especially with a solution.

The complexity and hydrophobic nature of produce surfaces make it easy for microorganism to bind to the surface and be protected against liquid solutions (Lee *et*

al. 2004). Seo and Frank (1999) treated lettuce with aqueous chlorine (20 mg/L) and found many live *E. coli* O157:H7 in the stomata and on cut edges after treatment. Similarly Han and Linton (2001) proved the better efficiency of ClO_2 gas versus aqueous solution in injured and uninjured green peppers.

Du et al (2002) demonstrated that ClO_2 gas inactivated microorganisms in the calyx and cavity of apples, which are parts of the fruits difficult to sanitize with liquid ClO_2 solution. Gaseous ClO_2 was also studied as a sanitizing agent, under laboratory conditions, on inoculated apples (Du *et al.* 2003), green peppers (Han *et al.* 2000a; Han *et al.* 2000b; Han *et al.* 2001a), lettuce (Lee *et al.* 2004), tomatoes, cabbage, carrots, and peaches (Sy *et al.* 2005b), strawberries (Han *et al.* 2004), and blueberries and raspberries (Sy *et al.* 2005a) as shown in Appendix 1.

2.4.4. Generation systems for gaseous ClO_2

Chlorine dioxide gas is considered highly unstable and is explosive in concentrations exceeding 10% in air at atmospheric pressure. Therefore it cannot be shipped in the gaseous form, and must be generated in situ. (Knapp and Battisti 2001; Keskinen and Annous 2011). The principal source for ClO_2 production is sodium chlorite ($NaClO_2$), that brings some advantages as an easy application and its high purity (Gordon *et al.* 2007). Currently there are three ways to generate ClO_2 usually starting with $NaClO_2$ (USEPA 1999; Keskinen and Annous 2011):

1) $2\ NaClO_2 + Cl_2\ (gas) = 2\ ClO_2\ (gas) + 2\ NaCl$

2) $2\ NaClO_2 + HOCl = 2\ ClO_2\ (gas) + NaCl + NaOH$

3) $5\ NaClO_2 + 4\ HCl = 4\ ClO_2\ (gas) + 5\ NaCl + 2H_2O$

The best way to generate ClO_2 will depend on the desired quantity to be produced, the allowed byproducts, and if it will be in the gas or aqueous form (Knapp and Battisti 2001). In this literature review will be emphasized the production of ClO_2 gas that can be produced in a constant flow concentration and other systems that have a varying flow during gas production.

2.4.4.1 Constant flow concentration

In the continuous flow generation, after the target concentration is reached, the concentration of ClO_2 gas stays constant throughout the treatment; as it is consumed, more gas is generated to equilibrate the system.

2.4.4.1.1. ClO_2 gas generator

The equipment generates gas in an automated system mixing two components, a solid (sodium chlorite) and a gas mixture of chlorine and nitrogen (2:98%). The chemical reaction is shown.

$$2\ NaClO_2\ (solid) + Cl_2\ (gas) = 2\ ClO_2\ (gas) + 2\ NaCl\ (solid)$$

The flow is controlled by sensors, integrated UV-VIS photometric system that keep constant concentration inside the chamber during the treatment and it also can control the relative humidity with a special probe, the system can be visualized in Figure 2.4 (Czarneski and Lorcheim 2005).

The generator is commercially available and being used for facilities sanitization as laboratories, surgical suits, equipment and also transport vans in pharmaceutical, healthcare and food areas. All those applications are approved by USEPA. The use of direct gas for sanitizing fruit and vegetables is not a current application.

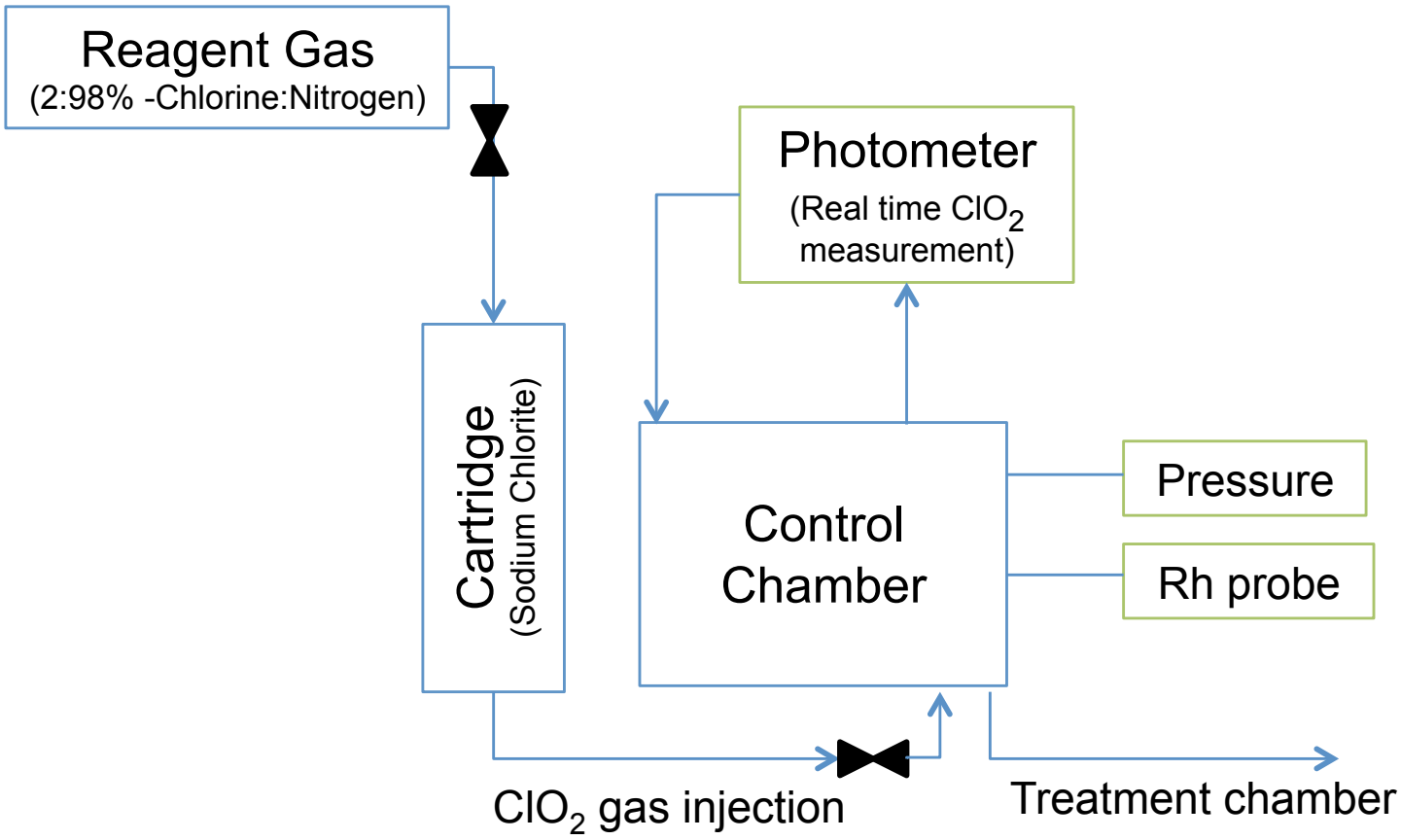

Figure 2.4 - Diagram of Chlorine Dioxide gas generator (Minidox) and control system adapted from (Czarneski and Lorcheim 2005)

2.4.4.2. Other systems

2.4.4.2.1 Liquid solution

A concentrated ClO_2 solution is maintained inside a sealed chamber and as it is volatile will vaporize, creating a ClO_2 atmosphere (Mahovic *et al.* 2009). The gas distributes evenly and completely within the space, similar to what happens with oxygen inside a close cell. Every time the gas is consumed more ClO_2 will be generated from the solution, achieving the correct partial pressure and creating a constant concentration.

2.4.4.2.2 Sachet

In this system the concentration of gas being delivered varies with time. The concentration increases until a maximum concentration is achieved, and then starts decreasing. This way to generate ClO_2 is done using a sachet and it is considered a non-continuous process. The sachet developed by TriNova (ICA TriNova Newnan, GA) produce ClO_2 gas by combining two dry solids, one containing sodium chlorite and other an acid (HCl, H_2SO_4, citric, acetic, etc.), following the reaction:

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

The components are mixed inside a permeable pouch that allows the gradual release of gas either into water or air. The sachet has the convenience of generating

gas during storage and delivery of food products as it is small and easy to manage (Lee *et al.* 2004).

2.5. Measurement of chlorine dioxide concentration

Major applications of ClO_2 are in the paper industry and drinking water treatment. Therefore, most of the existing quantification methods are geared to the determination of ClO_2 concentration in solution. When ClO_2 is found in gas form it is normally captured by distilled water or potassium iodide solution prior to its concentration determination (USEPA 1999).

Due to the high reactivity of ClO_2 it is difficult to find an appropriate detection method to accurately measure its concentration (Vaida and Simon 1995). The volatility of the compound from a solution must also be considered in order to have good and fast measurement (Kaczur and Cawlfield 2000). Some techniques were developed to measure ClO_2 concentration in water, such as amperometric titration, ion chromatography, and colorimetric methods such as ultraviolet-vis spectrophotometry (Keskinen and Annous 2011).

Most of the determination methods 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).

2.5.1 Amperometric titration

The Amperometric titration uses electrical potential to measure the concentration of a specific compound. The most common method to determine ClO_2 is by iodometric analysis; it is based on a titration where the solution is pre treated with potassium iodide, a compound that oxides depending on the solutions pH (Aieta *et al.* 1984; Kaczur and Cawlfield 2000).The products will vary according to the specific pH of the solution, as shown in the reactions below (Aieta *et al.* 1984; Kaczur and Cawlfield 2000):

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

$2ClO_2 + 2I^- = I_2 + 2ClO_2^-$ pH 7

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

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

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

This titration can be done by different titrants, depending on the specific product to be determined. One common titrant is phenylarsine oxide - PAO (C_6H_5AsO) that reacts with iodine ion following the reaction below (Aieta *et al.* 1984; Kaczur and Cawlfield 2000):

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

There are two standard methods outlined for this titration, 4500-ClO_2·C (outlined in Appendix 2) and 4500-ClO_2·E which are practical method developed for drinking water. The first method can measure free chlorine, chloramines, chlorite and ClO_2 separately, but can be influenced by free halogens, organic chloramines, NCl_3, and copper. The other standard method, 4500-ClO_2·E, is a similar titration with an extra step, nitrogen purging; it will measure chlorine, ClO_2, chlorite, and chlorate. This method can be affected by pH values lower than 4, dissolved oxygen, manganese, copper, and nitrate (Greenberg *et al.* 1992; Netramai 2010).

Another possible titrant for the iodometric titration is sodium thiosulfate ($Na_2S_2O_3$) that will also react with iodine (Kaczur and Cawlfield 2000). This method is outlined by ICA Trinova (ICA TriNova LLC 2006) and is presented in Appendix 3. The reaction involved in this process is:

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

2.5.2 Colorimetric method

The colorimetric method measures the concentration by correlating the color intensity with concentration of the specific compound. The more intense color will have a higher concentration of the compound. This method is based on indicators that are

oxidized by ClO_2, changing the solution's color. The intensity of the color is measured by absorbance (Agency for Toxic Substances and Disease Registry Division of Toxicology and Envirommental Medicine 2004).

There are different colorimetric methods but all follow a standard method, 4500-ClO_2.D for water and wastewater, they differ depending on the indicator used (Greenberg *et al.* 1992). The most popular indicator is N,N-diethyl-p-phenylenediamine (DPD), measured at 550 nm it has some limitations as oxychlorine species and Mn^{2+} interfere in the measurement, and is not recommended for measuring low concentrations of ClO_2 (Gordon and Rosenblat 1995; Kaczur and Cawlfield 2000). Chlorphenol red is another indicator that is not affected by chlorine but suffer interference from chlorite, it is best used for determining concentration between 0.05 - 2.5 mg/L of ClO_2 (Sweetin *et al.* 1996; Kaczur and Cawlfield 2000; 2004; Pepich *et al.* 2007).

EPA Method 327.0 is another colorimetric method developed that uses lissamine B and horseradish peroxidase and is able to be measured by visible spectrophotometer. It shown a better sensitivity than other methods and few interferences (Pepich *et al.* 2007).

2.5.3 Ion Chromatography

Ion chromatography is used to analyze samples of drinking water. The EPA specifies the ion chromatography in Method 300.0 or 300.1 - "Determination of inorganic

anions in drinking water by Ion Chromatography". It is done using an anion separator column (Dionex - AS9), where the ions are separated as they have different interaction with the column content and leave the column at different times, carried by the effluent components (Pfaff 1993; Hautman and Munch 1997). The ions are measured with a special detector (conductivity cell) placed at the end of the column. 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 *et al.* 2011b). But with this method it is not possible to detect OCl^-, HOCl and chloramines and can suffer influence by compounds with similar retention time or contaminants in water, glassware or other instruments (Pfaff 1993; Hautman and Munch 1997; Netramai 2010).

2.5.4 Other methods

Gas chromatography can be used to determine chlorine and ClO_2 in both, gas or aqueous form. UV spectroscopy is a common method to measure ClO_2 in both forms as it can detect a concentration range of 0.05 to 10 g/L (Kaczur and Cawlfield 2000).

Electrochemical gas sensor is another method to be used as a continuous measurement system. The concentration is determined by and electrical system where the device produces an electrical signal proportional to the amount of gas it is in contact (Henderson 1999; Netramai 2010). Netramai (2010) determined the ClO_2 permeability using this system and concluded that it is reliable and easy to use and set up.

2.6. Factors affecting chlorine dioxide efficacy

Some factors can affect the efficacy of ClO_2 gas in inactivating microorganisms on produce surfaces. Han et al (2001a) concluded that ClO_2 concentration delivered, exposure time, relative humidity (RH) and temperature could affect the inactivation of *E. coli* O157:H7 on green peppers treated with ClO_2. Concentration of ClO_2 during treatment was shown to be the most influential factor, followed by time, RH and temperature (Han *et al.* 2001a) . RH and ClO_2 gas concentration showed a synergetic behavior: increasing both factors resulted in greater log reduction of E. *coli* (Han *et al.* 2001a). Similarly, Benarde et al (1967) exposed *E. coli* to ClO_2 at different temperatures (5-32^{o}C) and concentrations (0.25 to 0.75 mg ClO_2/L) and found that increasing both factors also increased the killing rate of ClO_2 gas.

The amount of produce relative to the amount of gas applied, or the volume of the treatment chamber relative to the gas concentration also influence the efficacy of ClO_2 as they determine the amount of gas that will be circulating around the treated surfaces and effectively came into contact with the microorganisms (USEPA 1999; Yuk *et al.* 2006; Gómez-López *et al.* 2009). Another factor to be considered is ClO_2 degradation, which will depend on the amount of organic matter and the intensity of incident light. ClO_2 degradation will decrease the amount of ClO_2 available to act as sanitizer (Gómez-López *et al.* 2009).

Another variation in the sanitization effectiveness of ClO_2 is the surface integrity of the produce and the location of the target microorganisms. These factors were investigated by Han et al (2000), who compared the inactivation of *E. coli* O157:H7 on injured and uninjured green peppers. Injured green peppers had a smaller log reduction compared to uninjured ones, because injured surfaces can provide nutrients for the survival of microorganisms and can also hide bacteria and protect them from sanitization (Han *et al.* 2000a; Gómez-López *et al.* 2009).

2.7. Absorption of chlorine dioxide

Chlorine dioxide is absorbed by cell structures, pigments and microflora existing on produce surfaces, when in contact with them it oxidize forming chlorite (ClO_2^-), the major reaction (USEPA 1999; Netramai 2010). Richardson *et al*. (1998) studied how by-products were formed in water treated with ClO_2, could be equivalent to by-products formed in food surface, and found that only two were chlorinated and no halomethanes were present.

During treatment ClO_2 and ClO_2^- can be absorbed by the product, this was shown by Han et al. (2004), who found residues of ClO_2 and ClO_2^- on strawberries after 1 week of treatment. Some factors believed to affect the absorption of ClO_2 are cuts and bruises that can occur during processing and handling, which creates areas where the cells are exposed and become more susceptible to degradation by microorganisms, deterioration, and biochemical reactions (Allende *et al.* 2006; Rico *et al.* 2007). Another factor believed to influence ClO_2 absorption is the presence of water, as ClO_2 is very soluble and can be absorbed as a dissolved gas into damaged cells absorbed (Ishi 1958).

Netramai (2010) investigated the influence of cuts and water on the surface of produce during treatment with gas ClO_2. Shredded lettuce was compared with whole leaves; the amount of ClO_2^- absorbed was 10 times higher in cut lettuce than in whole

leaves when treated with 3.0 mg/L for less than 45 min and 6.0 mg/L for 15 min (Netramai 2010). The presence of water did not significantly affect the absorption of ClO_2 in lettuce but the increase of ClO_2 concentration and exposure time increased the residual by-products found on lettuce surface (Netramai 2010).

2.8. Effects on sensory quality, physiology and nutrients after chlorine dioxide treatment

The effects of aqueous and gas ClO_2 treatment on produces are still not well understood. More research is required to determine the physiological, toxicological mechanism and nutrient stability in treated fruits and vegetables (Gómez-López *et al.* 2009). Treatments with aqueous ClO_2 (3 and 5 ppm for 5 min) did not change sensory characteristics of lettuce, cantaloupe, apples and strawberries when evaluated in a triangle test (Rodgers *et al.* 2004). In contrast, Gomez-Lopez et al.(2008) reported that lettuce treated with 20 mg/L of aqueous ClO_2 for 5 min differed in sensory evaluation from lettuce treated with water in a triangle test.

When using ClO_2 in the gas form most studies showed that sensory quality of fruits and vegetables was affected (Gómez-López *et al.* 2009). Different fruits and vegetables exposed to ClO_2 gas showed quality change such as browning on cut cabbage and lettuce (treated with 2.7mg ClO_2/L for 12.3 to 20 min) and brown spots on the skin and a slight decrease in overall quality on apples (treated with 4.1 mg ClO_2/L) (Sy *et al.* 2005b). Tomatoes are an different case, after treatment with 2.7mg ClO_2/L for 12 min slight increase in quality, especially appearance, color, and aroma was observed (Sy *et al.* 2005b). Also white cabbage and lettuce treated with 0.4-0.6 mg/L of ClO_2 gas

for 5 min showed different sensory evaluation when compared with water washed produce in a triangle test (Gómez-López *et al.* 2008).

The formation of quinines due to oxidation of phenols is responsible for browning as it polymerizes and forms melanin, a brown pigment (Gómez-López *et al.* 2009). ClO_2 can also react with oligosaccharides, leaving a bleached surface on fruits and vegetables (Gómez-López *et al.* 2009). This effect was observed by Netramai (2010) when treating lettuce for more than 60 min with concentrations of 2 – 10 mg ClO_2/L in gas form. Bleaching is due to the reaction between chlorophyll, the plant green pigment, and gas ClO_2 (Gómez-López *et al.* 2009; Netramai 2010).

There are a few studies on the effect of ClO_2 treatment on nutritional quality. ClO_2 can react with phenolics and ascorbic acid, impacting the content of these components in the treated produce (Gómez-López *et al.* 2009).

2.9. Toxicology and regulations of chlorine dioxide

In the exposure to any hazardous substance it is important to consider factors that determine the degree of hazard and the precautions to be taken. The factors to be considered are: exposure amount (dose), exposure duration, the manner of exposure, whether there were any other chemicals involved, age, sex, and health situation of the exposed person (Agency for Toxic Substances and Disease Registry Division of Toxicology and Envirommental Medicine 2004)

Most studies of ClO_2 toxicity were done on drinking water as it is the main application for this disinfection method. Since ClO_2, is a very strong oxidizing agent it, tends to react very fast with most compounds, but it does not form compounds, that are a concern for human health, as chloramine (Rico *et al.* 2007; Bhagat 2010). Richardson *et. al.* (1998) reported that no halomethans and very small amounts of chlorinated by-products were founded in water treated with ClO_2 and concluded that most of the by-products formed from ClO_2 oxidation include oxygen, the list of formed by-products is shown in Table 3 (Richardson *et al.* 1998). Furthermore, since 70% of ClO_2 is transformed to ClO_2^-, it is important to take into consideration the toxic effect of both components (Qingdong *et al.* 2006).

Animal tests were conducted in order to determine potential adverse impacts of ClO_2 exposure on birth defects, brain development, and cancer development, they

indicated 5 mg/kg/day as being the lowest observed adverse-effect(Agency for Toxic Substances and Disease Registry Division of Toxicology and Enviromental Medicine 2004). Qingdong (2006) tested the effect of ClO_2 and its by-products in water ingested by rats during 90 days at a concentration 120 times higher than for humans (553 mg/L of ClO_2, ClO_2^- and ClO_3^-). The study showed that the ingestion did not affect weight gain, food utilization, indexes of blood and serum, liver/bodyweight, and kidney/bodyweight (Qingdong *et al.* 2006). Some studies were done with humans; 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). Also, no health effects were found in populations living in areas where water was treated with ClO_2 for 12 weeks (Gómez-López *et al.* 2009).

The exposure to ClO_2 in air can be of concern to human health as it first acts in the respiratory and ocular organs. A person exposed to air containing ClO_2 can have eye, throat, nose, and lung irritation. If ClO_2 is ingested or breathed in large amounts it can cause mouth, esophagus and stomach irritation; if the amount is large enough it can cause systemic damage as it affects blood cells and decreases the quantity of circulating oxygen (Agency for Toxic Substances and Disease Registry Division of Toxicology and Enviromental Medicine 2004). Additional studies are necessary to evaluate the carcinogenic effect of ClO_2, but previous studies have shown no dermal

Table 2.2 - ClO_2 by-products from water sanitization (Richardson et al. 1998)

Compound	Chemical classification
Butanoic acid	Carboxylic acid
Pentanoic acid	Carboxylic acid
Hexanoic acid	Carboxylic acid
Heptanoic acid	Carboxylic acid
2-Ethylhexanoic acid	Carboxylic acid
Octonic acid	Carboxylic acid
Undecanoic acid	Carboxylic acid
Tridecanoic acid	Carboxylic acid
Tetradecanoic acid	Carboxylic acid
Hexadecanoic acid	Carboxylic acid
2-*tert*-Butylmaleic acid	Carboxylic acid
2-Ethyl-3-methylmaleic acid	Carboxylic acid
1,1,3,3-Tetraclhoro-2-propanone	Chlorine containing compound
(1-Chloroethyl)dimethylbenzene	Chlorine containing compound
2,3,4 – Trimethylcyclopent-2-en-1-one	Ketone
2,6,6 – Trimethyl-2-cyclohexane-1,4-dione	Ketone
3-Ethyl styrene	Aromatic compound
2-Ethyl styrene	Aromatic compound
Ethylbenzaldehyde	Aromatic compound
Naphthalene	Aromatic compound
2-Methyinaphthalene	Aromatic compound
1-Methyinaphthalene	Aromatic compound
Hexanedioic acid, dioctyl ester	Ester

cancer risk (USEPA 2006), and studies in mice did not detect the development of any tumors after exposure to ClO_2 (Condie 1986).

There are some controversies about the advantages and adverse effects of ClO_2 and studies are still in progress to gather more information about its use. Because the uncertainty each institution determined different parameters for ClO_2 solution to be used as a sanitizer. The EPA determined a maximum ingestion level of 0.8 mg/L for ClO_2 and 1.0 mg/L for chlorite in treated drinking water (USEPA 2006; Gómez-López *et al.* 2009; Center for Disease Control and Prevention 2011). The Food and Agricultural Organization / World Health Organization (FAO/WHO) determined a daily intake of 0.03 mg/kg of body weight of chlorite and 0.01 mg/kg of chlorate (Gómez-López *et al.* 2009).

The Food and Drug Administration (FDA) approved the use of ClO_2 not exceeding 3 mg/L in water to be used in sanitizing process of fresh produces (Gómez-López *et al.* 2009). The approval was based on the fact that the substance degrades some time after treatment, leaving low residue in the produce. This was shown by Netramai (2010), who left treated shredded lettuce (6 mg ClO_2/L for 15 min) at room temperature for 15 and 60 minutes and reported a decrease in residual ClO_2 and ClO_2^- after this period (Netramai 2010). Some other regulations in different countries are given in Table 2.3.

The FDA approved, in 2001, the incorporation of agents in packaging material that will react and produce ClO_2 in the headspace to be used for packaged meats, poultry and seafood. The addictive cannot exceed 17.5 micrograms chlorite/in^2 of package film (USFDA 2001).

Despite the fact that ClO_2 gas was proven to be very effective in sanitizing fresh produce, FDA and EPA approval for its use in the gaseous form is pending due to lack of knowledge about its potential toxic effects and interaction with produce tissue.

Table 2.3 - Regulations for Chlorine Dioxide in different countries- Modified from (Tianjin Shareclean Science & Technology Company 2009)

Year	Country	Regulatory Agency	Usage Range
1985	USA	FDA	Food equipment sterilization
1985	EU	European Commission	Drinking water disinfection, food industry, medical, environment and public areas disinfection and sterilization
1987	Germany	—	Drinking water disinfection
1987	UK	Ministry of Health	Drinking water disinfection, hospital, environment and public areas disinfection and sterilization
1987	USA	EPA	Food processing plants, breweries, restaurants, environmental disinfection; Hospitals
1987	Australia	Ministry of Health	No. 926 food additive
1987	China	Ministry of Health	Food industry, medical, pharmaceutical and public areas disinfection and sterilization
1988	Japan	Ministry of Food Health	Drinking water disinfection
1989	USA	EPA	Storage water disinfection; livestock, disinfection and deodorizing
1992	—	WHO	Drinking water disinfection
1996	China	Ministry of Health	Food additives, fruits and vegetables preservation
2002	USA	FDA	Food processing equipment, pipe, crafts and arts equipment, especially in milk processing plant
2005	China	Ministry of Health	Drinking water disinfection

2.10. Packaging systems

Another approach to prevent foodborne illness is to use a specific package that includes an antimicrobial agent in combination with modified atmosphere packaging (MAP) (Appendini and Hotchkiss 2002; Netramai 2010). The headspace of the MAP package includes gases such as oxygen and carbon dioxide in different proportions such that the respiration of the fresh produce is slowed, delaying the microbial reproduction processes and the produce ripening, and increasing the produce shelf life. It can be used to pack fruits, vegetables, meat, etc. (Bhagat 2010; Netramai 2010).

Antimicrobial packaging is used to prolong shelf life and to increase the safety of produce by reducing or inhibiting the growth of microorganisms (Appendini and Hotchkiss 2002). In order to fulfill this function it is necessary to add some component inside the package or in the polymer that has an antimicrobial effect (Appendini and Hotchkiss 2002). Silver substitute zeolite and antimicrobial enzymes can be added to packages to interact with the food surface where the microorganisms are established (Appendini and Hotchkiss 2002; Netramai 2010).

Volatile antimicrobials can be added inside the package headspace through a pad soaked with solution that will vaporize or a sachet with solid chemical that will react and produce a sanitizing atmosphere. This vapor approach has an advantage as the antimicrobial can easily interact and penetrate in the food inactivating microorganism without direct contact with the antimicrobial generator, as the case of using polymer or generator system that for sanitation is necessary direct contact of the food to the agent

(Kim *et al.* 1999; Kaczur and Cawlfield 2000; Appendini and Hotchkiss 2002; Netramai 2010).

As mentioned earlier, volatile antimicrobial agents will be effective as long as the bactericide is in contact with the fresh produce (Du *et al.* 2002; Du *et al.* 2003; Ellis *et al.* 2006). To ensure direct contact with the overall produce surface, a uniform gas distribution inside the package is necessary, reaching all difficult areas and all the packed product (Netramai 2010). The importance of gas distribution was demonstrated by Shin (2007) and *Ellis et al*. (2006), they observed different log reduction in the chicken parts and change in color due to direct contact with the antimicrobial agent.

Netramai (2010) developed a study to identify the best design for a ClO_2 antimicrobial package. The best alternative reduced the distance that the gas needed to travel in order to reach the areas to be disinfected (Netramai 2010). With the new design it was possible to apply a lower dose of gas and improve the appearance of the treated product (Ellis *et al.* 2006; Gómez-López *et al.* 2009; Netramai 2010).

Another important issue related to volatile antimicrobial agents to be added inside packages is their interaction with the polymer materials. Mass transfer needs to be evaluated in order to ensure that the gas remains in the headspace. The interaction between ClO_2 and some polymers were studied by Netramai (2010), who assessed permeability, diffusion and solubility coefficients of the different materials.

In order to develop the best antimicrobial package using ClO_2, it is necessary to combine information about packaging internal design, the interaction between package material and ClO_2 gas and ClO_2 absorption on the fresh produce. Also consider the amount of gas that degrades, and the diffusion of gas in air and within the produce.

The absorption of ClO_2 by fresh produce is a concern related to food safety. The residues left in the produce surface, after ClO_2 treatment, need to be explored before any regulation, related to the use of ClO_2 gas to sanitize fresh produce, is approved. Also studies with ClO_2 gas were done using different ClO_2 generation system and any of them showed the concentration profile of those systems and how they affect the absorption or microorganism log reduction. To address those issues this research has the objective to determine the residues on fresh produce surface after treated with different ClO_2 delivery systems and determine the different delivery systems concentration profile.

CHAPTER 3 – MATERIALS AND METHODS

The experiment was designed to assess the residues on lettuce and tomatoes surface after a sanitizing treatment using three different ClO_2 delivery systems, Minidox (gas generator), sachet and a ClO_2 solution. The residues were also evaluated in different gas concentration, exposure time and treatment temperature. All the experiments were realized in a stable testing environment with the same conditions for all replicates.

In order to compare the residues left in the produce surface by the different ClO_2 delivery systems was also necessary to determine the gas concentration profile generated by the different delivery systems in the utilized conditions.

3.1. Testing chamber

A glass chamber was customized by the glassblowing facility (Department of Chemistry, Michigan State University, East Lansing, MI) containing 12 L internal volume and used to expose the produce samples to ClO_2.

The glass (Figure 3.1) was composed of: a glass lid, a low density polyethylene screen to support the samples, two ports to sample or introduce gas, and a hermetic closure system composed of Viton® O-Ring and a metal clamp ring for sealing the system and preventing ClO_2 to escape.

Figure 3.1- Glass chamber for absorption study

3.2. Sample preparation

3.2.1. Lettuce

Romaine lettuce (*Lactuca sativa L. var. longifolia*), Andy Boy brand (Salinas, CA), packaged in a PE bag, was purchased from a local store in East Lansing, MI, and was stored at 4^{o}C for a maximum of 5 d.

For each experiment the lettuce was brought to room temperature and three leaves, one from the outer, middle and inner layer, were cut into 25 pieces (3.2 x 3.8 cm^{2}) weighing a total of 35-50 g. The pieces included the midrib and leaf areas without bruises. These 25 pieces composed one sample unit to be exposed to ClO_2. They were

placed in a PE supports, as shown in Figure 3.2, to maximize the exposed area and avoid leaves to overlap each other.

Figure 3.2 - Shredded lettuce disposed in the PE screen prior to treatment

3.2.2. Tomatoes preparation

Nature Sweet brand cherry tomatoes were purchased from a local store in East Lansing, MI. They were stored at 4^{o}C for a maximum of 7 d. Before the experiment they were washed with tap water, manually spin-dried and placed on absorbent paper to air-dry for 15 min. Samples consisted of a variable number of tomatoes, between 20 to 25 unities, and the total weight of each sample was 240-260 g. Tomatoes with bruises, cuts or soft skin were discarded. During treatment tomatoes were hold by a PE screen, as shown in Figure 3.3.

Figure 3.3 - Tomatoes placed inside the glass chamber being treated with ClO_2 generated by the sachet

3.3. ClO_2 gas generation

3.3.1. ClO_2 solution

A ClO_2 stock solution was prepared with a Solution Pack provided by ICA-TriNova LLC (Newnan, GA). The pack consisted of two dry chemicals, sodium chlorite ($NaClO_2$) and sulfuric acid (H_2SO_4), and a gas-permeable sachet (18 x 18 cm^2). The chemicals were mixed inside the sachet to activate the reaction, and submerged in 3.8 L of deionized water for 7 d at 4^oC to generate approximately 2 g of ClO_2.

The actual concentration of the stock solution was determined using a titration method outlined by ICA-TriNova LLC (Appendix 3) and immediately before each experiment the stock solution was diluted with DI water according to the specific experiment concentration and placed in the chamber to create a ClO_2 atmosphere. The solution system set up with the treated produce can be seen in Figure 3.4.

The solution concentration to be used in each experiment was calculated based in the equilibrium between the solution and the headspace, in order to achieve the desired ClO_2 headspace concentration for each treatment condition. Henry's constant reported by Ishi (1958) and Kaczur and Cawlfield (2000), was used for the determination of the solution concentration. The initial solution concentration for each treatment can be seen in Table 3.1 and the calculations for its determination could be followed in Appendix 4. In order to better control the system the solution concentration was quantified after each dilution (with the stock solution) to check its correct composition and ensure the correct ClO_2 gas concentration in the headspace. This quantification was made using the same procedure outlined by ICA-TriNova LLC (ICA TriNova LLC 2006).

Table 3.1 – ClO_2 solution concentration to be used in each experiment to achieve the target ClO_2 gas concentration in the chamber headspace, during experiment at 4 and 23°C

Target concentration of ClO_2 gas (mg/L)	**ClO_2 solution initial concentration for 23°C (mg/L)**	**ClO_2 solution initial concentration for 4°C (mg/L)**
1	29.20	54.68
3	87.75	164.03
6	175.20	328.05
10	292.20	546.75

Figure 3.4 - Lettuce and Tomatoes exposed with ClO_2 gas generated by a ClO_2 solution

3.3.2. ClO_2 sachet

ICA-TriNova LLC provided the Z-series sachet to produce gaseous ClO_2. The gas was produced by mixing two solids, sulfuric acid (H_2SO_4) and sodium chlorite ($NaClO_2$), inside a gas-permeable sachet (10 x 12 cm).

Equal weights of solids were mixed by shaking in a permeable sachet, which was immediately inserted in the bottom of the glass chamber while the produce sample was placed in the PE screen, as shown in Figure 3.5. After each trial the sachet was discarded. The amount of chemical necessary to generate the ClO_2 for the experiment was calculated based on a sachet profile presented in Appendix 5 and is presented in Table 3.2.

Figure 3.5 - Lettuce and Tomatoes exposed to gas ClO_2 generated by sachets

Table 3.2 – Amount of sachet precursor used to achieve the specific ClO_2 gas concentration in the chamber headspace

Target ClO_2 gas concentration in the headspace (mg/L)	**Sachet precursor (g)**	
	Lettuce	**Tomatoes**
1.0	4.0	-
3.0	12.0	9.4
6.0	24.0	18.8
10.0	-	31.3

For the experiment at 4°C the same amount of chemical were used in order to see only the effect of temperature.

3.3.3. Minidox - ClO_2 generator

A Minidox M (Clordisys, Lebanon, NJ) was used to generate constant concentration of ClO_2 gas. The glass chamber was connected to the Minidox via tubing, the gas enters the chamber throughout a bottom sample port and leaves through the top port, as shown in Figure 3.6. Concentration and relative humidity was controlled by the Minidox sensors, allowing automatic corrections throughout the treatment. The relative humidity was maintained around 85-95%.

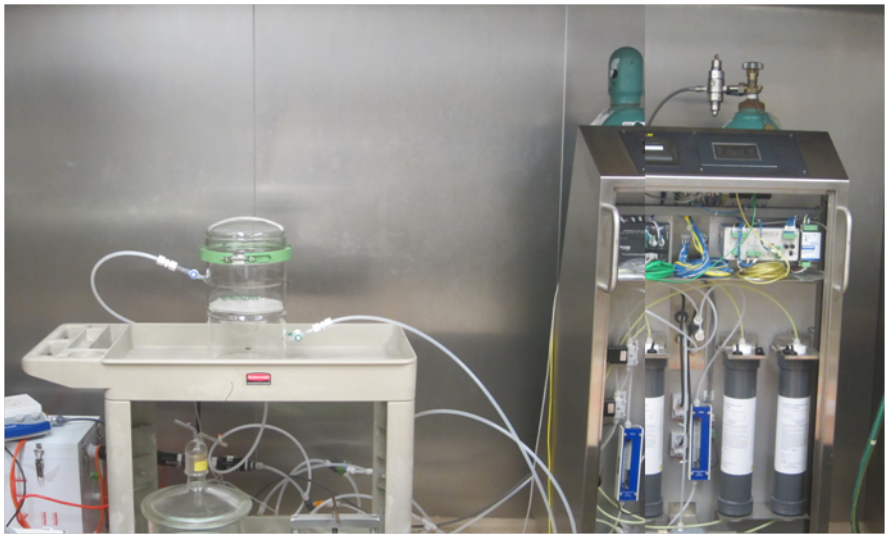

Figure 3.6 – Glass chamber connected to the ClO_2 Minidox equipment through tubing

3.4. Treatments conditions

Each produce was exposed to specific ClO_2 condition. Tomatoes were exposed to 3, 6 and 10 mg/L of ClO_2 at time intervals of 15, 30 and 60 min, for each concentration. Lettuce was exposed to equivalent concentration of 1, 3 and 6 mg/L of ClO_2 gas for the following intervals 5, 10, 15 and 30 min.

To determine the influence of temperature on ClO_2 absorption the treatments described above were repeated at 4°C and room temperature 23°C. For the 4°C experiment the chamber was placed in a refrigerator with temperature control, the chamber was maintained inside the refrigerator to achieve 4°C and the produce, at

ambient temperature, was placed in the system prior to exposure. The amount of ClO_2 and by-products were determined as described above.

All treatments were repeated three times. A diagram scheme shows the overall experiment (Figure 3.7).

3.5. Method for Quantification of residual ClO_2 and ClO_2^- on produce surface

Immediately after treatment samples were collected and washed in 300 mL of deionized water, inside a plastic bag, for 15 min in the dark, to avoid photodegradation (Han *et al.* 2004; Netramai 2010). The residues were quantified using the method 4500-ClO_2·C (Greenberg *et al.* 1992), a standard amperometric titration for examining water and wastewater, as modified by Netramai (2010) and presented in Appendix 2. This titration method allows separate determination of the amounts of chlorine, chloramines, ClO_2^-, and ClO_2.

A study was carried out in order to determine the detection limit of this procedure. A sample containing pure deionized water (without produce) was measured with the Phenylarsine oxide amperometric titration following the procedure outlined in Appendix 2. The measurement was repeated for eight samples and they were calculated to determine ClO_2 and ClO_2^-.

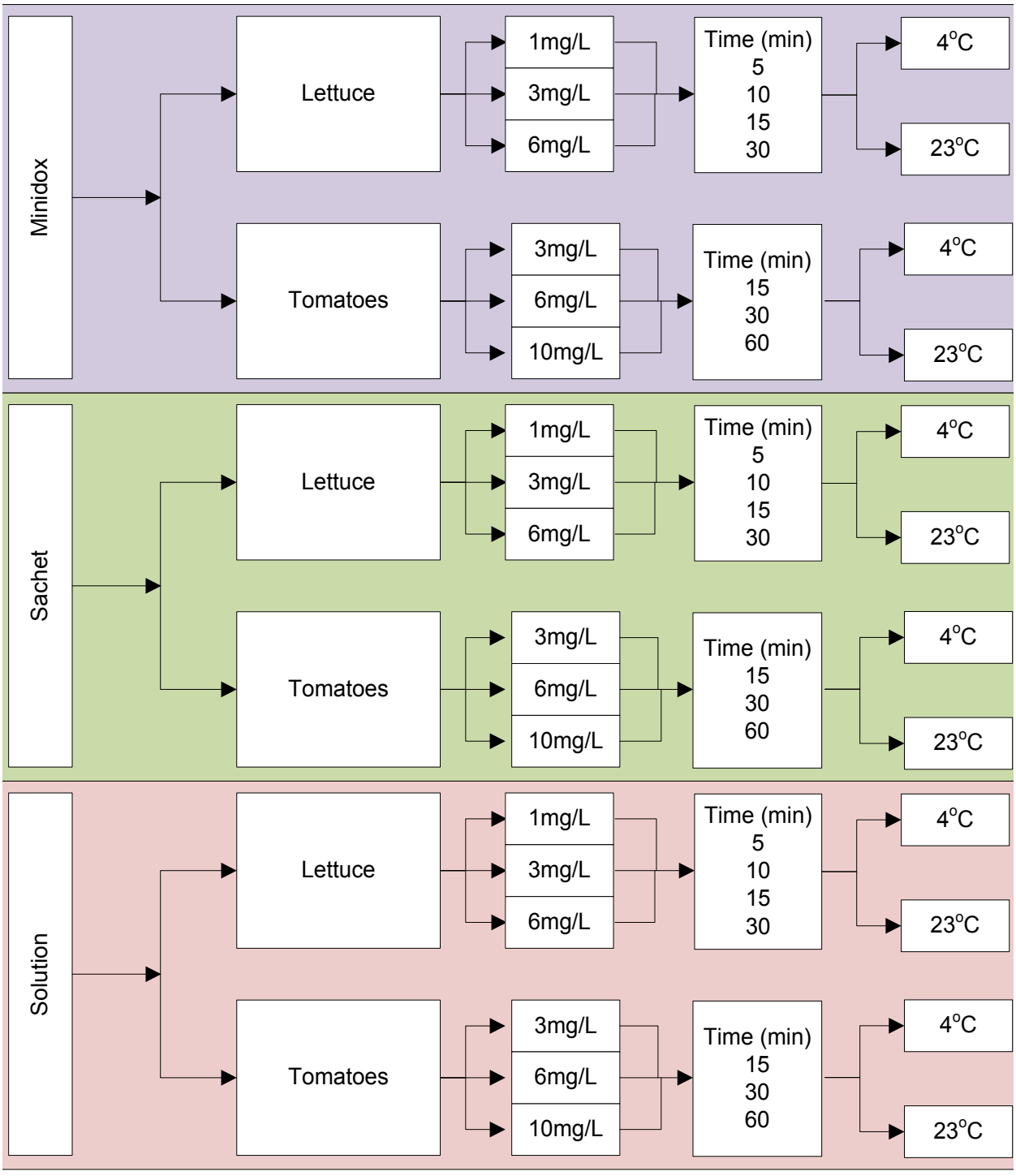

Figure 3.7 - Experiment Scheme

3.6. Concentration profile of each delivery system

Each of the delivery systems, sachet, solution and Minidox, generates ClO_2 differently. Each system will take different time to reach the target concentration. Therefore each delivery system has a specific concentration profile.

The residue of ClO_2 and by products on the fresh produce will depend on the concentration of ClO_2 gas that the produce is exposed to. In order to compare the residues of the three ClO_2 delivery systems in fresh produce it was necessary to determine concentration profile of ClO_2 gas throughout the treatment time.

In order to determine the concentration profile of each ClO_2 gas delivery system a UV spectrophotometer (Shimadzu UV-1800, Shimadzu Scientific Instruments, Columbia, MD) was used equipped with a 100 mm cuvette (Quartz spectrophotometer cell, cylindrical, Starna Cells, Atascadero, CA) and a recirculation pump (Masterflex - 600 rpm, 115 VAC – Cole-Parmer, Vernon Hills, IL)

It measures the absorbance of light, which can be correlated with concentration of ClO_2 using the Beer-Lambert Law. The wavelength used was 306 or 360 nm (depending on the concentration of ClO_2). All the systems were set up as described in section 3.3, with the same amount of chemical for the sachet case, the same solution concentration for the solution analyses, and the same equipment settings for the

Minidox. The difference is that the chamber was maintained empty (without produce) during these specific measurements. The chamber was covered and protected against light.

For sachet and solution delivery system the set up is shown in Figure 3.9. After each specific interval (5, 10, 15 and 30 min) the pump was turned on for 30 sec to recirculate the gas from the chamber to the UV spectrophotometer to determine the gas concentration.

For the Minidox M, the configuration was different and is shown in Figure 3.10. In this case the glass chamber had a third sampling port, from where the Minidox is delivering the gas. The same configuration was used for the experiment at 4^{o}C, the only difference is that the chamber was maintained in the refrigerator.

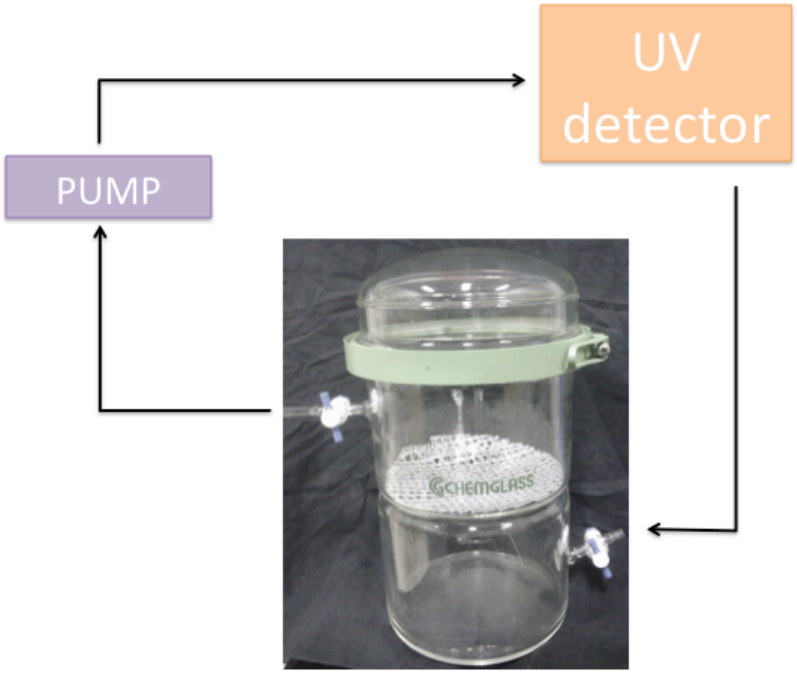

Figure 3.8 – Set up used for the determination of ClO_2 gas concentration profile for the solution and sachet

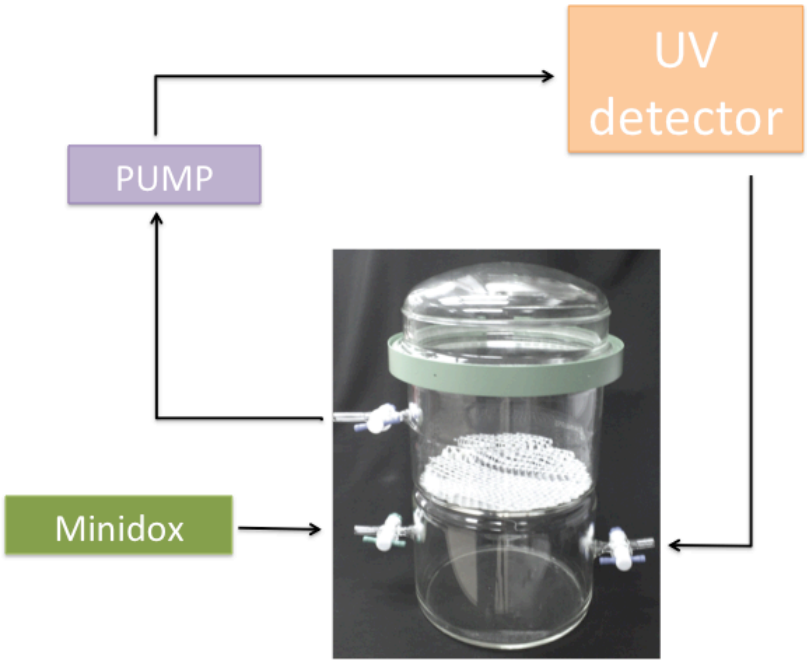

Figure 3.9 - Set up used for the determination of ClO_2 gas concentration profile for the Minidox

3.7. Statistical analysis

The results obtained form section 3.5 were analyzed using a factorial analysis of variance (ANOVA) using the software Statistical Analysis System (SAS). A 95% confidence interval was used for all the analysis and a Least Significant Difference (LSD) test was used to compare the means.

The results obtained from the residues on the produce surface were combined with the concentration profile of each system and a response surface analysis was used to model the residues found in the produce surface. This analysis was done using the software SAS with a 95% confidence interval.

CHAPTER 4 - RESULTS AND DISCUSSION

Three different ClO_2 generating systems, including the Minidox, sachet, and, solution, was used to study the residue of ClO_2 and ClO_2^- in shredded lettuce and cherry tomatoes. The study included the exposure of the two different fresh produce to ClO_2 generated by those different systems, under different concentrations, exposure times and temperatures. After each exposure the residues were quantified by amperometric titration and reported.

Lettuce and tomatoes were used in this experiment as they represent different produce surfaces, the first being a leafy green produce (romaine lettuce) with a irregular surface where high absorption was proven by the presence of high residues, on the other hand, tomatoes that has a smooth and plain surface covered by a waxy cuticle interacts differently with ClO_2.

All the delivery systems were studied separately to determine the concentration profile of ClO_2 gas in the chamber headspace over several time intervals. The concentration profiles represent the amount of ClO_2 gas that the fresh produce will be exposed during each specific treatment interval. The profile is specific for each delivery system since they delivery ClO_2 gas differently as mentioned in Chapter 3.

The second part consists in the determination of ClO_2 and ClO_2^- residues on the produce surface after treatment with the different delivery systems. After exposure of lettuce and tomatoes to specific ClO_2 gas concentration at specific time intervals and different temperature (4 and 23°C) the residues (ClO_2 and ClO_2^-), on the fresh produce surface, were quantified and compared based on the target concentrations, exposure time, delivery system, and treatment temperature.

Finally a model was developed where the absorption of ClO_2 by lettuce could be predicted based on the residues of the ClO_2 and by products on the produce surface in function of the ClO_2 delivery systems, ClO_2 concentration, exposure time and temperature.

4.1. Concentration profile of each ClO_2 delivery system

The ClO_2 headspace concentration generated by each delivery system was determined over time. The determination of the ClO_2 concentration profile as a function of time for each delivery system was carried out at 4 and 23°C.

Even though the system is air tight, totally close to any air to enter/scape, an amount of gas was lost during treatment due to reactions of auto-degradation, photo-

degradation and sorption by the LDPE screen (Vaida and Simon 1995; Netramai 2010) and all will be considered in this determination.

The ClO_2 concentration of the headspace was carried out using an UV spectrophotometer. The absorbance of ClO_2 was measured and the gas concentration was defined using the Beer-Lamberd Law:

$$A = \varepsilon * l * c \quad \text{(Beer-Lambert Law)}$$

Where A is absorbance, ε is the molar absorvity ($L.mol^{-1}.cm^{-1}$), *l* is path length (cm) and *c* is the concentration.

Although specific target ClO_2 concentration for the headspace was selected, each delivery system reaches the target concentration at a different time. The treatments were classified in concentrations groups of 1, 3, and 6 mg/L, as being the target concentration for each of the delivery system settings.

4.1.1. Minidox

The Minidox will produce a gas flow with constant concentration that is monitored by UV detector and adjust automatically if any deviation from the set point. The concentration of the gas inside the chamber increases over time, until it achieves the target ClO_2 concentration, same concentration than the flow coming from the Minidox. This concentration profile can be seen in Table 4.1.

The achieved concentration at 23°C is not exactly the target concentration due to the time that the system is working. The experiment at 4°C took more time to fill up the chamber; possible due to low diffusion rate of the gas at lower temperature (Chen and Othmer 1962).

Table 4.1 - Concentration profile of ClO_2 gas generated by the Minidox at 23 and 4°C in the empty glass chamber headspace

Target Concentration	Time (min)	ClO_2 concentration at 23°C (mg/L)*	ClO_2 concentration at 4°C (mg/L)*
1 mg/L	5	0.51	0.33
	10	0.68	0.50
	15	0.75	0.60
	30	0.77	0.73
3 mg/L	5	1.64	1.15
	10	2.20	1.76
	15	2.41	2.12
	30	2.51	2.51
6 mg/L	5	3.53	2.51
	10	4.63	3.79
	15	5.06	4.52
	30	5.34	5.34

* Results shown a mean of two replicates

4.1.1. Sachet

Table 4.2 shows the amount of ClO_2 produced by the sachet over time and at 23°C and 4°C and classified by the target ClO_2 concentration (gas concentration in the headspace to be achieved after 30 min exposure). The amount of chemical used in each experiment is the same as shown in Table 3.2.

The chemicals inside the sachet will take time to react and produce the target concentration of ClO_2 in the chamber, having a different concentration at the end of each treatment interval. For example, the concentration at 5 min will be different from the one at 10 min, as the sachet was designed to deliver the final concentration at the maximum exposure time. For example all the experiments using the sachet build to produce 1 mg/L in 30 min were classified in the 1 mg/L group.

The concentration of ClO_2 produced by the sachet at 4°C is lower than at 23°C throughout the different intervals. This is because the reaction rate of the two solids decreases as temperature decreases, generating less ClO_2 over time, and the diffusion of gas in air is lower (Chen and Othmer 1962).

Table 4.2 – Concentration profile of ClO_2 gas generated by the sachet at 23 and 4°C in the empty glass chamber headspace

Target Concentration	Time (min)	ClO_2 concentration at 23°C (mg/L)*	ClO_2 concentration at 4°C (mg/L)*
1 mg/L	5	0.21	0.12
	10	0.47	0.18
	15	0.60	0.27
	30	1.10	0.49
3 mg/L	5	0.50	0.31
	10	0.98	0.57
	15	1.39	0.74
	30	2.22	1.22
6 mg/L	5	0.89	0.69
	10	1.75	1.16
	15	2.51	1.53
	30	4.34	2.40

* Results shown a mean of two replicates

4.1.2. Solution

It is possible to generate a gas from a ClO_2 solution as it does not dissociate in the aqueous form (Mahovic *et al.* 2009). The solutions used to determine its profile were prepared with the concentrations shown in Table 3.1. The concentration of the gas generated in the headspace was measured with the UV spectrometer and the results are shown in Table 4.3.

As before, the concentrations of ClO_2 gas were classified in groups according to the target concentration of 1, 3, and 6 mg/L. As time increases the concentration of ClO_2 gas in the chamber headspace also increases.

For the solution experiment, the initial solution concentration (Table 3.1) was determined assuming and equilibrium between the solution and the gas in the headspace (Henry's Law). From the data it is possible to observe that the solution did not achieve the target concentrations (1, 3 and 6 mg/L), this is due to factors that are affecting the equilibrium, as: the diffusion in the solution, the gas diffusion, reaction rate, and also some autodegradation of ClO_2 or there was not time for the solution to achieve the equilibrium in the chamber.

The concentration of the gas in the headspace is higher at 4^oC, this is due to the fact that at this temperature the initial solution concentration was higher than the initial solution used at 23^oC (Table 3.1). Considering Henry's Law the solubility of the gas in the solution increase as temperature decrease, so more chemicals need to be added

into the solution in order to maintain the same ClO_2 concentration in the headspace (Prausnitz *et al.* 1998).

Table 4.3 - Concentration profile of ClO_2 gas generated by the solution at 23 and 4°C in the empty glass chamber headspace

Target Concentration	Time (min)	ClO_2 concentration at 23°C (mg/L)*	ClO_2 concentration at 4°C (mg/L)*
1 mg/L	5	0.16	0.26
	10	0.19	0.34
	15	0.21	0.38
	30	0.26	0.51
3 mg/L	5	0.44	0.59
	10	0.47	1.01
	15	0.53	1.13
	30	0.67	1.31
6 mg/L	5	0.60	1.02
	10	0.71	1.22
	15	0.81	1.40
	30	1.06	1.80

* Results shown a mean of two replicates

4.2. Limit of detection for the amperometric titration method

To determine the limit of detection pure deionized water was measured with a PAO amperometric titration. This setting was run with absence of ClO_2 and resulted in a measurement of 0.0356 ± 0.0072 mg of ClO_2 and ClO_2^-.

Based on a 95% confidence interval (two standard deviations) and with the value obtained with the deionized water it is possible to determine a limit of detection for the

procedure ad being 0.05 mg of ClO_2 and ClO_2^-. Values below this measurement cannot be distinguished between being a signal or noise.

The limit of detection can be specified for each of the tested produce by dividing it to the amount of produce tested in each case, this will be useful as the absorption results are in the same order of comparison. For the tomatoes the limit of detection will be 0.201 mg of ClO_2/kg of tomatoes and for lettuce will be 1.00 mg ClO_2/kg of lettuce. This values are different as the amount of produce used is different foe lettuce and tomatoes.

4.3. Residues found on shredded lettuce after ClO_2 treatment

The residues found in lettuce were analyzed using a factorial ANOVA with a 95% confidence interval that showed a four-way interaction between all parameters: delivery system, concentration, exposure time and temperature. The ANOVA table can be seen in Appendix 6. In this analysis the concentration groups of 1, 3 and 6 mg/L were considered in order to compare the results. The two temperatures were analyzed separately.

ClO_2 reacts with the organic matter present on the produce surface producing ClO_2^- (Han *et al.* 2004), the total residue represents the amount of ClO_2 and ClO_2^- left on the surface. All the determinations for lettuce were above the detection limit (1.00 mg

ClO_2/kg of lettuce), allowing the use of the amperometric titration method for the measurement of residues after ClO_2 treatment.

4.3.1. Residues on lettuce treated at 23°C

The residual ClO_2 in combination with ClO_2^- in shredded lettuce treated at 23°C is showed in Table 4.4. The residues were statistically compared between the delivery systems, by fixing time and concentration.

Significant different were observed in the residues found in lettuce surface treated each of the ClO_2 delivery systems. Across the different ClO_2 concentration levels and exposure times the delivery systems left different residues on the produce surface.

In general the residues on the lettuces after the treatments carried out with the sachets or the Minidox had a higher standard deviation than the residues found on lettuces treated by the solution. The lettuce treated with the Minidox had significantly higher ClO_2 and ClO_2^- residue than the ones treated with the solution, for all different concentrations and exposure times.

The residues from the treatment at 5 min using the sachet did not significantly differ from the solution treatment, but as the exposure time increased the residues recovered from the samples exposed to the sachet increased significantly, achieving the same levels as the residues found on the lettuce exposed to the Minidox at 30 min. The

exception is at 10 min exposure when all concentrations for the sachet and Minidox were not statistically different.

These results can also be explained by the difference in the actual concentration profile for each delivery system. The concentration of ClO_2 generated by the sachet and solution at 5 min of exposure are similar, what lead to no significance difference between the recovered residues. The same happens with the sachet and Minidox generation systems, after 30 min achieved similar ClO_2 concentrations, therefore the residues recovered from both delivery systems, at this time interval, are not significantly different.

As mentioned before, residues recovered from the treatment using sachet and Minidox did not differ from each other at the 10 min interval treatment for all the tested concentration groups (1, 3 and 6 mg/L) but are significantly different from the solution. It is possible that while the ClO_2 generated by the Minidox and sachet introduced to the chamber will diffuse trough air to the produce location, the ClO_2 generated by the solution have to diffuse thought water and air before it will achieve the lettuce, this will delay the absorption.

The effect of exposure time and concentration of ClO_2 on the residue (ClO_2 and ClO_2^-) on the lettuce surface is shown in Figure 4.1 to 4.3. For the lettuce exposed to ClO_2 generated by the Minidox, as exposure time increase a significant increase on the

residue for all different concentrations is observed, except for 1 mg/L where the residues for the treatment at 5 and 10 min did not differ. This is explained by the equivalent actual concentration of gas in the chamber at these times. But for the sachet delivery system the increase in time showed a significant increase on the residues except for 1 and 3 mg/L concentration where treatment at 10 min did not differ from 15 min. For generation of ClO_2 by the solution the effect on increasing exposure time was different for each concentration group. The increase in concentration was responsible for difference in residues at the different exposure times, as at 1 mg/L treatment the exposure for 5, 15 and 30 min was not significantly different but at 6 mg/L the same exposure times were different. This is due to the actual concentration in the 1 mg/L group increases in a lower rate compared to the actual concentration of the 6 mg/L group.

It is important to notice, regardless of the ClO_2 delivery system, the same behavior when concentration is increased by fixing the exposure time. For high exposure times, there is a significant difference in residues in lettuce treated with 1, 3 and 6 mg/L of ClO_2.

It is possible to see from the residue results that treating lettuce with the Minidox at 3 mg/L for 5 min is not significantly different from treating it with the sachet at 1 mg/L for 15 min. This could represent a benefit for the use of the sachet maintaining the lettuce quality as long as efficacy is equivalent.

The present results align with previous results, as indicated in the Table 4.4, the residues using the solution as ClO_2 delivery system, are comparable to the data reported by Netramai (2010). She reported that less than 7.17 and 9.37 mg ClO_2^- /kg lettuce were absorbed in treatment with 3 mg/L for 15 and 30 min respectively. When the concentration is increased to 6 mg/L for the same time periods, 9.4 and 15.23 mg ClO_2^-/kg lettuce was absorbed (Netramai 2010).

Table 4.4- Residue of ClO_2 and ClO_2^- on lettuce surface [mg ClO_2/kg of tomato] at different time intervals (min), concentrations (mg/L), delivery systems (Minidox, sachet and solution) and 23°C

Concentration Group	Time (min)	MINIDOX			SACHET			SOLUTION		
		Actual conc.[1] (mg/L)	Mean (mg ClO_2/ kg of lettuce)[2]	Sd[3]	Actual conc. (mg/L)	Mean (mg ClO_2/ kg of lettuce)	Sd	Actual conc. (mg/L)	Mean (mg ClO_2/ kg of lettuce)	Sd
1 mg/L	5	0.51	3.01 ^{A}	0.73	0.21	1.12 ^{B}	0.32	0.16	1.68 ^{B}	0.19
	10	0.68	4.37 ^{A}	0.91	0.47	4.78 ^{A}	1.47	0.19	1.01 ^{B}	0.14
	15	0.75	9.85 ^{A}	1.15	0.60	6.73 ^{B}	3.31	0.21	2.17 ^{C}	0.49
	30	0.77	13.12 ^{A}	0.73	1.10	10.90 ^{A}	1.86	0.26	4.00 ^{B}	0.34
3 mg/L	5	1.64	6.79 ^{A}	0.48	0.50	1.63 ^{B}	0.45	0.44	1.78 ^{B}	0.75
	10	2.20	9.47 ^{A}	1.45	0.98	8.18 ^{A,B}	1.84	0.47	6.13 ^{B}	1.62
	15	2.41	14.09 ^{A}	1.07	1.39	10.17 ^{B}	2.17	0.53	6.71 ^{C}	2.26
	30	2.51	21.12 ^{A}	2.59	2.22	19.90 ^{A}	2.20	0.67	9.00 ^{B}	2.24
6 mg/L	5	3.53	6.50 ^{A}	0.48	0.89	4.02 ^{B}	1.12	0.60	3.95 ^{B}	0.32
	10	4.63	11.32 ^{A}	2.17	1.75	10.53 ^{A}	0.71	0.71	5.40 ^{B}	2.08
	15	5.06	19.95 ^{A}	2.14	2.51	14.01 ^{B}	1.66	0.81	9.68 ^{C}	0.95
	30	5.34	26.91 ^{A}	1.85	4.34	24.18 ^{A}	2.21	1.06	14.82 ^{B}	3.31

Table 4.4 (cont'd)

[1] Actual concentration of ClO_2 in the empty chamber determined in section 4.2

[2] Within rows, means sharing the same superscript capital letter are not significantly different ($p>0.05$; $n=3$)

[3] Sd = standard deviation of the three measurements

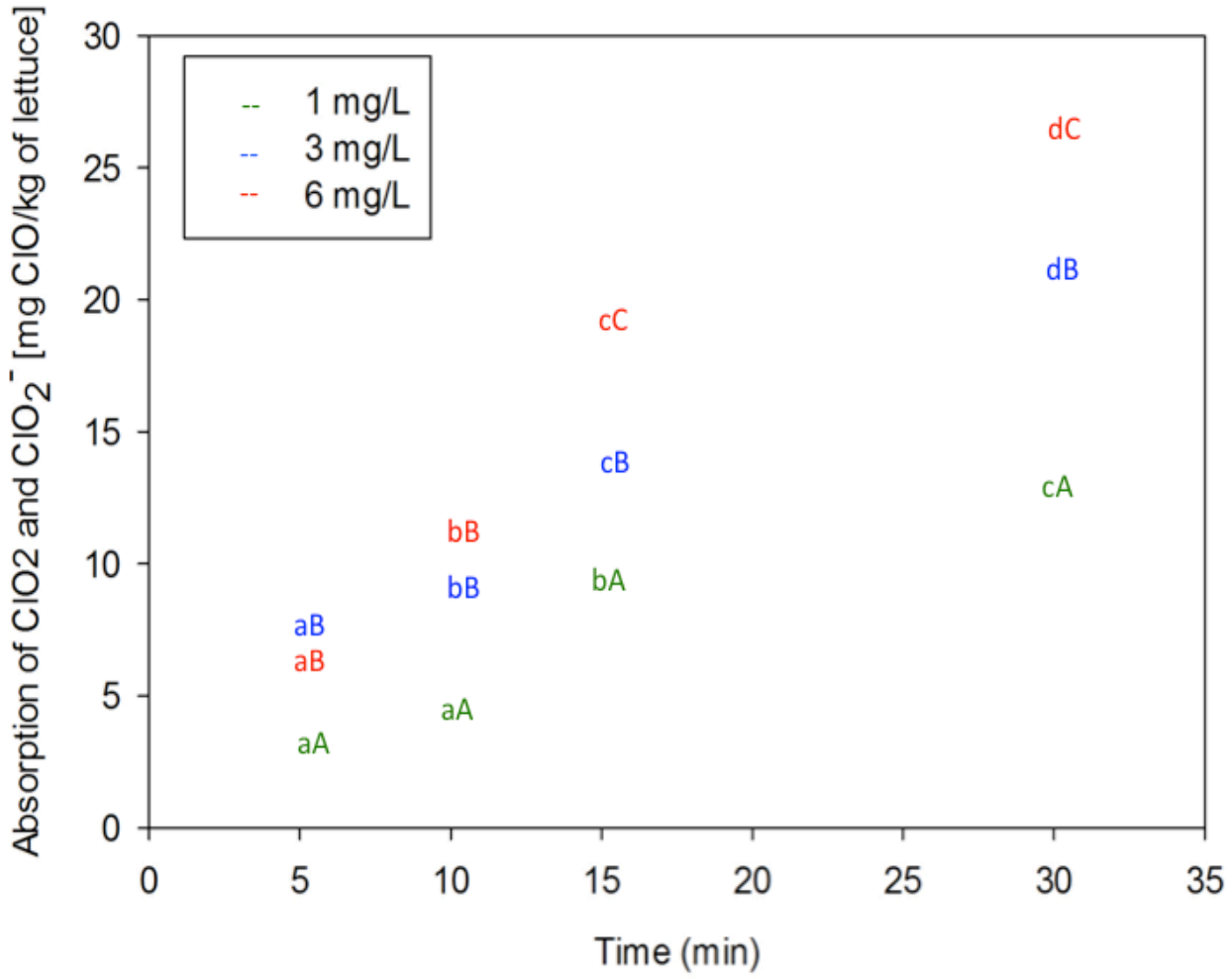

Figure 4.1 - Residue of ClO_2 and ClO_2^- when exposed to ClO_2 produced by the Minidox on shredded lettuce classified by the concentration group.

*The point sharing the same lower case letter indicate no significant difference between the time intervals at the same exposure concentration group (same color). Points sharing the same upper case letters indicate no significant difference between the exposure concentration group at the same time interval

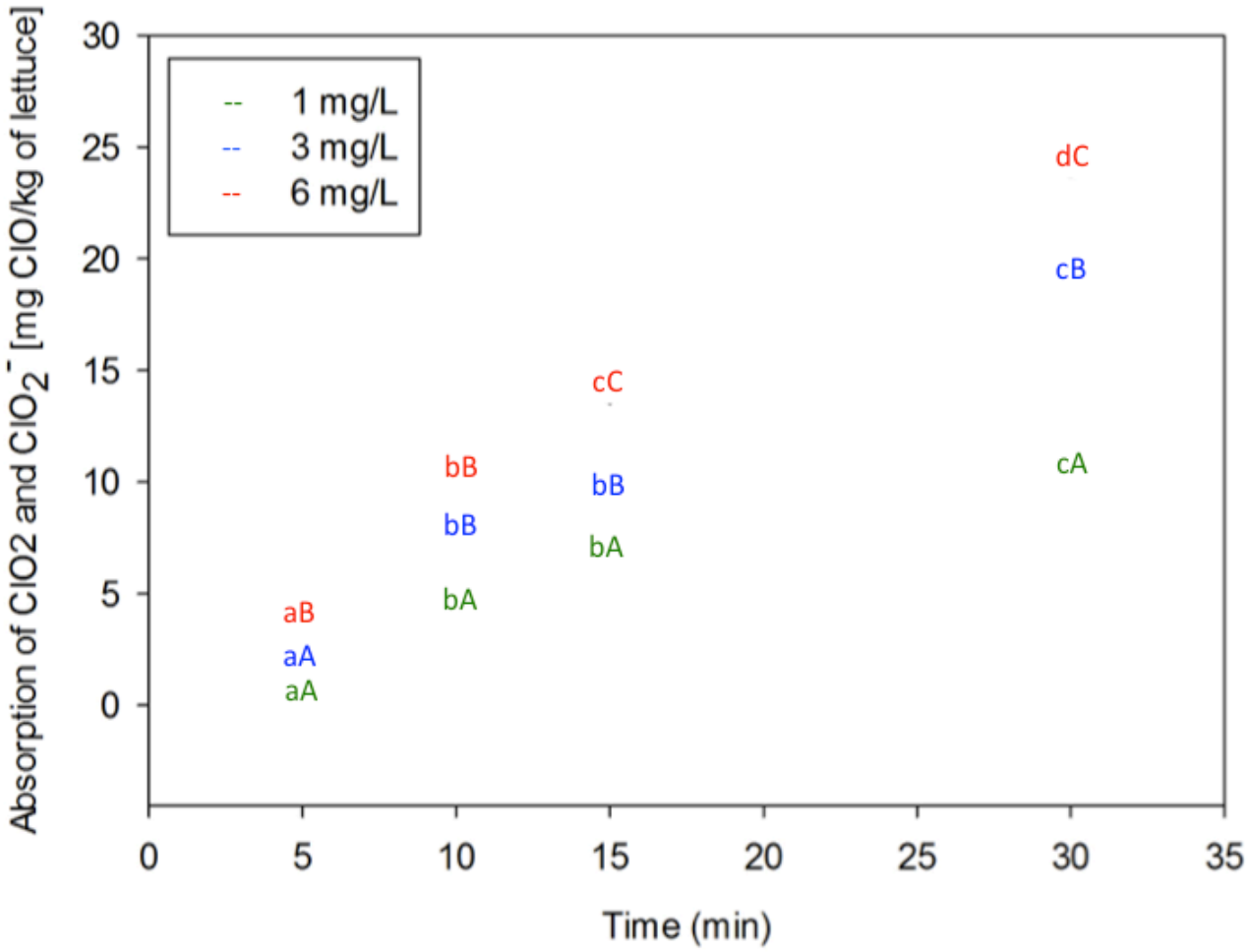

Figure 4.2 - Residued of ClO_2 and ClO_2^- when exposed by ClO_2 produced by the sachet on shredded lettuce classified by the concentration group

* The point sharing the same lower case letter indicate no significant difference between the time intervals at the same exposure concentration group (same color). Points sharing the same upper case letters indicate no significant difference between the exposure concentration group at the same time interval

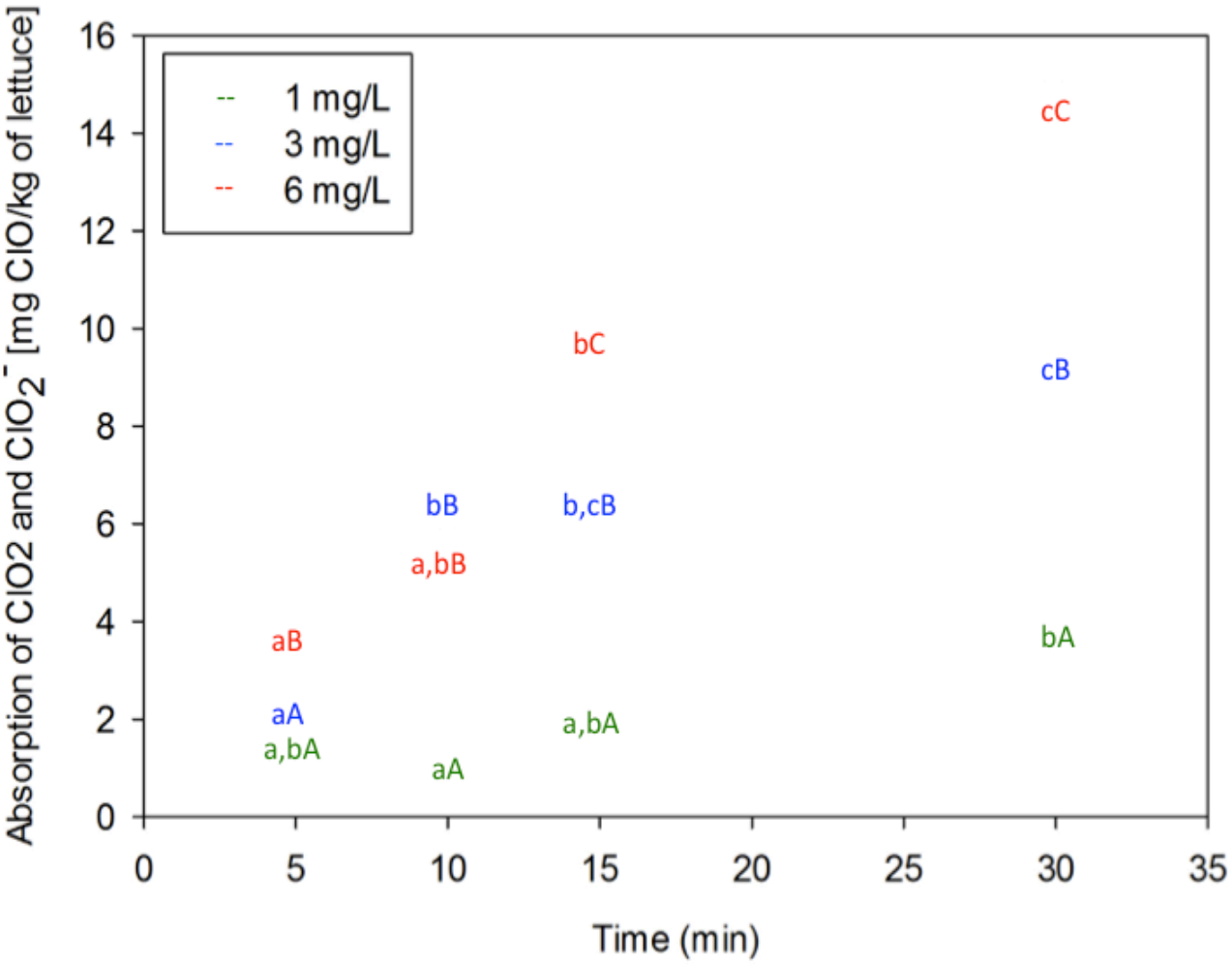

Figure 4.3 - Residue of ClO_2 and ClO_2^- when exposed by ClO_2 produced by the solution on shredded lettuce classified by the concentration group

*The point sharing the same lower case letter indicate no significant difference between the time intervals at the same exposure concentration group (same color). Points sharing the same upper case letters indicate no significant difference between the exposure concentration group at the same time interval

The residues in lettuce surfaces in the current experiment exceed the limits determined by EPA for drinking water, 0.8 mg/L of ClO_2 and 1 mg/L of ClO_2^- (USEPA 1999). Visual quality damage was observed in the lettuce surface after treatments with 1 mg/L for 15 min, 3 mg/L for 10 min and more than 5 min for 6 mg/L. The lettuce turned white and lost strength, becoming unacceptable for consumption, as possible to see in Figure 4.4. The bleaching could be attributed to oxidation of chlorophyll (Gómez-López *et al.* 2009).

It is important to notice that even thought the amount of ClO_2 found in the lettuce surface was high, it can decrease after storage, achieving safe amounts for human consumption. This was shown through a study by Han (2004) which found no ClO_2 residues and 0.07 mg Cl_2/kg of ClO_2^-after 1 week of the treatment with ClO_2 gas in strawberry surfaces. The same was observed by Netramai (2010). After leaving treated lettuce at room temperature for 60 min, levels of ClO_2 were undetectable and levels of ClO_2^- achieve less than 0.90 mg ClO_2^-/kg (Netramai 2010). But it is also important to investigate if any harmful by-products were formed during ClO_2 treatment in the produces surface (Han *et al.* 2004).

No regulatory agencies have approved the use of gaseous ClO_2 for sanitizing fresh produce because of the lack of information related to concentration of ClO_2 and the produce surface. The reported data refer to residues right after treatment, but more

research is still necessary to measure the behavior of the gas after treatment, and information of other by-products formed when ClO_2 reacts with plant tissue is needed. Also, with these results it is possible to see the difference in absorption when different delivery systems are used. This information is important for the creation of any new regulations, which will need to account for the different absorption qualities of the delivery systems.

‘

Figure 4.4 - Bleached lettuce after treatment with 6 mg/L for 10 min with the ClO_2 produced by the Minidox

4.3.2. Residues in lettuce treated at 4°C

The residues on shredded lettuce after treating with different ClO_2 delivery systems, at the specific concentration at different interval but at 4°C is shown in Table 4.5.

The residues on lettuce surfaces at lower temperature were lower than the residues found at higher temperature. The three delivery systems showed a equivalent residue profile for the 4°C treatment. The increase in concentration and exposure time also showed a different effect in the absorption for each delivery system.

The residues found on the lettuces treated with the solution were quite different than the residues found on lettuces treated with the Minidox and the sachet. The residues found on lettuce treated at higher concentration and for shorter intervals by the sachet and Minidox were significantly lower that the lettuce treated with the solution. But the highest concentration 6 mg/L for the longest interval, 30 min, in this treatment the lettuces treated with the Minidox also had the highest residue as it happened at 23°C and differ from the sachet and solution.

The impact on increasing ClO_2 concentration and exposure time on the residue can be seen in Figure 4.5 to 4.7 for each ClO_2 generation system. The behavior is different from that observed for 23°C. In the MInidox system, there was no difference in residues between the different exposure times when treated with 1 and 3 mg/L, except for the 30 min. In the treatment using the sachet, it was observed that as ClO_2

concentration in the headspace increases the difference between exposure times increases significantly. For example, at 6 mg/L all the exposure times are statistically different but at 1 mg/L only the treatments at 5 and 30 min differ from each other. In the case of the solution treatments, 5 and 30 min exposure times were statistically different in all tested concentrations.

Table 4.5 - Residue of ClO_2 and ClO_2^- on lettuce surface [mg ClO_2/kg of tomato] at different time intervals (min), concentrations (mg/L), delivery systems (Minidox, sachet and solution) and 4°C

Concentration group	Time (min)	MINIDOX			SACHET			SOLUTION		
		Actual conc.[1] (mg/L)	Mean (mg ClO_2/ kg of lettuce)[2]	Sd[3]	Actual conc. (mg/L)	Mean (mg ClO_2/ kg of lettuce)	Sd	Actual conc. (mg/L)	Mean (mg ClO_2/ kg of lettuce)	Sd
1 mg/L	5	0.33	1.35 ^{A}	0.36	0.12	1.19 ^{A}	0.32	0.26	2.51 ^{B}	0.53
	10	0.50	2.28 ^{A}	0.43	0.18	2.02 ^{A}	1.15	0.34	4.14 ^{A}	0.18
	15	0.60	2.88 ^{A}	0.35	0.27	2.94 ^{A}	0.84	0.38	4.97 ^{A}	0.39
	30	0.73	4.96 ^{A}	1.46	0.49	4.48 ^{A}	0.92	0.51	7.06 ^{A}	0.97
3 mg/L	5	1.15	3.78 ^{A}	1.27	0.31	1.45 ^{B}	0.24	0.59	5.43 ^{C}	1.28
	10	1.76	5.30 ^{A}	1.15	0.57	4.85 ^{A}	1.30	1.01	6.48 ^{A}	2.01
	15	2.21	4.57 ^{A}	1.28	0.74	7.23 ^{A}	1.28	1.13	7.18 ^{A}	3.31
	30	2.51	9.25 ^{A}	1.29	1.22	11.58 ^{A}	1.41	1.31	10.85 ^{A}	1.33
6 mg/L	5	2.51	4.62 ^{A}	0.55	0.69	4.65 ^{A}	0.66	1.02	6.28 ^{B}	0.65
	10	3.79	8.08 ^{A}	0.18	1.16	7.05 ^{A}	1.66	1.22	12.42 ^{B}	3.16
	15	4.52	9.62 ^{A}	1.45	1.53	9.84 ^{A}	2.48	1.40	11.40 ^{A}	0.33
	30	5.34	24.59 ^{A}	3.35	2.40	19.16 ^{B}	2.79	1.80	13.63 ^{C}	0.66

Table 4.5 (cont'd)

[1] Actual concentration of ClO_2 in the empty chamber determined in section 4.3

[2] Within rows, means sharing the same superscript capital letter are not significantly different ($p>0.05$; $n=3$)

[3] Sd = standard deviation of the three measurements

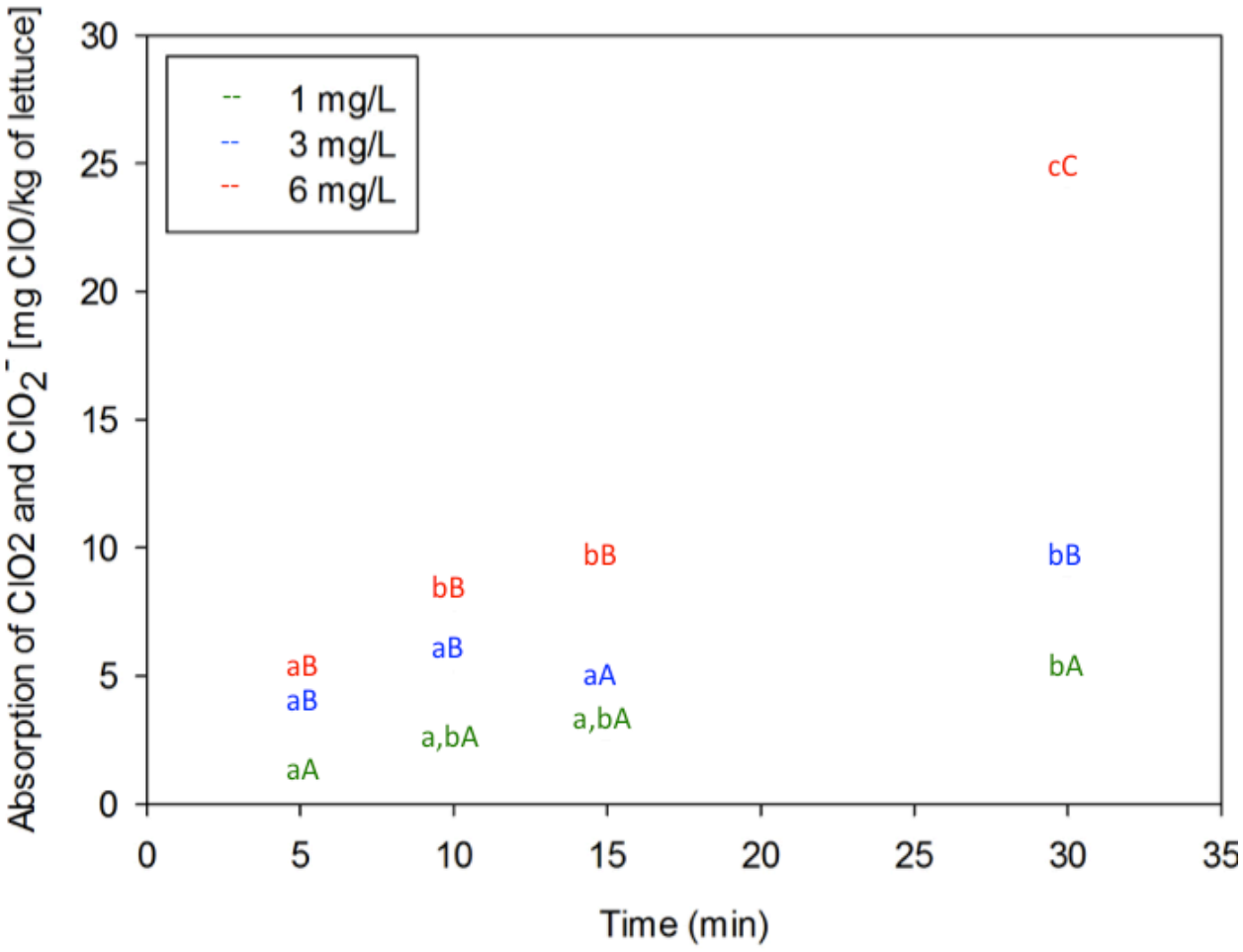

Figure 4.5 - Residue of ClO_2 and ClO_2^- when exposed by ClO_2 produced by the Minidox on shredded lettuce at 4°C, classified by the concentration group

*The point sharing the same lower case letter indicate no significant difference between the time intervals at the same exposure concentration group (same color). Points sharing the same upper case letters indicate no significant difference between the exposure concentration group at the same time interval

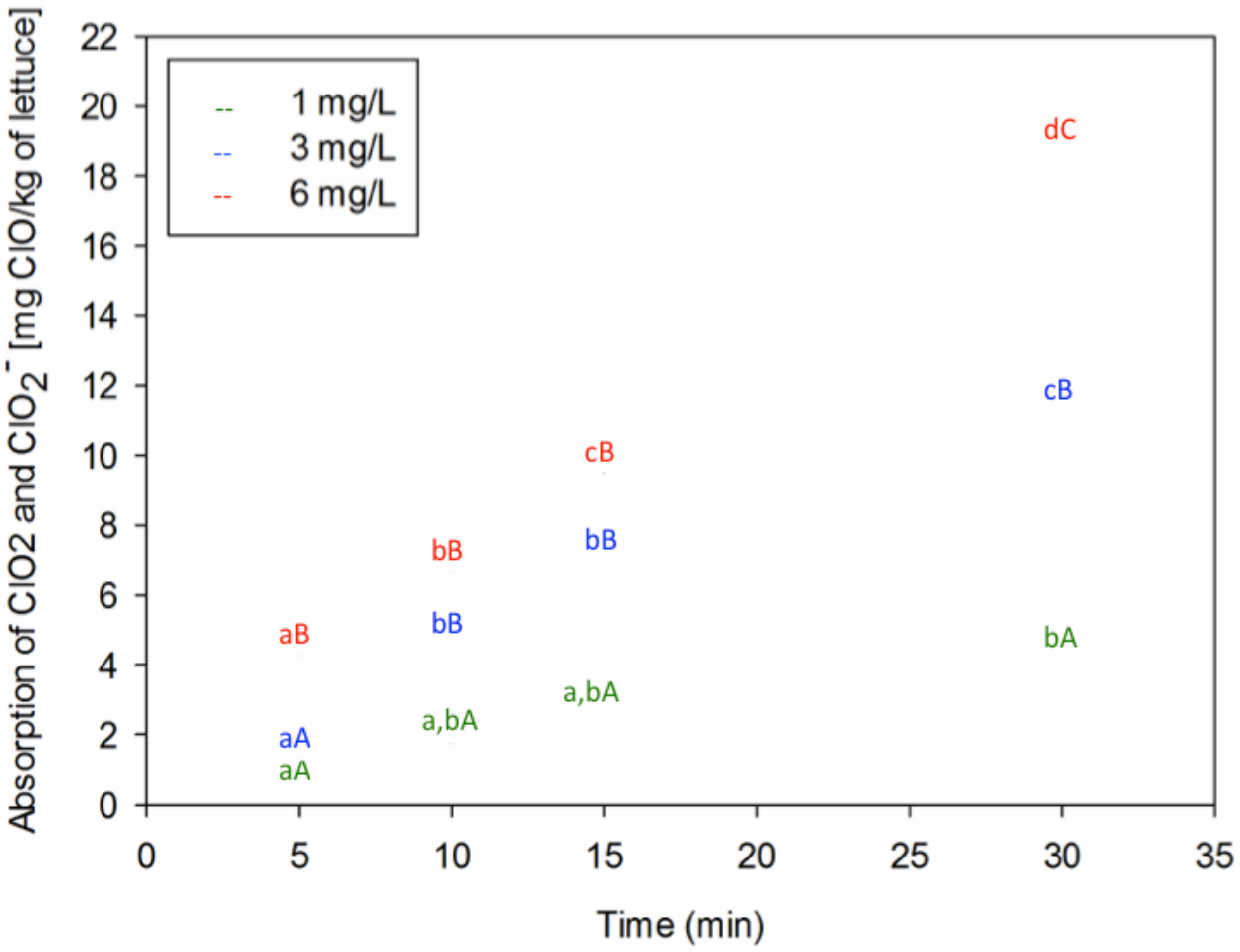

Figure 4.6 - Residue of ClO_2 and ClO_2^- when exposed by ClO_2 produced by the sachet on shredded lettuce at 4°C, classified by the concentration group

*The point sharing the same lower case letter indicate no significant difference between the time intervals at the same exposure concentration group (same color). Points sharing the same upper case letters indicate no significant difference between the exposure concentration group at the same time interval

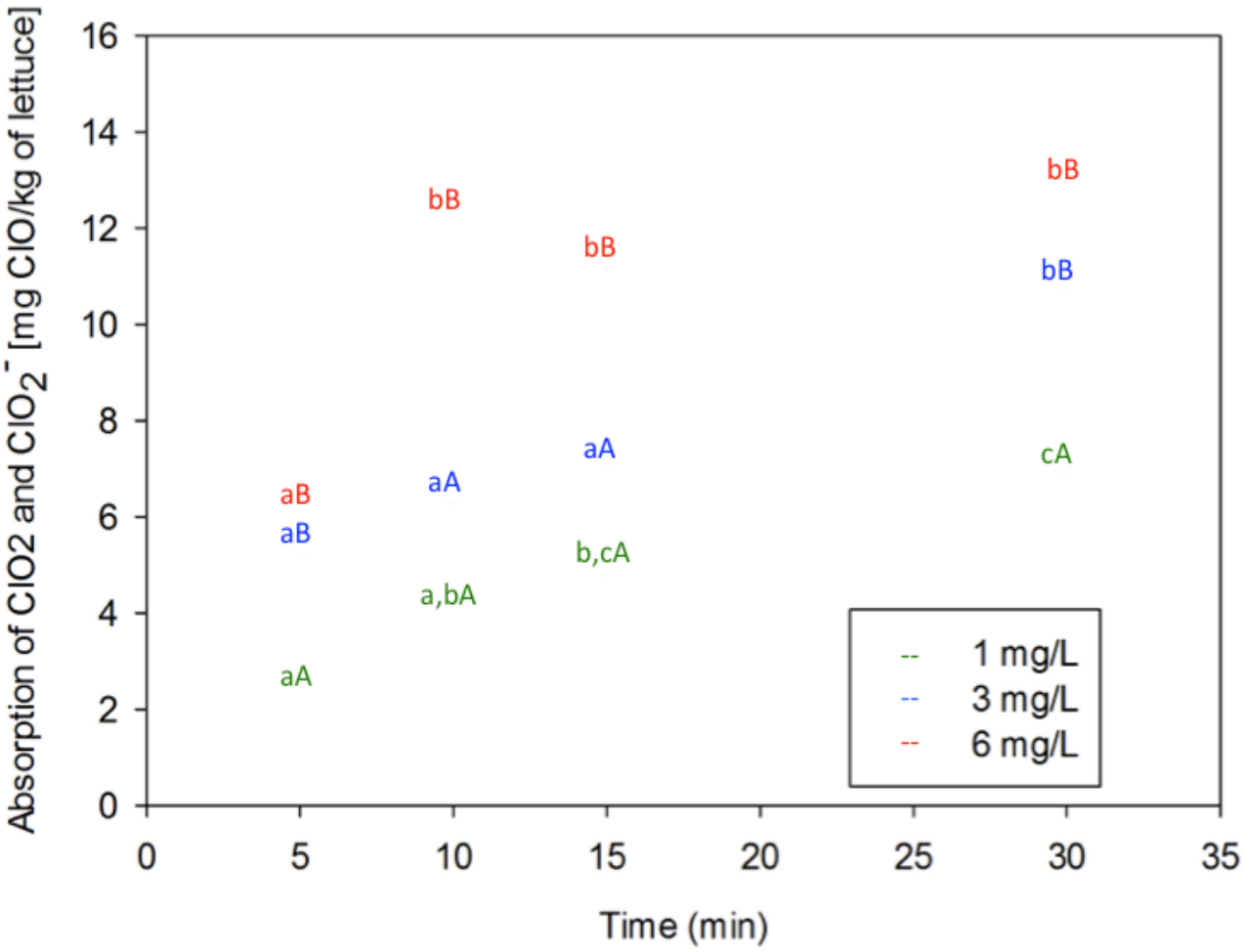

Figure 4.7 - Residue of ClO_2 and ClO_2^- when exposed by ClO_2 produced by the solution on shredded lettuce at 4°C, classified by the concentration group

*The point sharing the same lower case letter indicate no significant difference between the time intervals at the same exposure concentration group (same color). Points sharing the same upper case letters indicate no significant difference between the exposure concentration group at the same time interval

4.3.3 Comparison between treatment at 23 and 4°C

The effect of decreasing treatment temperature in the residues can be observed in Figure 4.8. For the sachet and Minidox generation system the decrease in temperature decreases the residues on lettuce surfaces. This could be due to the decrease on the respiration rate of lettuce with the decrease in temperature, lowering the gas exchange with the environment. In the case of the sachet, the difference could be due to the decrease in the ClO_2^- gas concentration caused by a slower reaction rate, as shown in section 4.1.1.

For the solution the decease in temperature did not significantly affect the amount of residues on the lettuce, except in the treatment at 1 mg/L for 10 min, 3 mg/L for 5 min and 6 mg/L for 5 and 10 min. The residues, after the treatment using solution as the delivery system, showed a bigger variability between treatments.

There is a difference in behavior between the solution and the other two delivery systems (as seen in Figure 4.8). The different behavior between the three delivery systems, when exposed to different temperatures, can be explained by the change in the initial setting of the solution and not for the sachet and MInidox. The sachet had the same amount of chemical in both situations (23 and 4°C) while the concentration of the solution changed between them; the initial solution concentration for treatment at 23°C was around 28 mg/L and for the treatment at 4°C was around 50 mg/L. This was discussed when the actual concentration profile for each experiment was determined

and the higher gas concentration observed for the solution at 4^{o}C compared to the 23^{o}C situation (section 4.1).

The diffusion rate of the gas in the headspace decreases with the decrease in temperature, what can be one of the explanations of the difference in the actual concentration of the gas in the headspace and in the absorption behavior at 23 and 4^{o}C. (Chen and Othmer 1962).

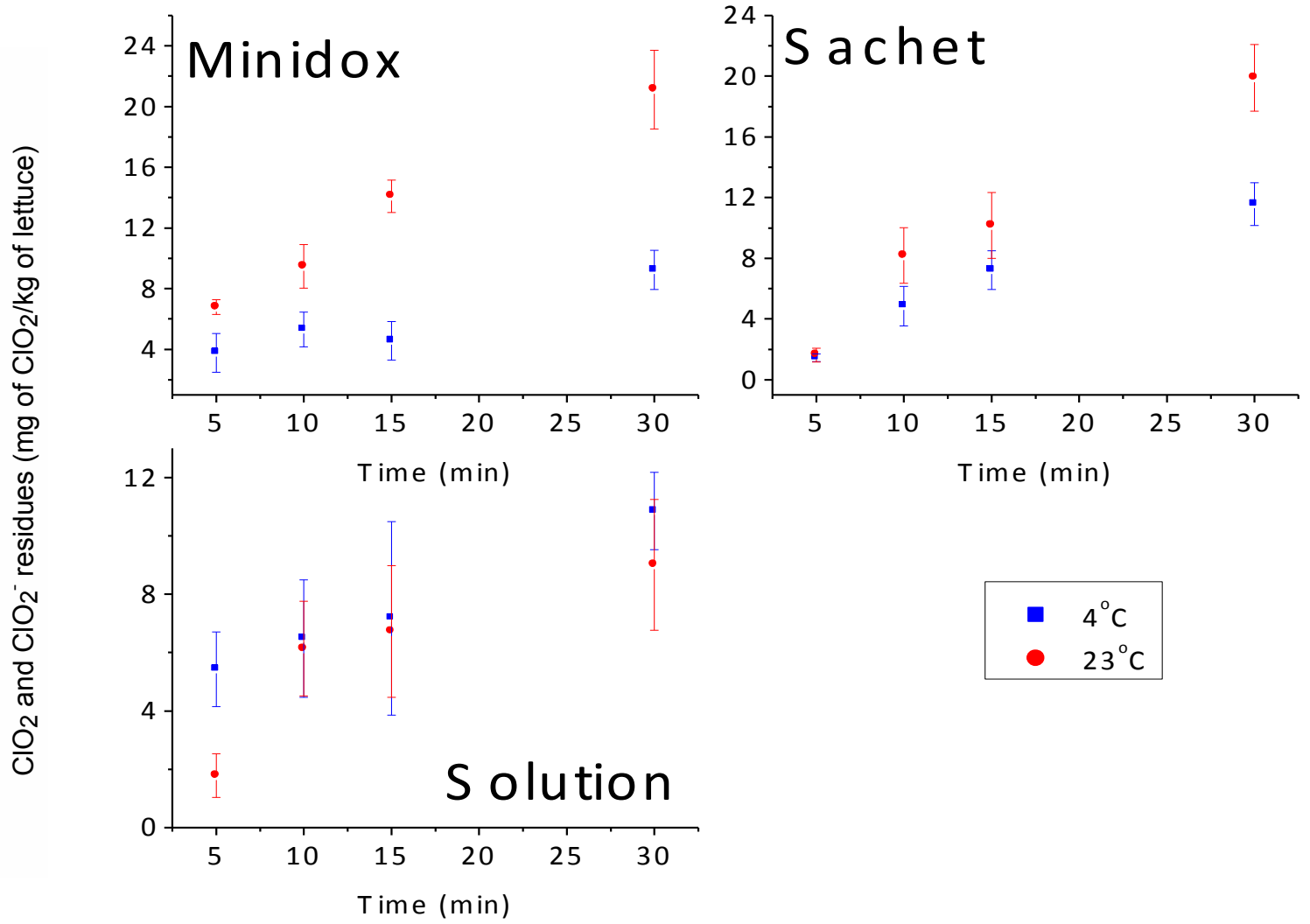

Figure 4.8 – Residual ClO_2 and ClO_2^- in lettuce surface when treated with the different delivery system, at 23 and 4°C and with a fixed ClO_2 concentration of 3 mg/L

4.4. Residues on cherry tomatoes surface after ClO_2 treatment

Residues of ClO_2 and ClO_2^- were quantified in tomato surfaces treated with the three different delivery systems, at specific ClO_2 concentrations, exposure times and temperatures. The results are shown in Table 4.6.

The residues found on tomatoes, no matter the delivery system, exposure time, concentration level or treatment temperature, are less then the detection limit of 0.201 mg ClO_2/ kg of tomato. In this situation the signal is within the margin of error of the amperometric system or noise level of the experimental procedure. The ClO_2 and ClO_2^- found in the tomato surface cannot be differentiated as part of the real signal or noise. In this case it is necessary to use a different measurement procedure, with a smaller detection limit, to have a better reliability in the absorption values. This also explains the large standard deviation from the data.

It is possible to ensure that the amounts of ClO_2 and ClO_2^- residue are very low in all three delivery systems because a waxy cuticle protects the tomato. The cuticle has very low gas permeability as it occurs only through pores and stomata (Vogg *et al.* 2004). However, the stem cavity of the tomato is made with the same tissue as the leafy green vegetables and might be the only region that is absorbing ClO_2 (Fennema 1996).

Also the concentration of gas inside the tissue will depend on tissue's solubility, diffusion and metabolic characteristics that will control the absorption (Fennema 1996).

The absorption of by-products in the tomato surface is in accordance with the EPA regulations for drinking water (0.8 mg/L of ClO_2 and 1 mg/L of ClO_2^-), suitable for consumption even after treatment. A change in color of the tomato stem cavity was observed after treatments with 6 mg/L for 30 min and 10 mg/L after 15 min, as is possible to see in Figure 4.9. This change did not affect the visual appearance of the fruits and may be imperceptible to consumers.

Trinetta (2011b) treated hydroponic tomatoes with 0.5 mg/L of gas ClO_2 for 10 min and did not find significant difference in residues between the control and treated tomatoes right after treatment, except for chlorite which increased 0.05 mg/kg of tomato. In this case was used an ion chromatography technique for the measurement of residues. Even though the measuring methodology and the tomato species were different the results were comparable.

Figure 4.9 – Appearance of cherry tomato before and after treatment with 10 mg/L for 15 min with the ClO_2 generated by the Minidox

Table 4.6 – Residue of ClO_2 and ClO_2^- on tomatoes surface [mg ClO_2/kg of tomato] at different time intervals (min), concentrations (mg/L), temperature (^oC) and delivery systems (Minidox, sachet and solution)

		MACHINE (10^{-2} mg ClO_2/kg of tomato)				SACHET (10^{-2} mg ClO_2/kg of tomato)				SOLUTION (10^{-2} mg ClO_2/kg of tomato)			
Concen-		23°C		4°C		23°C		4°C		23°C		4°C	
tration	Time (min)	Mean	sd[1]	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd
3 mg/L	15	3.193	2.268	1.144	1.982	4.149	3.638	1.694	2.032	1.861	1.748	6.590	2.649
	30	0.565	0.978	6.836	0.642	4.213	2.849	4.655	6.120	0.043	0.074	11.268	1.627
	60	0.364	0.630	5.666	1.227	1.870	1.708	3.523	6.019	3.026	1.434	5.699	1.063
6 mg/L	15	0.564	0.977	4.867	1.678	7.520	4.071	3.196	3.362	0.185	0.321	8.856	0.978
	30	1.194	2.068	5.012	2.913	4.653	5.065	2.261	1.782	0.849	1.471	7.283	2.540
	60	0.943	1.633	2.958	0.369	1.382	1.202	0.000	0.000	3.712	3.463	2.688	1.998
10 mg/L	15	5.880	9.696	1.111	0.574	0.566	0.568	3.611	3.351	3.235	5.604	6.431	0.626
	30	3.503	1.979	4.079	0.885	0.570	0.971	2.973	0.602	7.302	6.813	5.354	1.508
	60	4.680	1.811	9.836	1.827	3.248	2.913	3.700	4.654	4.724	2.159	5.020	1.381

[1] standard deviation of 3 replicates

4.5. Residuos of ClO_2 and ClO_2^- model for lettuce

An empirical model was designed to predict the absorption of ClO_2 and ClO_2^- in shredded lettuce exposed to ClO_2 produced by different delivery systems. The ClO_2 concentration, exposure time, and treatment temperature were the variables used to predict the model for each ClO_2 delivery system. A response surface analysis test was used to predict the regression coefficients that describe the model for each delivery system. The following general equation describes all the models:

$$y = a_0 + a_1x_1 + a_2x_2 + a_3x_1^2 + a_4x_2^2 + a_5x_1x_2$$

Where y is the total residue (ClO_2 and ClO_2^-), a_0 is a constant term, a_1 to a_5 are the variables coefficients, x_1 is the delivered concentration of ClO_2 and x_2 is the treatment exposure time.

The model terms were selected or rejected based on the P-value calculated with the surface analysis, with a 95% confidence level. A specific model was developed for each delivery system (Minidox, sachet and solution) and for each of the tested treatment temperatures (4 and 23°C), totaling 6 different models. The models were tested for the lack of fit and all of them were shown to be statistically significant (P-value > 0.05) indicating a good fit of the predicted model.

The data presented in Table 4.4 and 4.5 was used to predict all the models. In all of the developed models, the predictors were shown to have an effect on the response (residues), as the total model was shown to be significantly different from zero (Table 4.7). Also all the predictors were significant, so no term was dropped from the general equation. The R-squares, located on Table 4.7, show the percentage of variance that can be explained by the model. All R-squares are above 0.80.

A validation for the developed model was carried out in order to evaluate the mathematical model. The method used is presented in Appendix 7.

4.5.1. ClO_2 and ClO_2^- absorption model for a ClO_2 treatment generated by gas Minidox at 23°C

The equation for this model is:

$$Residue = -5.257 + 1.060t + 3.224c - 0.0192t^2 - 0.412c^2 + 0.0786tc$$

The response surface shows that as concentration and time increases, the absorption of ClO_2 and ClO_2^- increases (Figure 4.10).

The validation of the model was done with a treatment at 4 mg/L for 7.5 min, using the same experimental settings. Based on Appendix 8 the actual concentration of ClO_2 on the specific interval was 2.60 mg/L. This value was substituted in the model obtaining a residue of 8.74 mg of ClO_2 and ClO_2^-/kg, which is comparable to the

experimental results (8.60 ± 1.16 mg of ClO_2 and ClO_2^-/kg of lettuce), showing a good prediction of the theoretical model.

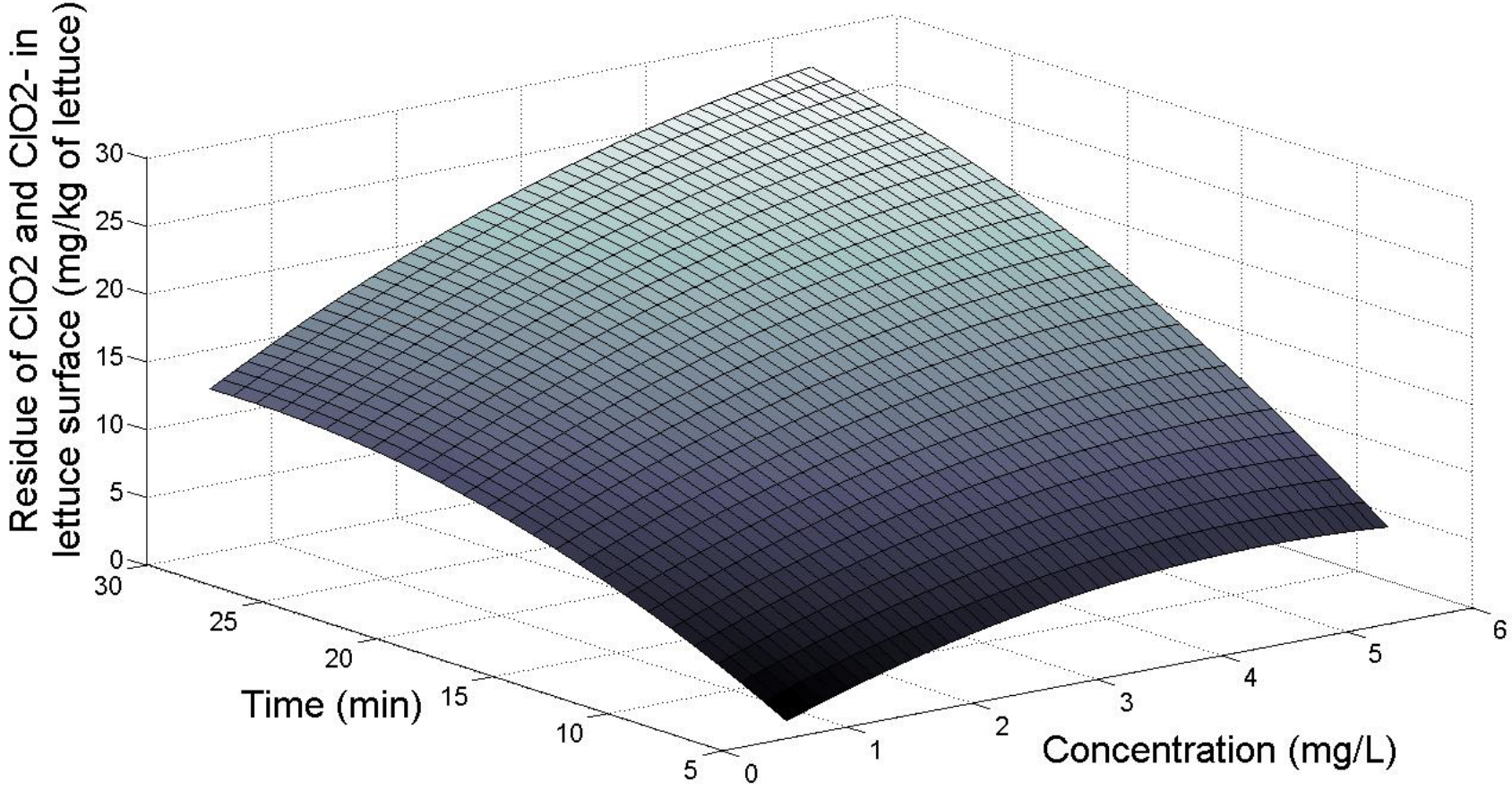

Figure 4.10 – Representation of the prediction model for residues in lettuce surface exposed to ClO_2 generated by gas Minidox at 23°C

4.5.2. ClO_2 and ClO_2^- absorption model for a ClO_2 treatment generated by Minidox at 4°C

The prediction model can be written as:

$$Residue = 5.849 + 0.363t - 1.673c + +0.00923t^2 + 0.436c^2 + 0.108ct$$

From the surface presented in Figure 4.11 is possible to observe the synergetic behavior of concentration and time, as both of those parameters increase the residues increase.

The validation of the model was done with a treatment at 4 mg/L for 7.5 min, using the same experimental settings. Based on Appendix 8 the actual concentration of ClO_2 on the specific time interval was 1.97 mg/L. This value was substituted in the model obtaining a residue of 3.64 mg of ClO_2 and ClO_2^-/kg, which is not comparable to the experimental results (5.12 ± 0.32 mg of ClO_2 and ClO_2^-/kg of lettuce), showing a deviation of the model in this case.

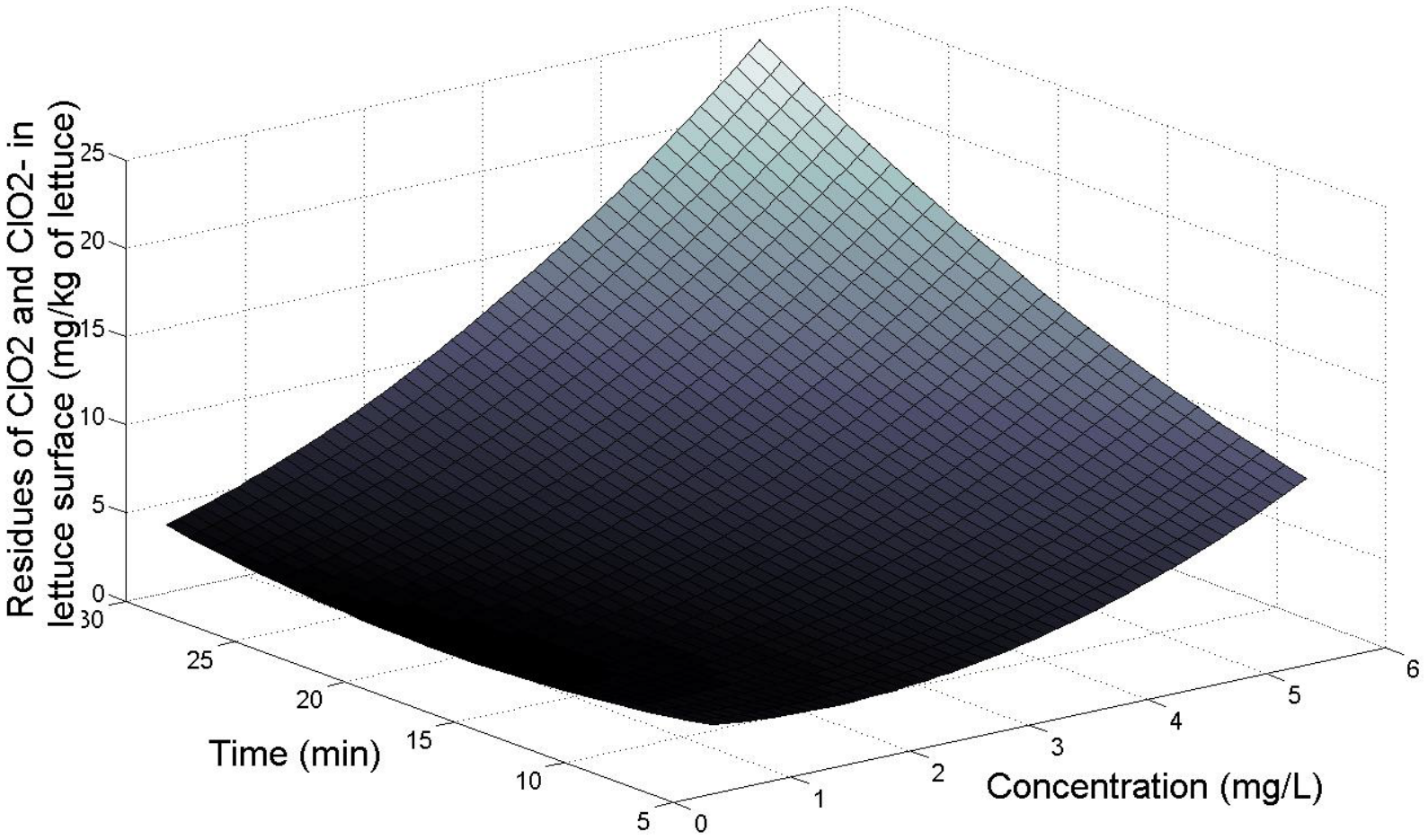

Figure 4.11 - Representation of the prediction model for residues in lettuce surface exposed to ClO_2 generated by the MInidox at 4°C

4.5.3. ClO_2 and ClO_2^- absorption model for a ClO_2 treatment generated by sachet at 23°C

The predicted equation that can be written as:

$$Residue = -2.057 + 0.457t + 5.327c - 0.0152t^2 - 1.783c^2 + 0.282ct$$

From the surface presented in Figure 4.12 is possible to observe the increase in time at lower concentration does not have a big effect in the residues, but as concentration increases the effect of time is stronger.

The validation of the model was done with a treatment at 4 mg/L for 7.5 min, using the same experimental settings. For this experiment was used 16 g of sachet and based on Appendix 8 the actual concentration of ClO_2 on the specific time interval was 1.14mg/L. This value was substituted in the model obtaining a residue of 6.70 mg of ClO_2 and ClO_2^-/kg, which is comparable to the experimental results (7.06 ± 0.74 mg of ClO_2 and ClO_2^-/kg of lettuce), showing a good prediction of the theoretical model.

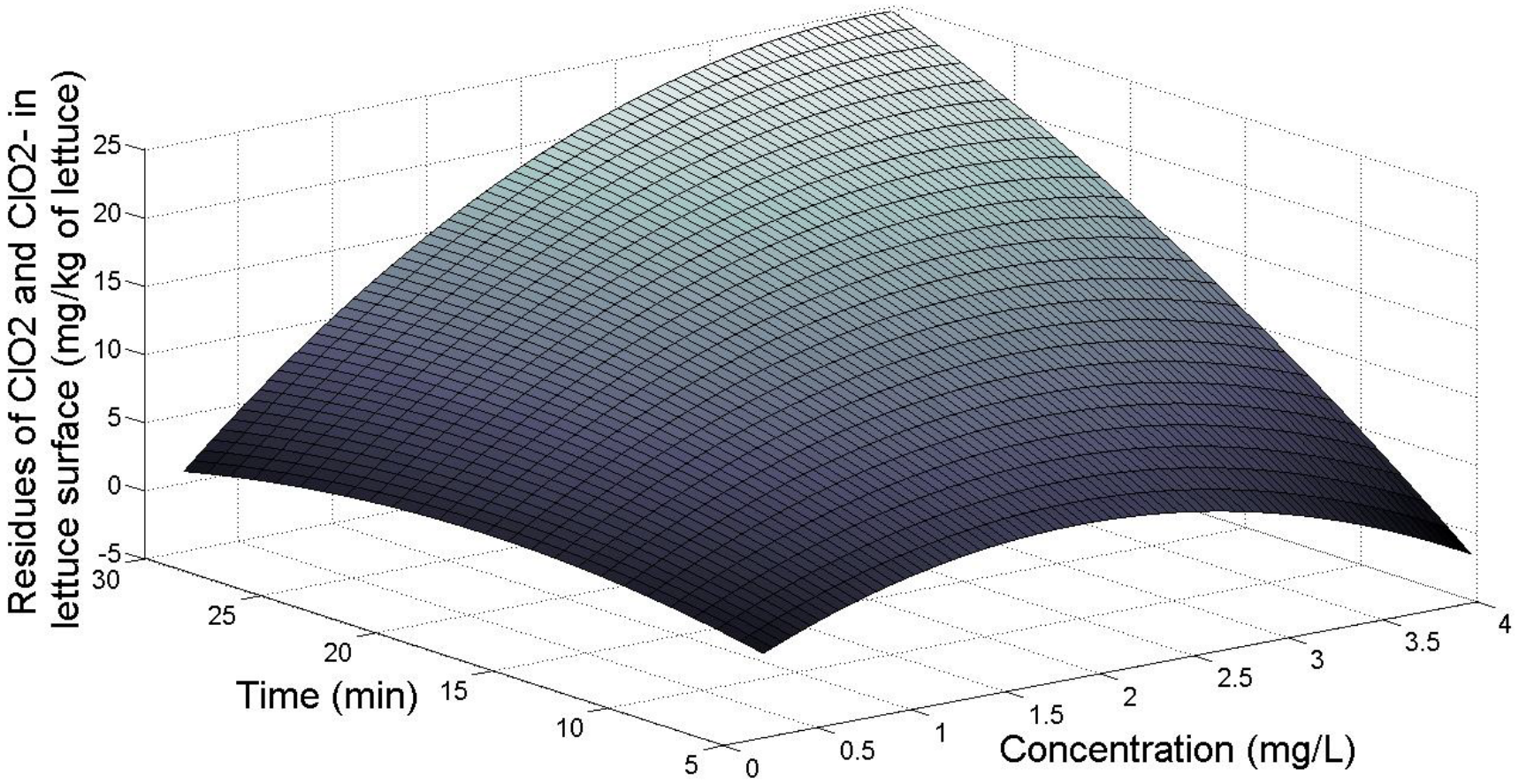

Figure 4.12 - Representation of the prediction model for residues in lettuce surface exposed to ClO_2 generated by the sachet at 23°C

4.5.4. ClO_2 and ClO_2^- absorption model for a ClO_2 treatment generated by sachet at 4°C

The predicted model that can be written as:

$$Residue = -0.412 + 0.214t + 4.879c - 0.00861t^2 - 2.013c^2 + 0.287ct$$

From the surface presented in Figure 4.13 is possible to observe it is similar from the residues surface for the sachet at 23°C. Also showing that as time and concentration increased together the residues in the surface increases.

The validation of the model was done with a treatment at 4 mg/L for 7.5 min, using the same experimental settings. For this experiment was used 16 g of sachet and based on Appendix 8 the actual concentration of ClO_2 on the specific time interval was 0.64 mg/L. This value was substituted in the model obtaining a residue of 4.39 mg of ClO_2 and ClO_2^-/kg, which is comparable to the experimental results (5.02 ± 0.83 mg of ClO_2 and ClO_2^-/kg of lettuce), showing a good prediction of the theoretical model.

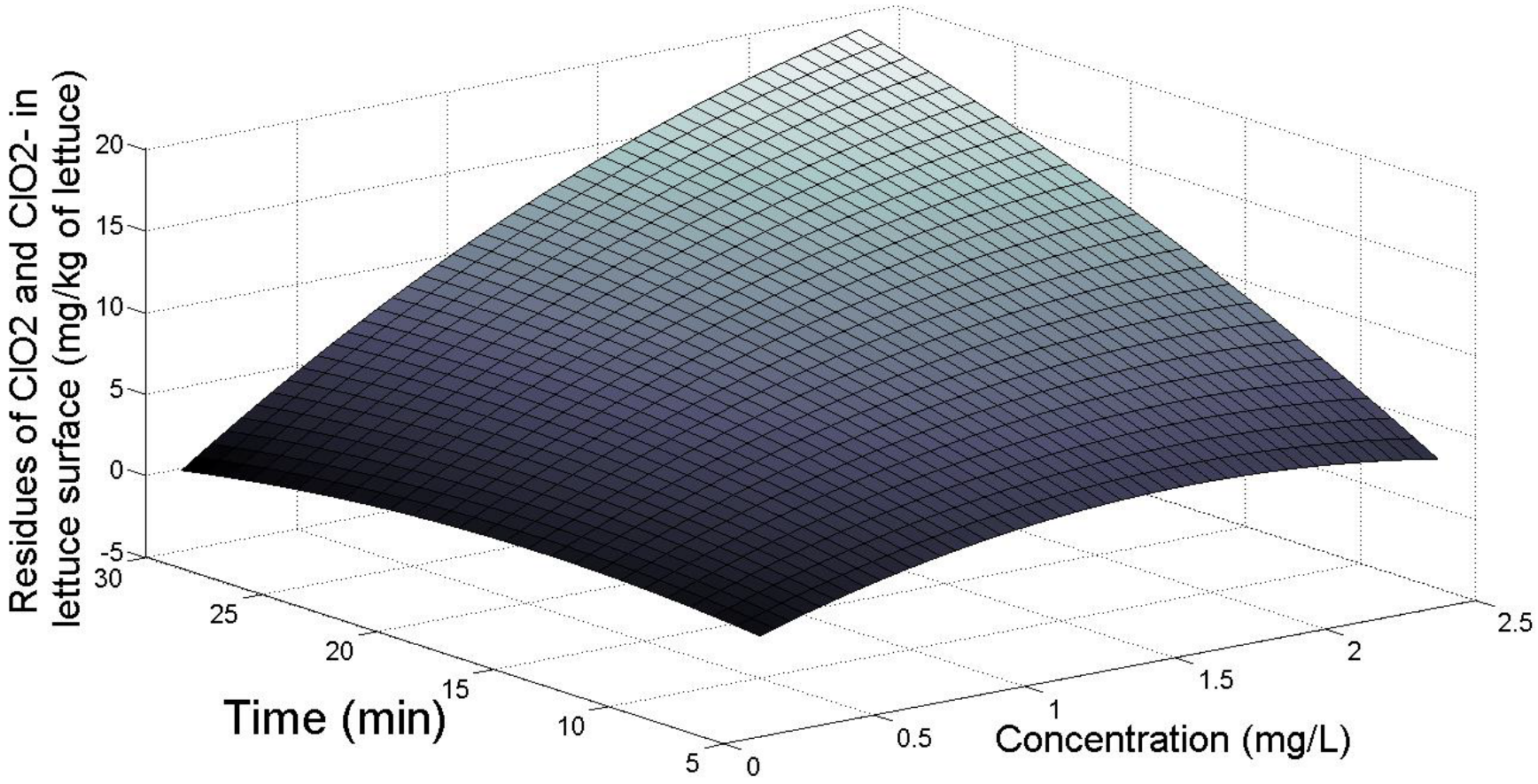

Figure 4.13 - Representation of the prediction model for residues in lettuce surface exposed to ClO_2 generated by the sachet at 4°C

4.5.4. ClO_2 and ClO_2^- absorption model for a ClO_2 treatment generated by the solution at 23°C

The predicted model that can be written as:

$$Residue = -2.529 + 0.335t + 7.226c - 0.00792t^2 + 0.621c^2 + 0.188ct$$

From Figure 4.14 is possible to observe that the effect of increasing concentration is higher then increasing time, as is possible to observe a bigger increasing angle for the concentration side.

The validation of the model was done with a treatment at 4 mg/L for 7.5 min, using the same experimental settings. For this experiment was used a solution with initial concentration of 116.8 mg/L and based on Appendix 8 the actual concentration of ClO_2 on specific time interval was 0.587 mg/L. This value was substituted in the model obtaining a residue of 4.82 mg of ClO_2 and ClO_2^-/kg, which is comparable to the experimental results (5.16 ± 0.31 mg of ClO_2 and ClO_2^-/kg of lettuce), showing a good prediction of the theoretical model.

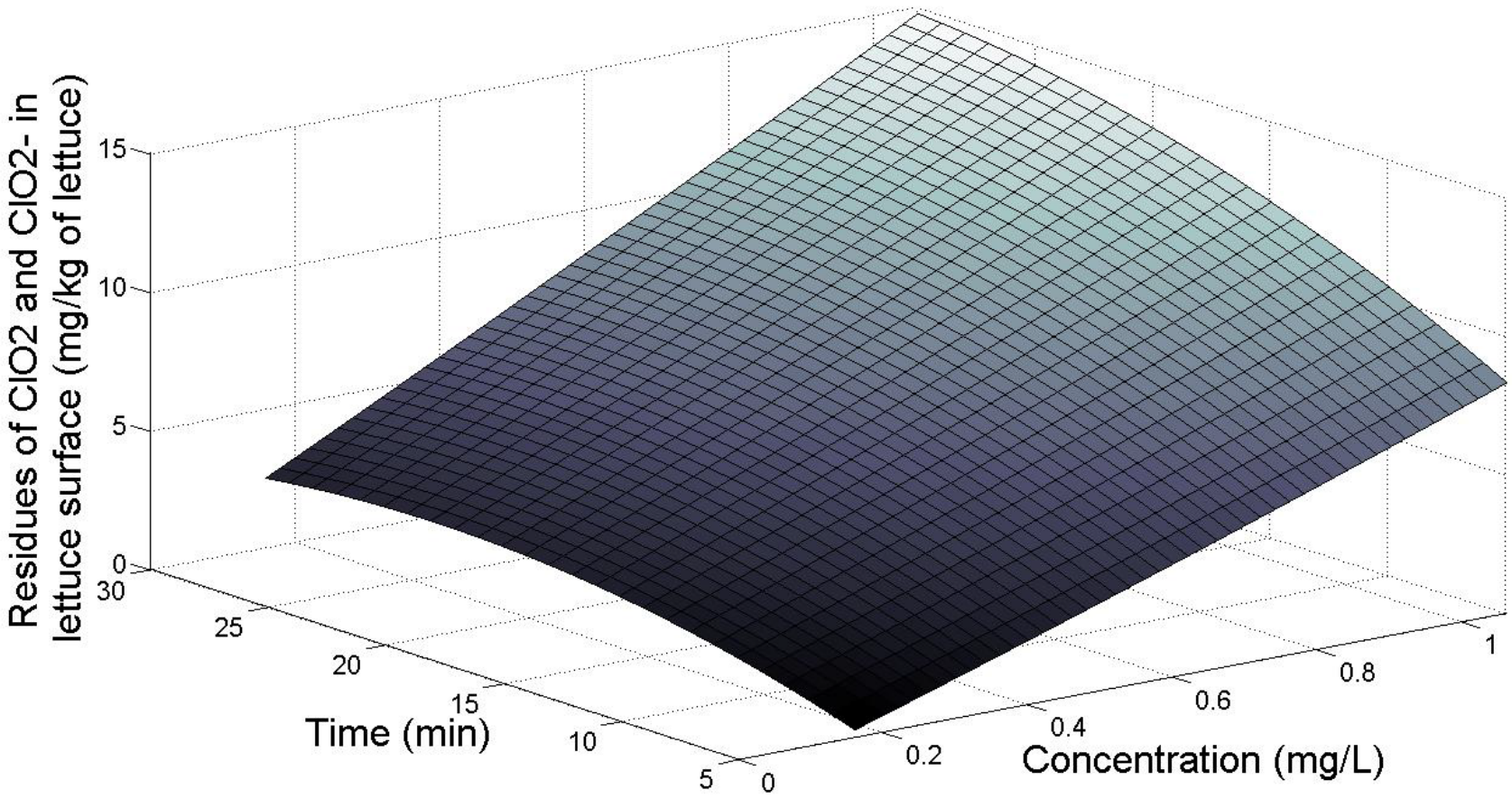

Figure 4.14 - Representation of the prediction model for residues in lettuce surface exposed to ClO_2 generated by the solution at 23°C

4.5.4. ClO_2 and ClO_2^- absorption model for a ClO_2 treatment generated by the solution at 4°C

The predicted model that can be written as:

$$Residue = 1.184 + 0.122t + 4.164c - 0.00252t^2 + 2.693c^2 - 0.174ct$$

From the surface presented in Figure 4.15 is possible to observe that as time and concentration increases absorption also increases, both in an almost linear way.

The validation of the model was done with a treatment at 4 mg/L for 7.5 min, using the same experimental settings. For this experiment was used a solution with initial concentration of 140.0 mg/L and based on Appendix 8 the actual concentration of ClO_2 specific time interval was 0.804 mg/L. This value was substituted in the model obtaining a residue of 5.31 mg of ClO_2 and ClO_2^-/kg, which is comparable to the experimental results (3.74 ± 0.88 mg of ClO_2 and ClO_2^-/kg of lettuce), showing a good prediction of the theoretical model.

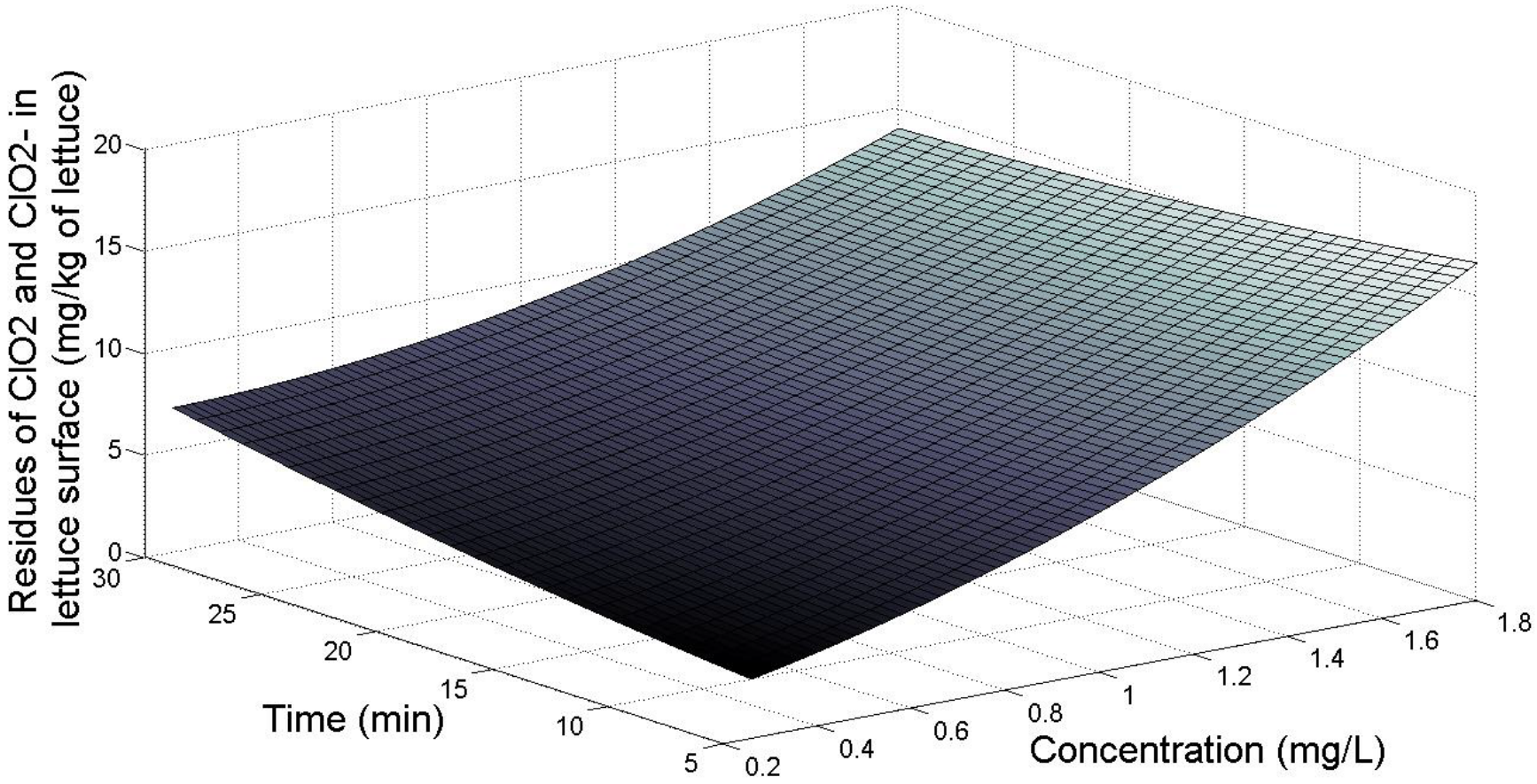

Figure 4.15 - Representation of the prediction model for residues in lettuce surface exposed to ClO_2 generated by the solution at 4°C

Table 4.7 – Evaluation of the total model parameters associated with the response surface analysis, total

ClO_2 Generation System	**Temperature (^oC)**	**Degrees of freedom**	**Sum of Squares**	**R-square**	**F Value**	**P Value**
Minidox	23	5	26942	0.9451	103.3	<0.0001
Minidox	4	5	28766	0.9492	112.0	<0.0001
Sachet	23	5	26135	0.9423	97.93	<0.0001
Sachet	4	5	17523	0.9425	98.43	<0.0001
Solution	23	5	9941.2	0.8648	38.38	<0.0001
Solution	23	5	5379.9	0.8001	24.02	<0.0001

CHAPTER 5 – CONCLUSIONS

Residue levels of ClO_2 and ClO_2^- were found in lettuce surfaces in larger amounts when the produce was treated with ClO_2 produced by the Minidox, followed by the sachet and, lastly, the solution.

The residues found in lettuce surfaces after all treatment conditions are high and may not be appropriate for human consumption, so it is necessary to study what happens to the produce after treatment to ensure that the produce is safe for human consumption. Also it is important to study the effect of ClO_2 on the sensory characteristics of the produce and the loss of nutrients and vitamins.

Tomatoes were shown to absorb very small amounts of ClO_2 and ClO_2^- and to be appropriate for ClO_2 sanitization. Compared to lettuce, the tomatoes' visual appearance changed very little, but it is still necessary to verify changes in vitamins, nutrients and sensory quality.

Comparing residues of ClO_2 and ClO_2^- for tomatoes and lettuce, it is possible to see that the type of plant tissue on the fresh produce surface affected the ClO_2 absorption. Both surfaces are covered by a cuticle but the tomato has a thicker

protective layer compared to lettuce, measuring 2000 μg/cm^2 and 450 to 800 μg/cm^2, respectively (Lamikanra *et al.* 2005).

The different concentration profiles of the delivery systems are responsible for the different residues (ClO_2 and ClO_2^-) on the produce surfaces. The different concentration profiles provide different pathways by which ClO_2 is generated and as a consequence each system will reach the target concentration at a specific time interval. This is part of the reason why it is necessary to establish what type of ClO_2 delivery system is used in the treatment when assessing the quality, safety or efficacy of ClO_2 on fresh produce.

The absorption models for each delivery system were validated and can be used for a reliable prediction of the absorption in lettuce surfaces. But this model is specific for lettuce and needs to be validated for other leafy green produce.

APPENDICES

APPENDIX 1: Antimicrobial effectiveness of ClO_2 in solution and gas form to inactivate microorganisms presented in fresh produce surface

Table A.1 - Summary of research studies on antimicrobial effectiveness of ClO_2 for fresh produce

Produce	Treatment	Microorganism	Results (Log reduction)	Reference
Apple	gas - 4mg/L for 10 min	*L. monocytogenes*	5.5 cfu/sample	(Du *et al.* 2002)
	solution - 3ppm for 5 min		≤ 5 cfu/g	(Rodgers *et al.* 2004)
	gas 4.8mg/L for 10 min	*E. coli* O157:H7	4.8 cfu/sample	(Du *et al.* 2003)
	solution 3 ppm for 5 min		≤ 5 cfu/g	(Rodgers *et al.* 2004)
Cabbage	gas 2.7mg/L for 20 min	*Salmonella* spp.	1.89 cfu/g	(Sy *et al.* 2005b)
	gas 2.7mg/L for 12.3 min	*E. coli* O157:H7	2.68 cfu/g	
	gas 2.7mg/L for 19.3 min	*L. monocytogenes*	3.31 cfu/g	
Carrot	gas 2.7mg/Lfor 20 min	*Salmonella* spp.	3.11 cfu/g	(Sy *et al.* 2005b)
	gas 2.7mg/L for 12.3 min	*E. coli* O157:H7	3.18 cfu/g	(Sy *et al.* 2005b)
	solution 5mg/L for 5 min		2.12 cfu/g	(Singh *et al.* 2002)
	gas 2.7mg/L for 19.3 min	*L. monocytogenes*	5.35 cfu/g	(Sy *et al.* 2005b)
Lettuce	solution 3ppm for 5 min	*L. monocytogenes*	≤ 5 cfu/g	(Rodgers *et al.* 2004)
	gas 2.7 mg/L for 19.3 min		1.23 cfu/g	(Sy *et al.* 2005b)
	gas 3.4 mg/L for 30 min		5 cfu/g	(Lee *et al.* 2004)
	solution 3 ppm for 5 min	*E. coli* O157:H7	≤ 5 cfu/g	(Rodgers *et al.* 2004)
	gas 2.7 mg/L for 12.3 min		0.72 cfu/g	(Sy *et al.* 2005b)
	gas 3.4 mg/L for 30 min		3.4 cfu/g	(Lee *et al.* 2004)
	solution 5ppm for 10 min	*Salmonella* spp.	> 1.5 log	(Huang *et al.* 2006)
	gas 2.7 mg/L for 20 min		1.21 cfu/g	(Sy *et al.* 2005b)

Table A-1 (cont'd)

Produce	Treatment	Microorganism	Results (Log reduction)	Reference
Strawberry	solution 3 ppm for 5 min	*L. monocytogenes*	≤ 5 cfu/g	(Rodgers *et al.* 2004)
	gas 0.6mg/L for 10 min		3.1 cfu/g	(Han *et al.* 2004)
	solution 3 ppm for 5 min	*E. coli* O157:H7	≤ 5 cfu/g	(Rodgers *et al.* 2004)
	gas 0.6mg/L for 10 min		4.1 cfu/g	(Han *et al.* 2004)
	gas 4.1mg/L for 30 min	*Salmonella* spp.	2.32 cfu/g	(Sy *et al.* 2005a)
Tomato	gas 4.1mg/L for 25 min	*Salmonella* spp.	4.33 cfu/sample	(Sy *et al.* 2005b)
	solution 5ppm for 6 sec		5 cfu/cm^2	(Pao *et al.* 2007)
	solution 5ppm for 6 sec	*S.enterica*	>5 log/mL	(Pao *et al.* 2007)
	1mg/kg of fruit for 2 hrs	*Salmonella* Typhimurium	≤ 7 log CFU/fruit	(Mahovic *et al.* 2009)

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 *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 ($(C_6H_5)AsO$) – 0.00564N
- Phosphate buffer solution (pH 7)
- Potassium Iodide (KI) solution – 5% (weight)
- Sodium Hydroxide (NaOH) – 6N, 0.02N and 0.002N
- Sulfuric Acid (H_2SO_4) – 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 H_2SO_4
    - 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 H_2SO_4
    - 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 (ICA TriNova LLC 2006). 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 V_s (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 V_a
- Discard the solution

Calculation

$$ClO_2\ (mg/L) = \frac{Va * N * 67500}{4 * Vs}$$

N= normality of Na_2S_2O

APPENDIX 4: Determination of the ClO_2 solution concentration to be used in each experiment

In this section is presented how to determine the initial solution concentration to be used to achieve an equivalent concentration of 1, 3, 6 and 10 mg/L of ClO_2 in the chamber headspace.

The calculation was done based in the equilibrium between the solution and the headspace following the Henrys Law. Figure 2.3 with the fitted curve was used to determine the Henry constant at a specific temperature.

$$y = 5.32E\text{-}06e^{3.65E\text{-}02x}$$

For experiment at 23°C and a desire concentration of 1mg/L of ClO_2 gas in the headspace we have:

y= 1.23E-5 atm ClO_2/(mg/L of solution) that is Henry's constant

From the ideal gas law, at 0°C the gas volume is 22.4 L/mol, transforming it to the studied temperature (23°C=296K) we have 24.29 L/mol of gas volume.

Calculation of the ClO2 partial pressure

$$\text{Partial pressure} = \frac{ClO_2 \text{ gas concentration} * \text{gas volume}}{ClO_2 \text{ Molar Mass}}$$

$$\text{Partial pressure} = \frac{1 \text{ mg/L} * 24.29 \text{ L/mol}}{118} = 3.598E\text{-}4 \text{ atm } ClO_2$$

67500 mg/mol

Calculation of the solution concentration

$$\text{Solution concentration} = \frac{\text{Partial pressure}}{\text{Henry's constant}}$$

$$\text{Solution concentration} = \frac{3.598\text{E-}4}{1.23\text{E-}05} = 29.2 \text{ mg/L}$$

The solution with a concentration of 29.2 mg/L will be used to achieve a gas concentration in the headspace of 1 mg/L of ClO_2. The initial solution concentration for the other situation used in this experiment is outlined in Table 3.1.

APPENDIX 5: Determination of Z-series sachet amount to be used in the experiment

In this section is presented how to determine the amount of chemical to be used in the sachet to achieve an equivalent concentration of 1, 3, 6 and 10 mg/L of ClO_2 in the maximum exposure time of the treatment, 30 min for lettuce and 60 min for tomato. All the calculations were based on the experimental determination.

The first stage was to determine the sachet profile, the amount of gas it is being produced by time using a fixed amount of chemicals. For that a glass jar (800 mL) with a tide seal closure was used as a reactor to measure the amount of gas produced by the sachet (Z-series - ICA TriNova LLC, Newnan, GA) over time. The sachet was prepared with 2 g of each chemical (precursor and activator), mixed and immediately hanged in the glass jar containing 60 mL of KI solution as shown in Figure A.1. The jar was sealed to avoid loosing gas and was protected from light to avoid photodegradation (Netramai 2010).

Figure A.1 -Glass jar with a hanged sachet - experiment setup

At specific intervals the sachet was discarded and the KI solution was titrated using a Sodium Thiosulfate titration outlined in Appendix 3. The determination was done at 23^{o}C. Each measurement was done in triplicate.

Table A.1 - ClO_2 generation with 2g of precursor

Time (min)	Average (mg ClO_2/g of precursor)	Standard Deviation
5	1.12	0.204
10	1.71	0.174
15	2.09	0.107
30	3.01	0.301
60	3.84	0.435

The amount of ClO_2 generated by 2 g of precursor and 2 g of activator over time at 23^{o}C is presented in Table A.2. Based on this table was possible to calculate the amount of chemicals to be used. For the experiment involving lettuce the maximum time

of the treatment was 30 min, in this time, according to experimental results, was released 3.01 mg of ClO_2/g of percursor. In order to generate an equivalent concentration of 1 mg/L inside the chamber (that contains 12 L) it is necessary to produce 12 mg of ClO_2 during the maximum 30 min of exposure.

1g of sachet precursor → 3.01 mg of ClO_2

X g of sachet precursor → 12.00 mg of ClO_2

X = 4.00 mg

For an equivalent concentration of 1 mg/L it is necessary 4.00 mg of precursor and 4.00 mg for activator.

The same calculation was done to determine the amount of sachet chemical to be used in order to have concentrations of 3 and 6 mg/L. The results are shown in Table A.2.

For tomatoes the same principle was used, but in this case the maximum treatment time was 60 min. In this period, according to the profile, ClO_2 produced was 3.83mg of ClO_2/g of percursor. In this case we had

1 g of sachet precursor → 3.83 mg of ClO_2

X g of sachet precursor → 12.0 mg of ClO_2

X = 3.13 mg

The amount of chemical used for tomatoes in the equivalent concentration of 3, 6, and 10 mg/L is also outlined in Table 3.2.

APPENDIX 6: ANOVA results for section 4.3 and 4.1

In this section is presented ANOVA table resulted from a factorial ANOVA with a 95% confidence interval.

Table A.2 – factorial ANOVA table for residues in lettuce surface

Effect	Num DF	Den DF	F value	Pr > F
Delivery system	2	144	62.18	<.0001
conc	2	144	414.13	<.0001
d.system*conc	4	144	3.95	0.0045
time	3	144	553.00	<.0001
d.system*time	6	144	21.18	<.0001
conc*time	6	144	40.73	<.0001
d.system*conc*time	12	144	3.98	<.0001
temp	1	144	106.41	<.0001
d.system*temp	2	144	113.74	<.0001
Conc*temp	2	144	4.35	0.0146
d.system*conc*temp	4	144	0.88	0.4790
Time*temp	3	144	33.68	<.0001
d.system*time*temp	6	144	8.61	<.0001
conc*time*temp	6	144	2.15	0.0510
d.system*conc*time*temp	12	144	2.93	0.0011

Where: DF=degrees of freedom, Num=numerator, Den=denominator, d.system = delivery systems, conc=concentration, temp=temperature

Table A.3 – Factorial ANOVA table for residues on tomato surface

Effect	Num DF	Den DF	F value	Pr > F
Delivery system	2	108	3.76	0.0264
conc	2	108	1.89	0.1557
d.system*conc	4	108	1.62	0.1737
time	2	108	0.25	0.7763
d.system*time	4	108	0.82	0.5130
conc*time	4	108	2.57	0.0420
d.system*conc*time	8	108	0.69	0.7036
temp	1	108	15.31	0.0002
d.system*temp	2	108	5.67	0.0046
Conc*temp	2	108	1.86	0.1601
d.system*conc*temp	4	108	3.21	0.0156
Time*temp	2	108	0.93	0.3963
d.system*time*temp	4	108	3.20	0.0159
conc*time*temp	4	108	2.50	0.0470
d.system*conc*time*temp	8	108	1.31	0.2465

Where: DF=degrees of freedom, Num=numerator, Den=denominator, d.system = delivery systems, conc=concentration, temp=temperature

APPENDIX 7: Mathematical model validation

An experiment was carried out in order to evaluate the mathematical model created to predict the residues on lettuce surface after ClO_2 treatment.

The same settings as described in Chapter 3 were used. The ClO_2 concentration and exposure time were chosen randomly, but in the range of values used to determine the model (1 to 6 mg/L and 5 to 30 min).

In order to obtain an equivalent condition for the different delivery systems, the setting used for each situation was:

- Minidox – equipment concentration flow of 4 mg/L of ClO_2
- Sachet – 16 g of precursor and activator
- Solution – initial concentration of 116.8 mg/L for 23^{o}C and 140 mg/L for 4^{o}C

The experiments were done at 23 and 4^{o}C and repeated three times. The residues determination followed the same procedure as outlined in Chapter 3.

APPENDIX 8: Prediction of the ClO_2 gas actual concentration in the chamber headsapce

A concentration curve was built, for each delivery system, in order to determine the actual concentration of ClO_2 when the initial settings of the experiment are changed (new concentration and exposure time).

In all situations the data presented in section 4.1 was used. The concentrations were divided by the delivery system parameters (gas flow concentration for the Minidox, amount of chemicals for the sachet, and solution initial concentration for the solution experiment) in order to obtain a relative concentration that was used to build a curve for each system. This curve was then fitted with a logarithm trendline giving an equation that can determine the relative concentration.

Minidox

In the case of the Minidox, the data in Table 4.1 (actual concentration) was divided by its respective target concentration (that is the concentration of ClO_2 shown by the equipment) obtaining the relative concentration. The relative concentration over time was plotted, as presented in Figure A.2 and A.3. Using a curve fitting procedure, a curve was built to describe the relation between the relative concentration and time and the equation is also presented in Figure A.2 and A.3.

In order to determine the actual concentration of ClO_2 in the headspace, at a specific time interval for the Minidox, the fitted equation will be used. The relative concentration will be obtained by the equation at the specific time interval (x) and the result is multiplied by the concentration shown in the Minidox equipment (target concentration).

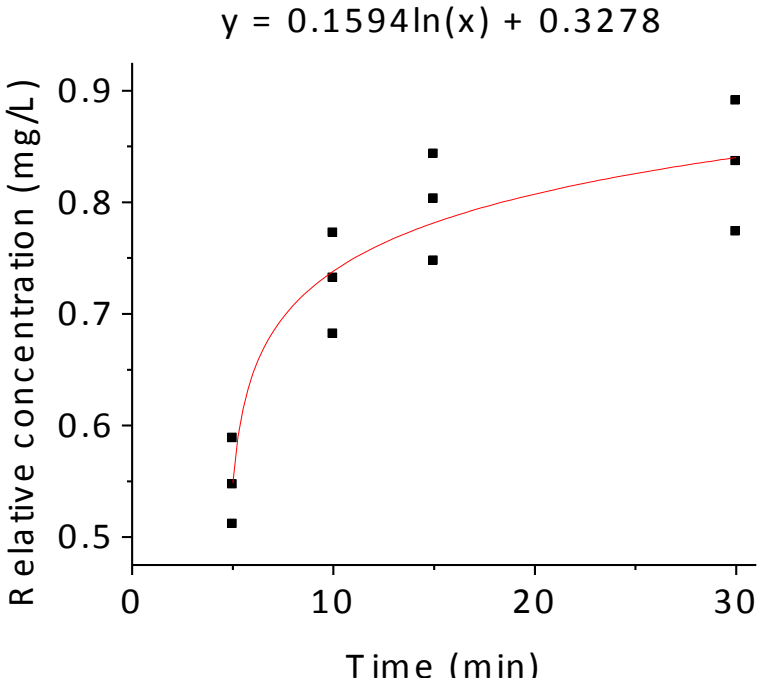

Figure A.2 - Curve for the Minidox at 23°C with the fitted equation

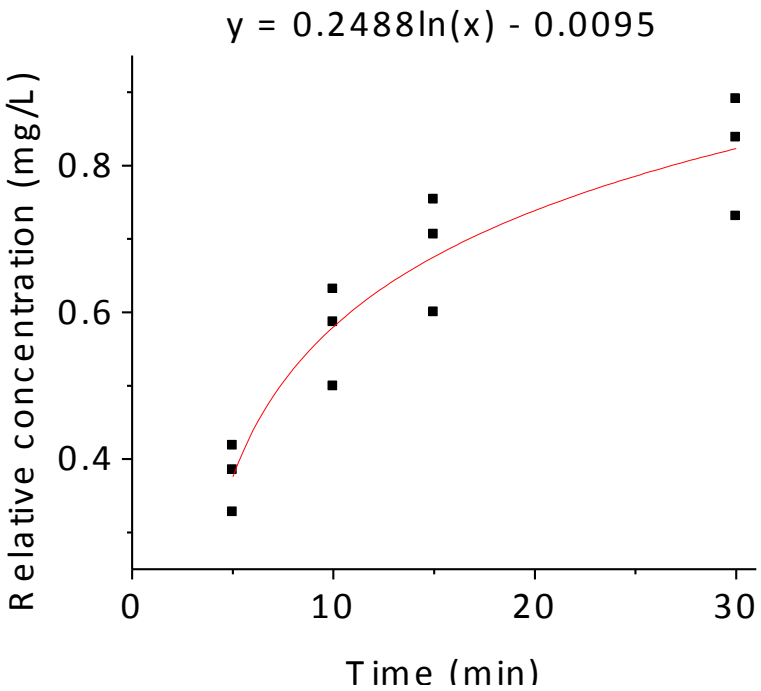

Figure A.3 - Curve for the Minidox at 4°C with the fitted equation

e.g.

For a treatment at 23°C with a target concentration of 4 mg/L and 7.5 min we have:

y = 0.1594ln(x) + 0.3278

y = 0.1594ln(7.5) + 0.3278 = 0.6490 that is the relative concentration

actual concentration = 0.6490*4 = 2.596 mg/L

Sachet

For this case the data in Table 4.2 (actual concentration) was divided by the amount of sachet chemicals used in each experiment, shown in Table 3.2. The fitted curve with the respective equation is presented in Figure A.4 and A.5.

The same procedure as the Minidox will be done to determine the actual concentration of ClO_2 in the chamber, but the result from the equation (relative concentration) will be multiplied by the amount of chemicals used in the experiment.

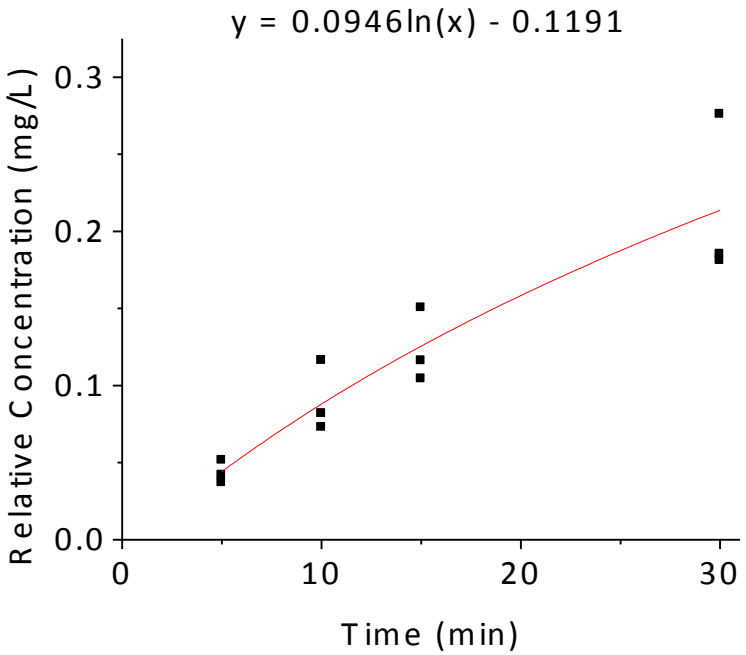

Figure A.4 - Curve for the sachet at 23°C with the fitted equation

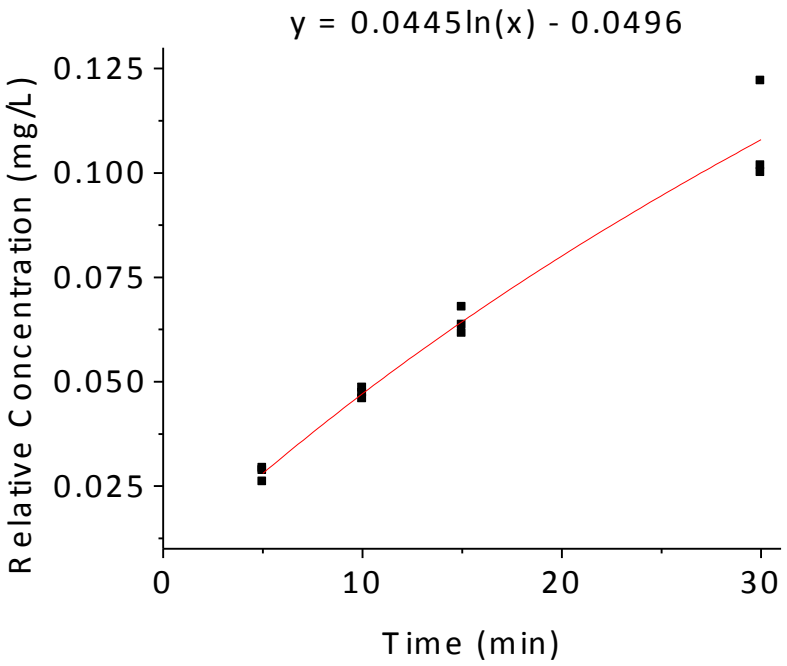

Figure A.5 - Curve for the sachet at 4°C with the fitted equation

Solution

For the solution, the data in Table 4.3 (actual concentration) was divided by the initial solution concentration presented in Table 3.1. The fitted curve with the respective equation is shown in Figure A.6 and A.7.

In order to obtain the actual ClO_2 concentration for the new setting, the same procedure used in the Minidox will be used, but the relative concentration will be will be multiplied by the initial solution concentration.

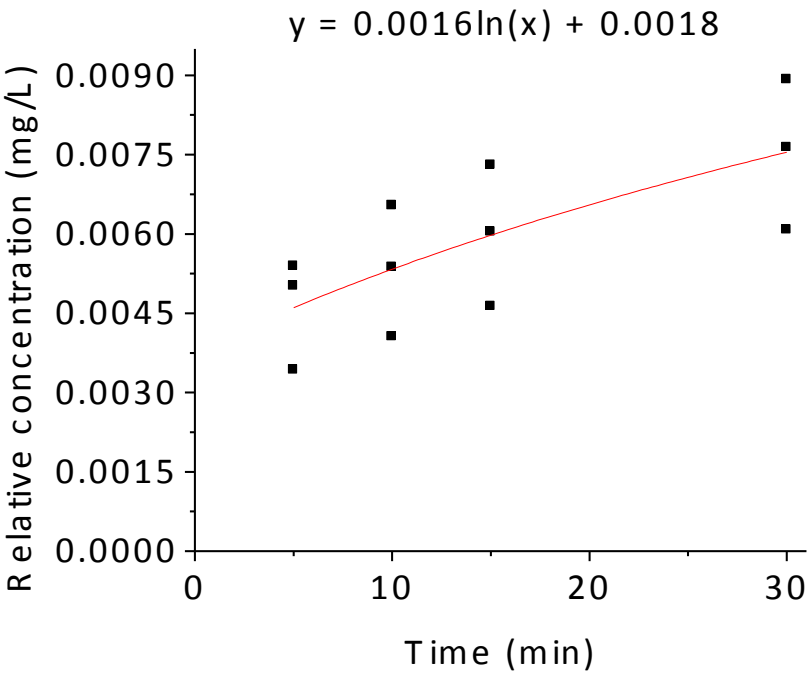

Figure A.6 - Curve for the solution at 23°C with the fitted equation

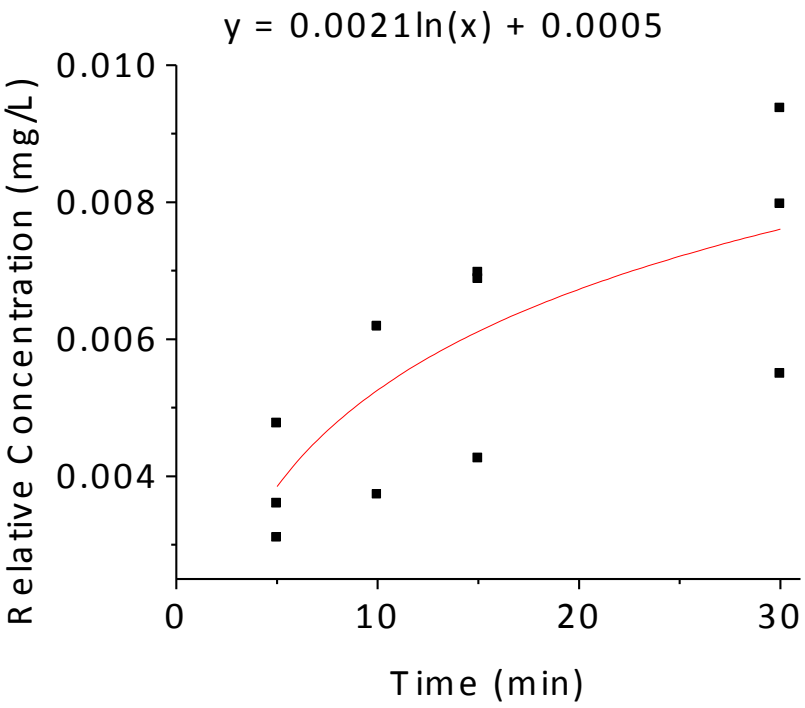

Figure A.7 - Curve for the solution at 4°C with the fitted equation

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