DEVELOPMENT OF A CHLORINE-BASED SANITATION STRATEGY TO ENHANCE SAFETY FOR FRESH PRODUCE

by

XI CHEN

(Under the Direction of Yen-Con Hung)

ABSTRACT

Chlorine-based sanitizers are widely used in fresh produce industry. However, the antimicrobial efficacy of chlorine-based sanitizers for fresh produce washing process could be compromised by their reaction with organic matter. Excess amount of residual chlorine can result in a large amount of hazardous disinfection by-products (DBPs), whereas deficiency of residual chlorine may not completely inactivate pathogens on produce. The overall objective of this study was to develop a disinfection strategy to ensure the microbial safety and minimize DBPs formation potential for fresh produce postharvest washing process. In the first study, the correlations between water quality parameters and chlorine demand of different types of fresh produce were determined. It was found that ultraviolet absorbance measured at 254 nm (UV254) was most correlated with chlorine demand of various fresh produce wash waters (R = 0.77) among all the tested parameters. In addition, various types of fresh produce can be divided into two clusters based on the Phenolics-to-Protein/ΔE or PPC ratio with the threshold PPC at 0.6. Chlorine demand of different types of produce in the same cluster was highly correlated with UV254. The second study undertaken revealed that organic load, initial chlorine concentration and sanitizer pH all significantly ($P \leq 0.05$) affected the chlorine demand of produce wash water. Equations to predict chlorine demand for different types of fresh produce, chlorine-based sanitizers, UV254, initial chlorine concentration and sanitizer pH were developed. In the third study, based on the findings from the first two studies, chlorine dosing strategy to estimate initial chlorine concentration needed to reach a targeted residual chlorine for produce washing process were developed and verified using an automatic produce washer. It showed that 10 mg L^{-1} of residual chlorine can maintain microbial inactivation efficacy and minimize the initial chlorine concentration. The findings in this project can also be useful for produce industry to enhance the microbial and chemical safety of fresh and fresh-cut produce.

INDEX WORDS: Fresh produce, Postharvest washing, Chlorine-based sanitizer, Prediction equation, Chlorine demand

DEVELOPMENT OF A CHLORINE-BASED SANITATION STRATEGY TO ENHANCE SAFETY FOR FRESH PRODUCE

by

XI CHEN

B.E., Sichuan University, China, 2011

M.S., Auburn University, 2013

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2017

DEVELOPMENT OF A CHLORINE-BASED SANITATION STRATEGY TO ENHANCE SAFETY FOR FRESH PRODUCE

by

XI CHEN

Major Professor:	Yen-Con Hung
Committee:	Mark A. Harrison
	Ching-Hua Huang
	Jose Reyes de corcuera

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
May 2017

DEDICATION

I dedicate this thesis to my parents for their unconditional support and encouragement during my PhD journey. Thanks to my wife for coming into my life, giving me joy, and changing my life forever.

ACKNOWLEDGEMENTS

I would like to take this opportunity to express my heartfelt gratitude to my major advisor, Dr. Yen-Con Hung, for his support, encouragement and patience during the past four years. It is such an honor to do my doctoral degree under his guidance. I also thank my other committee members, Dr. Mark Harrison, Dr. Qing-Hua Huang, and Dr. Jose Reyes for their time and assistance in the completion of my dissertation. My appreciation is extended to my friends and lab mates for their great help during my research and for their precious friendship.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Fresh Produce Safety

Fresh fruits and vegetables are essential parts of the human health diet and offer a wide range of vitamins, nutrients and fiber (Yeni, Yavaş, Alpas, & Soyer, 2016). Thus, the consumption of fresh produce in the United States has increased rapidly over the past two decades. The Food and Drug Administration (FDA, 2001) reported that the consumption of fruits and vegetables increased from 284 pounds per capita in 1987 to 319 pounds per capita in 1997, and the total fresh produce market also doubled from $34.6 billion to $70.5 billion during the same period. Another survey showed that by 2005 daily sales of fresh-cut produce reached 6 million packages in North American (Olaimat & Holley, 2012).

Foodborne illness is a major public concern in terms of the numbers of persons affected and economic loss. According to estimations from the Center for Disease Control and Prevent (CDC), there are 48 million people in the US that suffer from foodborne illnesses, resulting in 128,000 hospitalizations and 3,000 deaths annually (Scallan, 2011). In addition, it was estimated that approximately $ 77.7 billion every year was spent on investigating foodborne illnesses caused by pathogens (Scharff, 2012). An important portion of foodborne outbreaks is associated with the consumption of fresh produce in the US. The percentage of foodborne outbreaks caused by fresh produce increased from 0.7 % in the 1970s to 6 % in the 1990s (Sivapalasingam, Friedman, Cohen, & Tauxe, 2004). Another study found that produce was responsible for 27 % of outbreaks and 40 % of foodborne illness in 2014 (DeWaal, Xuman, & Plunkett, 2009). Every year, produce-associated foodborne outbreaks were estimated to cause approximately $ 1.4 billion economic losses and over 1 million illnesses in the US (Batz, Hoffmann, & Morris, 2011).

Compared with meat or poultry products, most fresh produce is minimally processed and eaten raw, which increases the potential risk of foodborne illness. Once fresh produce is contaminated, it is also very difficult to eliminate those pathogens (Yeni et al., 2016). Moreover, the growth of microorganisms can be facilitated by processing steps such as cutting, slicing, or peeling which damage the surface of fresh produce and release nutrients (Harris et al., 2003).

Escherichia coli is a gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia* (Fujisawa et al., 2000). Approximately 19 %, 40 %, and 48 % of outbreaks associated with fruits, seed sprouts and leafy vegetables in the US from 1990 to 2004 were caused by *E. coli*, respectively (Brandl, 2006). Based on disease syndromes and characteristics, five virulence groups of *E. coli* are recognized: enteroaggregative (EAggEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), enteropathogenic (EPEC), and enterotoxigenic (ETEC). *E. coli* O157:H7, an EHEC strain, is recognized as one of the most important and threatening foodborne pathogens related to fresh produce. *E. coli* O157:H7 infection can lead to hemolytic uremic syndrome (HUS), characterized by hemolytic anemia, thrombocytopenia, and renal injury

(Griffin & Tauxe, 1991). Batz et al. (2011) identified that fresh produce was the second most common vehicles for *E. coli* O157:H7 (18.4 %). *E. coli* O157:H7 outbreaks associated with produce in the US from 2010 to 2015 are summarized in Table 1-1 (CDC, 2017). Lettuce, spinach and strawberries are the most common fresh produce categories related to *E. coli* O157:H7 outbreaks. In history, the largest *E. coli* O157:H7 outbreak was occurred in Japan in 1996 and related to the raw radish sprouts, which caused more than 12,000 illnesses and 12 deaths (Michino et al., 1999). One of the largest *E. coli* O157:H7 outbreak related to fresh produce in the US and Canada occurred in 2006, in which 199 cases with 3 deaths were reported in 26 states (Olaimat & Holley, 2012). Besides *E. coli* O157:H7, microorganisms that have been frequently related to fresh produce outbreaks include hepatitis A virus, norovirus, *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella* spp., Non-O157 shiga toxin-producing *E. coli*, *Campylobacter* spp., *Shigella* spp. and *Clostridium* spp. (Beuchat, 2002; Buck, Walcott, & Beuchat, 2003; CDC, 2011; Olaimat & Holley, 2012; Sivapalasingam et al., 2004).

Various types of fresh produce including lettuce, spinach, sprouts, tomatoes, cantaloupe, strawberries, etc. have been reported to cause foodborne illness outbreaks in recent years (CDC, 2008; Gurtler, Bailey, Jin, & Fan, 2014; Warriner & Namvar, 2010). Several common pathogen-produce combinations are *E. coli* O157:H7-lettuce or spinach, *Salmonella*-cantaloupe or tomato, and *Cyclospora*-raspberries (DeWaal & Bhuiya, 2007; Lynch, Tauxe, & Hedberg, 2009).

Contamination of fresh produce with foodborne pathogens can occur either pre or postharvest (Beuchat, 2002). Some preharvest sources include soil, feces, irrigation water, pests and animals. *L. monocytogenes, Clostridium* spores and *B. cereus* spores are commonly found in soil (Beuchat & Ryu, 1997). The presence of other pathogens such as *E. coli* O157:H7, *Salmonella* spp., *C. jejuni* in soil likely results from feces or untreated sewage. Unless effective sanitizing procedures are applied, soil on the surface of fruits and vegetables may harbor these pathogens that remain viable through subsequent handling to the point of consumption.

On the other side, postharvest processing can also influence the microbial safety of fresh produce. Doyle & Erickson (2008) found that cutting or slicing not only increased the potential to contaminate produce, but enhanced bacterial growth as well. Washing had the potential to cause cross-contamination, especially when water is recirculated (Luo et al., 2011). Infected workers were considered the primary source of viruses that cause foodborne illness for the Ready-to-eat food (Berger et al., 2010).

Table 1-1. *E. coli* O157:H7 outbreaks related to consumption of fresh produce from 2010 to 2015 in the US.

Year	State	Illnesses	Hospitalizations	Deaths	Food Vehicle
2010	Maryland	7	4	0	Apple cider, unpasteurized
2011	Oregon	15	7	2	Strawberries
2011	Minnesota	6	1	0	Strawberries; watermelon
2011	Multistate	60	35	0	Romaine lettuce
2011	Minnesota	14	0	0	Apple cider, unpasteurized
2011	Multistate	26	5	0	Lettuce
2012	Multistate	24	0	0	Leaf lettuce
2012	California	12	1	0	Lettuce
2012	Multistate	52	0	0	Romaine lettuce

2012	Pennsylvania	9	7	0	Romaine lettuce
2012	Multistate	33	13	0	Prepackaged leafy green
2012	Massachusetts	8	4	0	Leaf lettuce
2013	Multistate	14	9	1	Prepackaged leafy green
2013	Michigan	20	7	0	Salad (tomato)
2013	Missouri	6	4	0	Lettuce-based salads
2013	Arizona	94	22	0	Lettuce
2013	California	5	5	0	Green leaf lettuce
2013	Pennsylvania	15	10	0	Prepackaged leafy green
2013	Florida	7	5	0	Kale
2013	Multistate	33	9	0	Romaine lettuce
2013	Colorado	9	1	0	Cucumber
2013	California	8	1	0	Green beans
2013	Connecticut	9	8	0	Lettuce
2014	Multistate	4	1	0	Spinach
2014	Multistate	16	13	0	Prepackaged salad
2014	Minnesota	57	9	0	Potato salad (celery)
2014	Multistate	11	2	0	Leaf lettuce
2015	Multistate	16	10	0	Romaine lettuce
2015	Colorado	16	5	0	Pork, green onion
2015	Minnesota	2	1	0	Apple cider, unpasteurized
2015	Multistate	19	3	0	Chicken salad (celery, onion)
2015	Multistate	5	3	0	Prepackaged salad

1.2 Treatments to ensure produce safety

In the last few decades many studies have been conducted concerning sanitizing fresh produce during processing. The efficiency of numerous physical and chemical technologies to enhance the safety and quality of fresh and fresh-cut produce have been covered in several reviews (Gil, Selma, Lopez-Galvez, & Allende, 2009; Gómez-López, Rajkovic, Ragaert, Smigic, & Devlieghere, 2009; Goodburn & Wallace, 2013; Olaimat & Holley, 2012).

1.2.1 Physical sanitation methods

One common physical methods to remove bacteria from fresh produce surface was brushing. Erickson, Liao, Cannon, & Ortega (2015) found that brushing under running water led to 0.25 to 2.11 log reduction of *E. coli* O157:H7 and 0.27 to 2.97 log reduction of *Salmonella* on cantaloupe, honeydew melon, and carrots. It also showed that brushing removed fewer pathogens from contaminated cantaloupe than from other produce, indicating that the produce surface roughness and structure had an impact on brush efficacy.

Ultrasound is another physical sanitizing method with the power to cause cavitation. During cavitation and subsequent collapse of microbubbles, large amount of energy is released to generate heat, pressure and free radicals, which can inactivate microorganisms (Gómez-López et al., 2014). In addition, ultrasound can break the produce surface tension and hence let sanitizer solution to reach bacteria that hide in the crack or folded surfaces (Afari, Hung, King, & Hu, 2016). The combination of ultrasound with chemical sanitizers to enhance the antimicrobial efficacy has been previously evaluated. Sagong et al., (2010) found that, compared to individual treatments, the combined treatment of ultrasound and organic acids resulted in additional 0.8 to 1.0 log reduction on *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* without causing significant quality change on lettuce. Afari et al. (2016) reported that between 0.54 and 1.67 log reductions in *E. coli* O157:H7 and *Salmonella* on lettuce were achieved by combined ultrasound (130 and 210 W) and electrolyzed oxidizing water depending on the treatment time.

Ultraviolet (UV) light is also a powerful technology to inactivate pathogens from produce surface, with the most effective wavelength being 200 - 280 nm (Olaimat & Holley, 2012). UV can be absorbed by protein and nucleic acids, resulting in lethal mutations on the DNA of bacterial cells. Pang & Hung (2016) evaluated the synergistic effect of combined applications of UV and ozonated water for removing *E. coli* O157:H7 on romaine lettuce. This combined treatment gave a 4.8-log reduction after 15 min treatment time, which was significantly higher than the UV or ozonated water alone. The advantages of using UV to treat food are inexpensive, easy-to-use, zero residual and non-selective lethal effect (Yaun, Sumner, Eifert, & Marcy, 2004). However, the poor penetrative capacity of UV light limits its applications in food sanitation.

The use of irradiation on fresh iceberg lettuce and spinach to control pathogens and extend shelf-life has been approved by the FDA (FDA, 2008). Irradiation has been found to greatly reduce foodborne pathogens on produce without negatively impacting the texture, color and nutritional quality of produce (Gil et al., 2009). Shim et al., (2012) found that expose of lettuce leaves to 1 kGy gamma irradiation led to a 3-log reduction of *S*. Typhimurium and *S. aureus* on each leaf. However, the application of irradiation in food industry is not very successful due to the public concern. Additional studies and education need to be conducted to allow irradiation technology be widely accepted by consumers for use in fresh produce processing (Goodburn & Wallace, 2013).

1.2.2 Chemical sanitation methods

Postharvest washing is an important method to reduce pathogen contamination on fresh produce (Gil et al. 2015). In most produce processing facilities, fresh fruits and vegetables are dipped into a washing tank to remove dirt, pesticide residues, debris, tissue fluids and microorganisms, as well as to rapidly cool the products (Van Haute, Sampers, Holvoet, & Uyttendaele, 2013). To enhance the antimicrobial efficacy, chemical sanitizers are commonly added into the wash water. Several sanitizers that can be used for washing fresh produce are shown in Table 1-2 (Huang, Hung, Hsu, Huang, & Hwang, 2008; Ölmez & Kretzschmar, 2009). Numerous studies have been conducted to evaluate the microbicidal efficacy of sanitizers on fresh produce during washing (Gil et al., 2009; Goodburn & Wallace, 2013). Some of the common chemical sanitizers for produce washing are sodium hypochlorite (NaOCl), electrolyzed oxidizing (EO) water, chlorine dioxide (ClO_2), ozone, peroxyacetic acid and hydrogen peroxide. Many literatures regarding the use of sanitizers concluded that washing with chemical sanitizers reduces the microbial populations on the surface of the produce by 1 to 3 log units (Beuchat, Adler, & Lang, 2004; Gómez-López et al., 2009; Guentzel, Lam, Callan, Emmons, & Dunham, 2008). However, if sanitizers in wash water are ineffective, produce washing can redistribute pathogens and cause cross-contamination. Luo et al. (2011) found that although no recovery of *E. coli* O157:H7 was found from wash water, washing treatment containing 5 mg L^{-1} free chlorine could not prevent transference of pathogens from inoculated to clean lettuce.

The efficacy of the disinfection treatments was greatly dependent on the type of fresh produce and the characteristics of the produce surfaces (cracks, crevices and texture). Wang, Feng, Liang, Luo, & Malyarchuk (2009) found that an increase in fruit surface roughness would introduce protection to microbes entrapped on fruit surface and reduce the washing efficacy. In addition, Pang & Hung (2015) reported that the combination treatment of electrolyzed water and UV-ozonated water for inactivating *E. coli* O157:H7 was more effective on romaine lettuce than on iceberg lettuce. This can be explained by the fact that bacteria tend to aggregate near and into stomata of iceberg lettuce leaves, while no such attraction of bacteria to stomata was found on romaine lettuce leaves (Kroupitski, Pinto, Brandl, Belausov, & Sela, 2009).

The inactivation efficacy was also affected by the type of foodborne pathogens. Kim, Hung, & Brackett (2000) compared the resistance of *E. coli* O157:H7, *L. monocytogenes*, *B. cereus* and *B. cereus* spores to electrolyzed oxidizing water and chemically modified water. *B. cereus* spores were shown to be the most resistant pathogen, followed by *B. cereus*. *E. coli* O157:H7 and *L. monocytogenes* were the most sensitive pathogens among the tested microorganisms. In addition, Jadeja, Hung, & Bosilevac (2013) reported that the different strains of same serotype can differ in their resistance toward an intervention, and the resistance of shiga toxin-producing *E. coli* (STEC) to EO water from high to low was *E. coli* O157 ≥ O103 ≥ O26 ≥ O111 ≥ O121 ≥ O45 ≥ O145.

Additionally, washing conditions affected the pathogens inactivation on fresh produce. Many sanitizers such as HOCl and EO water were found to have greater activity at acidic pH than at alkaline pH (Park, Hung, & Chung, 2004). Dipping time, sanitizer concentration and produce-to-water ratio all affected the microbial inactivation efficacy and shelf-life of produce (Afari, Hung, & King, 2015; Fett, 2002; Pangloli & Hung, 2013; Pirovani, Guemes, & Piagnetini, 2001). Moreover, the hurdle washing process, which is to use a combination of technologies to attack the microorganism in different ways, can have better efficacy than traditional single treatment (Goodburn & Wallace, 2013). Afari et al. (2016) reported that near neutral electrolyzed (NEO) water combined with 210 W ultrasonication for 15 min led to 4.4 log reduction of *E. coli* O157:H7, which was significantly higher than the 2.7 log reduction of NEO water alone.

Table 1-2. Advantages and limitations of chemical sanitizers for fresh produce washing process.

Sanitizers	Advantages	Limitations
Chlorine	Inexpensive Easy to use Strong stability	Hazardous by-products Activity pH dependent Corrosive
Electrolyzed oxidizing water	Non-corrosive Non-toxic No residual Inexpensive	Activity pH dependent Hazardous by-products
Chlorine dioxide	High microbicidal efficacy Efficacy not affected by pH	Require on-site generation High initial investment cost
Ozone	High microbicidal efficacy Short contact time No residual No hazardous by-products	Require on-site generation Toxic High initial investment cost
Peroxyacetic acid	Efficacy not affected by organic load Effective in pH 5-9 No hazardous by products	Corrosive to skin and equipment Loss effectiveness in presence of metals
Hydrogen peroxide	No residual Easy to use Inexpensive	Low antimicrobial efficacy Long contact time Negatively impact produce quality

1.3 Chlorine-based sanitizers

Chlorine-based sanitizers are defined as the sanitizers that contain chlorine as the main antimicrobial component. The common chlorine-based sanitizers are NaOCl, EO water and ClO_2. Due to the low cost, good effectiveness against foodborne pathogens and minimal impact on the produce quality (Van Haute et al., 2013), chlorine-based sanitizers are widely used in fresh produce washing process in the US.

1.3.1 Sodium hypochlorite

Approximately 76 % of the respondents in an industry survey reported the use of NaOCl on fruit and vegetable decontamination (Seymour, 1999). The main active component in NaOCl solution is hypochlorous acid (HOCl). HOCl is a weak acid ($pK_{HOCl,\ 25\ ^0C} = 7.54$) which can dissociate into hydrogen ions (H^+) and hypochlorite ions (OCl^-) in the reversible reactions as follow (Morris, 1966):

$$HOCl \longleftrightarrow H^+ + OCl^-$$

The combination of Cl_2, HOCl and OCl^- in the solution is generally known as free chlorine. Free chlorine composition in HOCl solution is depended on the pH and temperature of HOCl solution (Deborde & von Gunten, 2008). The distribution of Cl_2, HOCl and OCl^- as a function of pH at 25 °C was shown in Figure 1-1. Chlorine gas becomes increasingly dominant below pH 3. Due to the relatively low solubility of Cl_2 in water, a large portion of free chlorine can be lost at this pH during storage through off-gassing of Cl_2. As a strong oxidant, Cl_2 is also toxic to human and corrosive to equipment surfaces. Therefore, chlorine solution is rarely adjusted to pH lower than 2 for produce washing process.

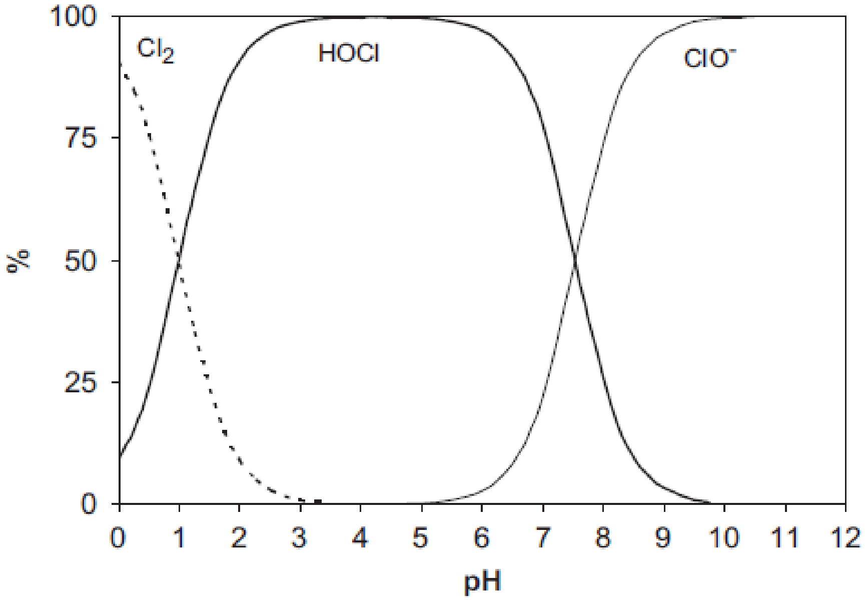

Figure 1-1. Distribution of main aqueous chlorine species from pH 0 to 12 (Adapted from Deborde & Gunten, 2008).

At pH > 7.5, OCl^- is dominated in chlorine solution. Although OCl^- with lower oxidation reduction potential (ORP, 600-700 mV) is more stable than HOCl and Cl_2, the low ORP limits its antimicrobial efficacy (Koseki, Yoshida, Isobe, & Itoh, 2004). As a result, the foodborne pathogen reduction of chlorine solution at alkaline pH is usually lower than chlorine solution at acidic pH.

At pH 2.5 – 7.5, HOCl is the dominated form of free chlorine species. HOCl is the preferred free chlorine specie for disinfection due to its high oxidation potential (ORP > 900 mV) and low impact on human and environment.

In practice, chlorine is used in residual concentrations at 50-200 mg L^{-1} with a 1-2 min dipping time (Beuchat et al., 2004). The pH of HOCl solution is adjusted to 6.0-7.5 as well. Recent studies using HOCl to inactivate microorganisms on fresh produce are shown in Table 1-3. Sapers, Miller, & Mattrazzo (1999) found that washing apples with chlorine solution (200 mg L^{-1} free chlorine) at pH 6.4 for 1 min significantly decreased the population of *E. coli* strains by 2.1 log CFU g^{-1}, when compared with unwashed samples. Stopforth, Mai, Kottapalli, & Samadpour (2008) reported that for mixed leafy green inoculated with *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes*, immersing in chlorinated water (50 mg L^{-1} free chlorine) at pH 6.5 for 60 to 90 s achieved reduction by 2.1 to 2.8 log CFU g^{-1}.

1.3.2 Electrolyzed oxidizing water

Electrolyzed oxidizing (EO) water has been reported as an alternative sanitizer with a strong microbicidal effect (Pangloli & Hung, 2013). The mechanism of EO water generator is shown in Figure 1-2. EO water is the product of the electrolysis of sodium chloride solution in an electrolysis cell, within which the anode and cathode are separated by a membrane. During electrolysis, anode side produces EO water containing HOCl, HCl with low pH (~2.5) and high ORP (> 1000 mV); cathode side generates electrolyzed reduced (ER) water containing NaOH with high pH (10.0-11.5) and low ORP (-800 to -900 mV) (Huang et al., 2008). The lethal effect of EO water appears to be due to the high ORP, low pH and active components such as HOCl.

Recent studies using EO water to inactivate microorganisms on fresh produce are shown in Table 1-4. Koseki et al. (2004) demonstrated that EO water could be used for microbial decontamination of cucumbers. Cucumbers treated with EO water at pH 2.6 with 30 mg L^{-1} free chlorine by dipping for 10 min showed an aerobic mesophilies reduction by 1.4 log CFU per cucumber, which was higher than the 1.2 log reduction treated by NaOCl at pH 9.3 with 150 mg L^{-1} free chlorine. Hung, Tilly, & Kim (2010) washed strawberries and broccoli with EO water (pH of ~2.7, and free chlorine of 25-100 mg L^{-1}) for 1 and 5 min, and found that EO water was either more than or as effective as chlorinated water in killing *E. coli* O157:H7 on strawberries and broccoli.

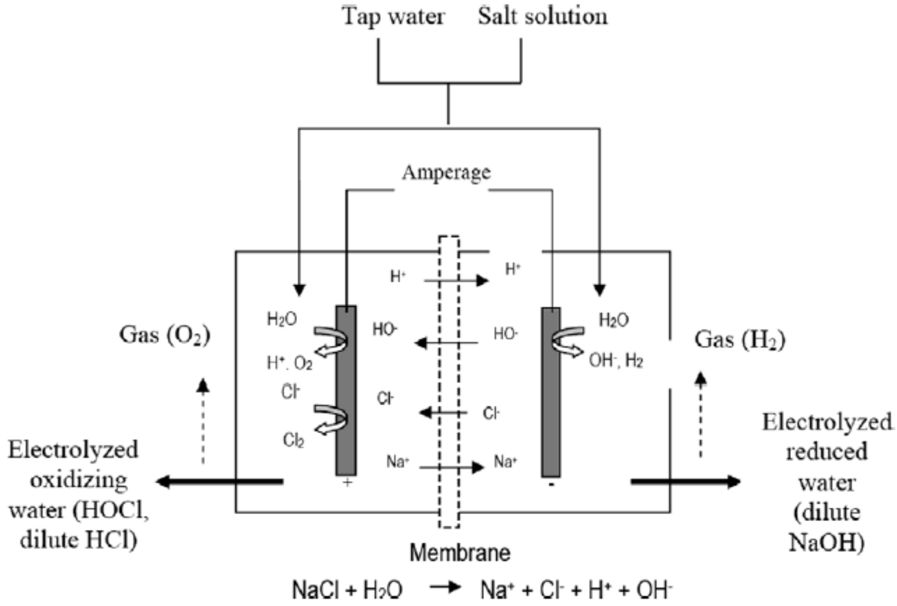

Figure 1-2. Schematics of electrolyzed water generator (Adapted from Huang et al., 2008)

1.3.3 Chlorine dioxide

ClO_2 is approved by FDA for washing fresh and fresh-cut produce for up to 3 mg L^{-1} (FDA, 2002). The antimicrobial effect of ClO_2 was shown to be related to non-specific oxidative damage of the cell membrane on bacteria cells, which lead to the destruction of membrane and leakage of cellular (Berg, Roberts, & Matin, 1986). ClO_2 is one of the few compounds that exists most entirely as monomeric free radicals, which makes its antimicrobial efficacy independent of temperature, pH, chloride and ionic strength (Amy et al., 2000). In addition, the oxidizing capacity of ClO_2 is as 2.5 times that of HOCl, so it generally requires lower concentration of ClO_2 than that of HOCl to achieve a similar microbial reduction (Gómez-López et al., 2009). However, concentrated chlorine dioxide vapor is potentially explosive. Because of this, ClO_2 must be generated on-site and cannot be stored and transported under the pressure.

The efficacy of ClO_2 for inactivating microorganisms on different fruits and vegetables has been studied by many researchers (Table 1-5). Keskinen, Burke, and Annous (2009) compared the efficacy of chlorine and ClO_2 in decontaminating *E. coli* O157:H7 from iceberg lettuce during washing. ClO_2 treatment containing 200 mg L^{-1} chlorite ion at pH 8.0 reduced *E. coli* O157:H7 populations on iceberg lettuce and romaine lettuce by 1.45 and 0.83 log CFU g^{-1}, respectively, which was more efficient than NaOCl treatment containing 200 mg L^{-1} free chlorine at pH 8.0.

Table 1-3. Inactivation of microorganisms on fresh produce by NaOCl.

Produce	Microorganism	Free chlorine (mg L^{-1})	pH	Time (min)	Log reduction	Reference
Apple	*E. coli*	200	6.4	1	2.1	Sapers, et al., 1999
Blueberries	*E. coli* O157:H7	100	9.64	1	4.41	Pangloli, & Hung, 2013
	E. coli O157:H7	100	9.64	5	4.81	
Broccoli	*E. coli* O157:H7	100	8.8	1	1.26	Hung, et al., 2010
	E. coli O157:H7	100	8.8	5	1.60	
Cucumber	Aerobic mesophilic	150	9.3	10	1.5	Koseki, et al., 2004
	Coliform	150	9.3	10	1.6	
	Fungi	150	9.3	10	2.2	
Iceberg lettuce	*E. coli* O157:H7	20	8.0	2	0.81	Keskinen, et al., 2009
Leafy greens	*E. coli* O157:H7	50	6.5	1	2.4	Stopforth, et al., 2008
	L. monocytogenes	50	6.5	1	2.2	
	S. enterica	50	6.5	1	2.2	
Romaine lettuce	*E. coli* O157:H7	20	8.0	2	0.52	Keskinen, et al., 2009
	E. coli O157:H7	200	7.87	1	1.31	del Carmen Velázquez, et al., 2009
	Yersinia enterocolitica	200	7.87	1	2.10	
Spinach	*E. coli* O157:H7	100	10.6	3	2.01	Rahman, Ding, & Oh, 2010
	L. monocytogenes	100	10.6	3	2.20	
Strawberries	*E. coli* O157:H7	55	8.2	1	1.24	Hung, et al., 2010
	E. coli O157:H7	55	8.2	5	1.51	
Tomato	*E. coli* O157:H7	200	7.87	1	2.56	del Carmen Velázquez, et al., 2009
	Yersinia enterocolitica	200	7.87	1	4.77	

Table 1-4. Inactivation of microorganisms on fresh produce by EO water.

Produce	Microorganism	Free chlorine (mg L^{-1})	pH	Time (min)	Log reduction	Reference
Blueberries	*E. coli* O157:H7	30	2.63	1	3.90	Pangloli, & Hung, 2013
	E. coli O157:H7	30	2.63	5	4.44	
Broccoli	*E. coli* O157:H7	100	2.6	1	1.51	Hung, et al., 2010
	E. coli O157:H7	100	2.6	5	1.78	
Cucumber	Aerobic mesophilic	30	2.6	10	1.5	Koseki, et al., 2004
	Coliform	30	2.6	10	1.8	
	Fungi	30	2.6	10	2.1	
Iceberg lettuce	*E. coli* O157:H7	50	2.6	2	0.68	Keskinen, et al., 2009
	S. Typhimurium	155	7.5	5	3.0	Afari, et al., 2015
Leafy greens	*E. coli* O157:H7	50	6.5	1	2.4	Stopforth, et al., 2008
	L. monocytogenes	50	6.5	1	2.0	
	Salmonella spp.	50	6.5	1	2.3	
Romaine lettuce	*E. coli* O157:H7	50	2.6	2	0.50	Keskinen, et al., 2009
	S. Typhimurium	155	7.5	5	2.2	Afari, et al., 2015
Spinach	*E. coli* O157:H7	50	2.54	3	2.4	Rahman, Ding, & Oh, 2010
	L. monocytogenes	50	2.54	3	2.8	
Strawberries	*E. coli* O157:H7	50	2.7	1	1.17	Hung, et al., 2010
	E. coli O157:H7	50	2.7	5	1.58	
	Aerobic mesophilic	30	2.6	10	1.6	Koseki, et al., 2004
	Coliform	30	2.6	10	> 1.1	
	Fungi	30	2.6	10	1.7	
Tomato	*S.* Typhimurium	155	7.5	5	7.6	Afari, et al., 2015

Table 1-5. Inactivation of microorganisms on fresh produce by ClO_2.

Produce	Microorganism	ClO_2 concentration (mg L^{-1})	Time (min)	Log reduction	Reference
Apple	*Enterobacter sakazakii*	100	1	≥ 4.49	Kim, et al., 2006
Blueberries	*L. monocytogenes*	3	30	1.30	Wu & Kim, 2007
	Pseudomonas aeruginosa	3	30	1.31	
	S. Typhimurium	3	30	1.52	
	S. aureus	3	30	1.92	
	Yersinia enterocolitica	3	30	0.62	
Broccoli sprouts	*E. coli* O157:H7	50	5	1.66	Kim, Kim, & Song, 2009
	L. monocytogenes	50	5	1.24	
	S. Typhimurium	50	5	1.54	
Cantaloupe	*E. coli* O157:H7	5	10	4.6	Mahmoud, Vaidya, Corvalan, & Linton, 2008
	L. monocytogenes	5	10	4.3	
	S. Poona	5	10	> 5	
Iceberg lettuce	*E. coli* O157:H7	20	2	1.06	Keskinen, et al., 2009
Romaine lettuce	*E. coli* O157:H7	20	2	0.44	Keskinen, et al., 2009
Strawberry	*E. coli* O157:H7	5	2	1.8	Mahmoud, Bhagat, & Linton, 2007
	L. monocytogenes	5	2	2.4	
	S. enterica	5	2	1.9	
Tomato	*S. enterica*	20	1	5	Pao, Kelsey, Khalid, & Ettinger, 2007
	Erwinia carotovora	10	1	5	

1.4 Reactions of chlorine with organic compounds

One concern of using chlorine-based sanitizer is their reaction with organic matter. During the produce washing process, organic compounds accumulate in produce wash water due to the recycling of water (Barrear, Blenkinsop, & Warriner, 2012). The presence of organic compounds can lead to a rapid depletion of active component in chlorinated wash solution, which may compromise the microbial inactivation efficacy of washing treatments. When a 10 % of organic load was presented in the processing water, there was no significant difference for inactivating *E. coli* O157:H7 on lettuce leaves among water control, peroxyacetic acid at 10 and 20 mg L^{-1}, and chlorine at 30 mg L^{-1} (Zhang, Ma, Phelan, & Doyle, 2009). For ClO_2, the bactericidal effect of ClO_2 on wastewater increased by up to threefold when the chemical oxygen demand (COD) concentration was decreased by half (Ayyildiz, Ileri, & Sanik, 2009).

Kinetic and mechanism of HOCl toward organic compounds is well-discussed by Deborde & Gunten (2008). The reactivity of HOCl towards organic compounds is usually selective, and there are three kinds of possible reactions: (i) oxidation reaction, (ii) addition reaction to unsaturated bonds, (iii) electrophilic substitution reactions at nucleophilic sites. The reactivity of chlorine with common functional groups from high to low is: reduced sulfur moieties > primary and secondary amines > phenols, tertiary amines » double bonds, other aromatics, carbonyls, amides. Due to their relatively high concentration and reactivity, protein and phenolic are the two main organic compounds that react with chlorine during produce washing (Waters & Hung, 2014).

Reactions of chlorine with organic compounds would eventually generate disinfection by-products (DBPs). Most chlorine DBPs are formed through oxidation and substitution reactions (Deborde & Gunten, 2008). Two major classes of DBPs are trihalomethanes (THMs) and haloacetic acids (HAAs). The four common THMs include chloroform, bromoform, bromodichloromethane, dibromochloromethane; the five common HAAs include chloroacetic acid, dichloroacetic acid, trichloroacetic acid, bromoacetic acid, and dibromoacetic acid (Serodes, Rodeiguez, Li, & Bouchard, 2002). Besides, there are a variety of other classes of DBPs, such as haloacetonitriles, haloketones, haloaldehydes, and chloropicrin. In contract to chlorine, ClO_2 reacts with organic compounds only by oxidation, so it does not produce much organochlorine compounds. Fan & Sokorai (2015) reported that little trichloromethane levels ($\leq 3\ \mu g\ L^{-1}$) was detected from ClO_2 solution even at concentration up to 200 mg L^{-1} compared with the trichloromethane level (40 µg L^{-1}) in solutions of chlorine (200 mg L^{-1} free chlorine) mixed with lettuce extract. Instead, the major DBPs of chlorine dioxide are chlorite and chlorate (Van Haute, Tryland, Eschdero, Vanneste, & Sampers, 2017).

A wide variety of factors such as chlorine concentration, organic load, pH, temperature and contact time can affect the DBPs formation during produce washing process (Fleischacker & Randtke, 1983). Specifically, the kinetic of chlorination reaction is second order with a first order reaction with free chlorine concentration and another first order reaction with total organic compound concentration (Deborde & Gunten, 2008). Therefore, the increasing free chlorine concentration and organic load would increase the DBPs formation. In terms of the pH effect, the overall organochlorine compound formation decreases with increasing pH. For specific DBPs, increasing pH has been associated with increasing THMs and decreasing HAAs (Hansen, Willach, Mosbæk, & Andersen, 2012). The high rate of THMs formation at alkaline pH can be explained by the fact that OCl^- is easier than HOCl to electrophilically attack the nucleophilic organic compounds. The higher temperature in wash solution can supply more energy to chlorination reaction, so the formation rate of THMs and HAAs increases with temperature. Moreover, THMs and HAAs appeared to rapidly form during the first 4-5 h of chlorination process, followed by a reduction in the formation rate. It was also observed that near 90 % of the THMs was formed within the first 24 h of chlorine addition to natural water (Uden & Miller, 1983). More recent study shows that more than 60 % of chlorine demand of romaine lettuce wash water occurred within first 5 min over a 90-min observation period (Weng et al., 2016). Besides, the presence of bromide ion and ammonia in wash solution can affect the formation of DBPs as well (Deborde & von Gunten, 2008).

Many DBPs has been associated with short-term and long-term toxicity to experimental animals and humans. Epidemiological studies demonstrated an association between exposure to DBPs and bladder cancer or colon cancer (Rahman, Driscoll, Cowie, & Armstrong, 2010; Villanueva et al., 2004). The EPA estimated that 2-17 % of urinary bladder cancer cases was caused by DBPs (Hrudey, 2009). For specific DBPs, chloroform is one of the most prevalent THMs for chlorine disinfection. The acute chloroform intoxication via inhalation was reported to cause cardiomyocyte fragmentation and waviness indicative of acute heart failure (Harada, Ichiyama, Ikeda, Ishihara, & Yoshida, 1997). The tumorigenicity of chloroform during long-term exposure was caused by the chloroform-induced cytolethality and regenerative cell proliferation (Larson, Wolf, & Butterworth, 1994). In terms of HAAs, the dichloroacetic acid and trichloroacetic acid have been shown to produce developmental, reproductive, neural and hepatic effects in experimental animals. HAAs can also induce genomic DNA damage and mutagenicity in cells through mediating the generation of reactive oxygen species (Pals, Ang, Wagner, & Plewa, 2011). The Maximum contamination level (MCL) of DBPs currently regulated in drinking water by the US Environmental Protection Agency (EPA) are listed in Table 1-6 (EPA, 2006).

So far there is no regulation about DBPs related to fresh produce or produce wash water, and studies on DBPs formation during produce washing are limited. Gómez-López et al. (2010) compared the THMs formation for lettuce washing process from NaOCl (100 mg L^{-1}) and ClO_2 (3.7 mg L^{-1}). It shows that the formation of THMs was only detected in process water from NaOCl treatment (217 ± 38 µg L^{-1}). Van Haute et al. (2013) found that the total trihalomethanes (TTHMs) reached 124.5 µg L^{-1} after 1 h of washing in water with a COD of 1000 mg L^{-1}, while no TTHMs were detected on the fresh-cut lettuce after washing. Fan & Sokorai (2015) reported that the concentration of THMs in wash water increased from 155 to 284 µg L^{-1} after repeated use of 1 L of 100 mg L^{-1} chlorine to wash 6 batches of 100 g of fresh-cut iceberg lettuce, and there was 21 µg L^{-1} of THMs formation in simulated iceberg lettuce wash water for every 100 mg L^{-1} increase in free chlorine concentration.

Table 1-6. The Maximum contamination level of DBPs regulated in drinking water.

DBPs	MCL (μg L^{-1})
TTHMs (total trihalomethanes)	80
Chloroform, bromoform, bromodichloromethane, and dibromochloromethane	
HAA5 (Haloacetic acids)	60
Monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, trichloroacetic acid, monobromoacetic acid, and dibromoacetic acid	
Chlorite	1000
Bromate	10

1.5 Chlorine dosing strategy for fresh produce washing process

The antimicrobial efficacy of produce washing process was largely depended on the free chlorine residual in processing water (Gil et al., 2009). On the other hand, excess amount of free chlorine may react with organic compounds in produce wash water to generate hazardous DBPs (Fan & Sokorai, 2015).

Because of the accumulation of organic matter during continuous washing process, maintaining chlorine residual in washing solution to a specific level is always a challenge. Currently some commercial produce washing systems rely on measuring ORP and pH to monitor chlorine residual, while the reliability of these parameters are questionable (Zhou, Luo, Nou, & Millner, 2014). Many researchers therefore developed models to predict "chlorine demand", which is the chlorine loss as a consequence of reacting with organic compounds. Pirovani, Piagnetini, Guemes, & Arkwright (2004) developed models to predict total and free chlorine loss during shredded lettuce washing process at different initial chlorine concentration, water-to-produce ratio and time. Waters & Hung (2014) developed chlorine demand prediction equations for various produce wash waters using total protein and phenolics contents as the organic load indicators. Zhou et al. (2014) used COD as the organic load indicator to develop an algorithm to maintain the free chlorine residual for lettuce washing process. The major limitation of these models is that many parameters that were selected as the organic load indicator require hours of reaction time and complicated analysis protocols (e.g. COD and total phenolics). In addition, some of these studies were designed using only one type of fresh produce, recommendation on relation between parameters and organic load/chlorine demand may not be valid for other type of fresh produce.

In the author's opinion, model for commercial produce washing process need to be able to predict chlorine demand for various types of fresh produce and for different processing conditions such as different initial chlorine concentration, organic load and sanitizer pH. All the parameters in the model also need to be easily measured or on-line monitored with simple protocols, short analysis time and low instrument/reagent cost.

1.6 Research objectives

The overall objective of this study was to develop a chlorine-based disinfection strategy to achieve high microbial inactivation efficacy and minimize DBPs formation potential for fresh produce postharvest washing process. There were three specific objectives in this study:

1. To determine the relationship between chlorine demand and produce wash water quality parameters, and to explore the feasibility of using wash water quality parameters to estimate chlorine demand for different types of produce
2. To evaluate the effects of sanitizer pH, initial chlorine concentration and organic load on chlorine demand, and to develop prediction equations of various produce wash waters at different processing conditions
3. To develop a chorine dosing strategy for produce washing process to achieve high microbial inactivation and minimize residual chlorine in produce wash water

References

Afari, G. K., Hung, Y. C., & King, C. H. (2015). Efficacy of neutral pH electrolyzed water in reducing *Escherichia coli* O157: H7 and *Salmonella* Typhimurium DT 104 on fresh produce items using an automated washer at simulated food service conditions. *Journal of Food Science, 80*(8), M1815-M1822.

Afari, G. K., Hung, Y. C., King, C. H., & Hu, A. (2016). Reduction of *Escherichia coli* O157: H7 and *Salmonella* Typhimurium DT 104 on fresh produce using an automated washer with near neutral electrolyzed (NEO) water and ultrasound. *Food Control, 63,* 246-254.

Amy, G., Bull, R., Craun, G. F., Pegram, R., Siddiqui, M., & Organization, W. H. (2000). Disinfectants and disinfectant by-products.

Ayyildiz, O., Ileri, B., & Sanik, S. (2009). Impacts of water organic load on chlorine dioxide disinfection efficacy. *Journal of Hazardous Materials*, 168(2), 1092-1097.

Barrera, M. J., Blenkinsop, R., & Warriner, K. (2012). The effect of different processing parameters on the efficacy of commercial post-harvest washing of minimally processed spinach and shredded lettuce. *Food Control*, 25(2), 745-751.

Batz, M. B., Hoffmann, S. A., & Morris, J. G. (2011). Ranking the risks: the 10 pathogen-food combinations with the greatest burden on public health. Emerging Pathogens Institute, University of Florida.

Berg, J., Roberts, P., & Matin, A. (1986). Effect of chlorine dioxide on selected membrane functions of *Escherichia coli*. *Journal of Applied Bacteriology, 60*(3), 213-220.

Berger, C. N., Sodha, S. V., Shaw, R. K., Griffin, P. M., Pink, D., Hand, P., & Frankel, G. (2010). Fresh fruit and vegetables as vehicles for the transmission of human pathogens. *Environmental Microbiology, 12*(9), 2385-2397.

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

Beuchat, L. R., Adler, B. B., & Lang, M. M. (2004). Efficacy of chlorine and a peroxyacetic acid sanitizer in killing *Listeria monocytogenes* on iceberg and romaine lettuce using simulated commercial processing conditions. *Journal of Food Protection, 67*(6), 1238-1242.

Beuchat, L. R., & Ryu, J.-H. (1997). Produce handling and processing practices. *Emerging Infectious Diseases, 3*(4), 459.

Brandl, M. T. (2006). Fitness of human enteric pathogens on plants and implications for food safety. *Annual Review of Phytopathology, 44*, 367-392.

Buck, J., Walcott, R., & Beuchat, L. (2003). Recent trends in microbiological safety of fruits and vegetables. *Plant Health Progress, 10*(1), 1094.

Centers for Disease Control and Prevention. (2008). Investigation of outbreak of infections caused by *Salmonella* Litchfield.

Centers for Disease Control and Prevention. (2011). Investigation update: multistate outbreak of human *Salmonella* I 4,[5], 12: i:-infections linked to alfalfa sprouts. *On the World Wide Web at http://www. cdc. gov/salmonella/i4512i-*

/021011/index. html (February 20, 2011).

Centers for Disease Control and Prevention. (2017). Foodborne Outbreak Online Database (FOOD). *On the World Wide Web at http://wwwn.cdc.gov/foodborneoutbreaks*. *(February 28, 2011)*.

Deborde, M., & von Gunten, U. (2008). Reactions of chlorine with inorganic and organic compounds during water treatment - Kinetics and mechanisms: A critical review. *Water Research, 42*(1-2), 13-51.

del Carmen Velázquez, L., Barbini, N. B., Escudero, M. E., Estrada, C. L., & de Guzmán, A. M. S. (2009). Evaluation of chlorine, benzalkonium chloride and lactic acid as sanitizers for reducing *Escherichia coli* O157: H7 and *Yersinia enterocolitica* on fresh vegetables. *Food Control, 20*(3), 262-268.

DeWaal, C. S., & Bhuiya, F. (2007). Outbreaks by the numbers: fruits and vegetables 1990–2005. International Association for Food Protection annual meeting, Lake Buena Vista, FL.

DeWaal, C. S., Xuman, A. T., & Plunkett, D. (2009). Outbreak alert! Analyzing foodborne outbreaks 1998–2007. Washington, DC: Center for Science in the Public Interest.

Doyle, M., & Erickson, M. (2008). Summer meeting 2007–the problems with fresh produce: an overview. *Journal of applied microbiology, 105*(2), 317-330.

Environmental Protection Agency. (2006). National primary drinking water regulations: Stage 2 disinfectants and disinfection byproducts rule. *Federal Register, 71*, 387-493.

Environmental Protection Agency. (2009). Water systems, disinfection byproducts, and the use of monochloramine. *On the World Wide Web at https://www.epa.gov/sites/production/files/2015-09/documents/how_does_epa_regulate_disinfection_byproducts_dbps.pdf (March 10, 2017).*

Erickson, M. C., Liao, J., Cannon, J. L., & Ortega, Y. R. (2015). Role of brushes and peelers in removal of *Escherichia coli* O157: H7 and *Salmonella* from produce in domestic kitchens. *Journal of Food Protection, 78*(9), 1624-1631.

Fan, X., & Sokorai, K. J. (2015). Formation of trichloromethane in chlorinated water and fresh-cut produce and as a result of reaction with citric acid. *Postharvest Biology and Technology, 109*, 65-72.

Fett, W. (2002). Factors affecting the efficacy of chlorine against *Esherichia coli* O157: H7 and *Salmonella* on alfalfa seed. *Food Microbiology, 19*(2-3), 135-149.

Fleischacker, S. J., & Randtke, S. J. (1983). Formation of organic chlorine in public water supplies. *Journal (American Water Works Association)*, 132-138.

Food and Drug Administration. (2001). Analysis and evaluation of prevention control measures for the control and reduction/elimination of microbial hazards on fresh and fresh-cut produce, Center for Food Safety and Applied Nutrition. *On the World Wide Web at https://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/ucm090977.htm (March 10, 2017).*

Food and Drug Administration. (2002). Secondary direct food additives permitted in food for human consumption. *Code of federal regulations. Title, 21.*

Food and Drug Administration, HHS. (2008). Irradiation in the production, processing and handling of food. Final rule. *Federal register*, 73(164), 49593.

Fujisawa, T., Sata, S., Aikawa, K., Takahashi, T., Yamai, S., & Shimada, T. (2000). Modification of sorbitol MacConkey medium containing cefixime and tellurite for isolation of *Escherichia coli* O157: H7 from radish sprouts. *Applied and Environmental Microbiology, 66*(7), 3117-3118.

Gil, M. I., Selma, M. V., Lopez-Galvez, F., & Allende, A. (2009). Fresh-cut product sanitation and wash water disinfection: Problems and solutions. *International Journal of Food Microbiology*, *134*(1-2), 37-45.

Gómez-López, V. M., Gil, M. I., Allende, A., Blancke, J., Schouteten, L., & Selma, M. V. (2014). Disinfection capacity of high-power ultrasound against *E. coli* O157: H7 in process water of the fresh-cut industry. *Food and Bioprocess Technology*, *7*(12), 3390-3397.

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

Goodburn, C., & Wallace, C. A. (2013). The microbiological efficacy of decontamination methodologies for fresh produce: a review. *Food Control, 32*(2), 418-427.

Griffin, P. M., & Tauxe, R. V. (1991). The epidemiology of infections caused by *Escherichia coli* O157: H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. *Epidemiologic Reviews, 13*(1), 60-98.

Guentzel, J. L., Lam, K. L., Callan, M. A., Emmons, S. A., & Dunham, V. L. (2008).

Reduction of bacteria on spinach, lettuce, and surfaces in food service areas using neutral electrolyzed oxidizing water. *Food Microbiology*, 25(1), 36-41.

Gurtler, J. B., Bailey, R. B., Jin, T. Z., & Fan, X. (2014). Reduction of an *E. coli* O157: H7 and *Salmonella* composite on fresh strawberries by varying antimicrobial washes and vacuum perfusion. *International Journal of Food Microbiology, 189*, 113-118.

Hansen, K. M., Willach, S., Mosbæk, H., & Andersen, H. R. (2012). Particles in swimming pool filters–Does pH determine the DBP formation? *Chemosphere*, 87(3), 241-247.

Harada, K., Ichiyama, T., Ikeda, H., Ishihara, T., & Yoshida, K. (1997). An autopsy case of acute chloroform intoxication after intermittent inhalation for years. *Nihon Hoigaku Zasshi= The Japanese Journal of Legal Medicine, 51*(4), 319-323.

Harris, L., Farber, J., Beuchat, L., Parish, M., Suslow, T., Garrett, E., & Busta, F. (2003). Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh-cut produce. *Comprehensive Reviews in Food Science and Food Safety, 2*(s1), 78-141.

Hrudey, S. E. (2009). Chlorination disinfection by-products, public health risk tradeoffs and me. *Water Research, 43*(8), 2057-2092.

Huang, Y.-R., Hung, Y.-C., Hsu, S.-Y., Huang, Y.-W., & Hwang, D.-F. (2008). Application of electrolyzed water in the food industry. *Food Control, 19*(4), 329-345.

Hung, Y. C., Tilly, P., & Kim, C. (2010). Efficacy of electrolyzed oxidizing (EO) water and chlorinated water for inactivation of *Escherichia coli* O157: H7 on strawberries and broccoli. *Journal of Food Quality, 33*(5), 559-577.

Jadeja, R., Hung, Y.-C., & Bosilevac, J. M. (2013). Resistance of various shiga toxin-producing *Escherichia coli* to electrolyzed oxidizing water. *Food Control, 30*(2), 580-584.

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

Kim, C., Hung, Y.-C., & Brackett, R. E. (2000). Efficacy of electrolyzed oxidizing (EO) and chemically modified water on different types of foodborne pathogens. *International Journal of Food Microbiology, 61*(2), 199-207.

Kim, H., Ryu, J.-H., & Beuchat, L. R. (2006). Survival of *Enterobacter sakazakii* on fresh produce as affected by temperature, and effectiveness of sanitizers for its elimination. *International Journal of Food Microbiology, 111*(2), 134-143.

Kim, Y., Kim, M., & Song, K. (2009). Efficacy of aqueous chlorine dioxide and fumaric acid for inactivating pre-existing microorganisms and *Escherichia coli* O157: H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on broccoli sprouts. *Food Control, 20*(11), 1002-1005.

Koseki, S., Yoshida, K., Isobe, S., & Itoh, K. (2004). Efficacy of acidic electrolyzed water for microbial decontamination of cucumbers and strawberries. *Journal of Food Protection, 67*(6), 1247-1251.

Kroupitski, Y., Pinto, R., Brandl, M., Belausov, E., & Sela, S. (2009). Interactions of

Salmonella enterica with lettuce leaves. *Journal of Applied Microbiology, 106*(6), 1876-1885.

Larson, J. L., Wolf, D. C., & Butterworth, B. E. (1994). Induced cytolethality and regenerative cell proliferation in the livers and kidneys of male B6C3F1 mice given chloroform by gavage. *Toxicological Sciences, 23*(4), 537-543.

López-Gálvez, F., Allende, A., Truchado, P., Martínez-Sánchez, A., Tudela, J. A., Selma, M. V., & Gil, M. I. (2010). Suitability of aqueous chlorine dioxide versus sodium hypochlorite as an effective sanitizer for preserving quality of fresh-cut lettuce while avoiding by-product formation. *Postharvest Biology and Technology*, 55(1), 53-60.

Luo, Y., Nou, X., Yang, Y., Alegre, I., Turner, E., Feng, H., Abadias, M., & Conway, W. (2011). Determination of Free Chlorine Concentrations Needed to Prevent *Escherichia coli* O157:H7 Cross-Contamination during Fresh-Cut Produce Wash. *Journal of Food Protection, 74*(3), 352-358.

Lynch, M., Tauxe, R., & Hedberg, C. (2009). The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. *Epidemiology and Infection, 137*(03), 307-315.

Mahmoud, B., Vaidya, N., Corvalan, C., & Linton, R. (2008). Inactivation kinetics of inoculated *Escherichia coli* O157: H7, *Listeria monocytogenes* and *Salmonella* Poona on whole cantaloupe by chlorine dioxide gas. *Food Microbiology, 25*(7), 857-865.

Mahmoud, B. S., Bhagat, A., & Linton, R. H. (2007). Inactivation kinetics of inoculated *Escherichia coli* O157: H7, *Listeria monocytogenes* and

Salmonella enterica on strawberries by chlorine dioxide gas. *Food Microbiology, 24*(7), 736-744.

Michino, H., Araki, K., Minami, S., Takaya, S., Sakai, N., Miyazaki, M., Ono, A., Yanagawa, H. (1999). Massive outbreak of *Escherichia coli* O157: H7 infection in schoolchildren in Sakai City, Japan, associated with consumption of white radish sprouts. *American Journal of Epidemiology, 150*(8), 787-796.

Morris, J. C. (1966). The acid ionization constant of HOCl from 5 to 35. *The Journal of Physical Chemistry, 70*(12), 3798-3805.

Olaimat, A. N., & Holley, R. A. (2012). Factors influencing the microbial safety of fresh produce: a review. *Food Microbiology, 32*(1), 1-19.

Ölmez, H., & Kretzschmar, U. (2009). Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. *Lwt-Food Science and Technology, 42*(3), 686-693.

Pals, J. A., Ang, J. K., Wagner, E. D., & Plewa, M. J. (2011). Biological mechanism for the toxicity of haloacetic acid drinking water disinfection byproducts. *Environmental Science & Technology, 45*(13), 5791-5797.

Pang, Y. H., & Hung, Y. C. (2016). Efficacy of slightly acidic electrolyzed water and UV-ozonated water combination for inactivating *Escherichia coli* O157: H7 on romaine and iceberg lettuce during spray washing process. *Journal of Food Science, 81*(7), M1743-M1748.

Pangloli, P., & Hung, Y.-C. (2013). Reducing microbiological safety risk on blueberries through innovative washing technologies. *Food Control, 32*(2), 621-625.

Pao, S., Kelsey, D., Khalid, M., & Ettinger, M. (2007). Using aqueous chlorine dioxide to prevent contamination of tomatoes with *Salmonella enterica* and *Erwinia carotovora* during fruit washing. *Journal of Food Protection, 70*(3), 629-634.

Park, H., Hung, Y.-C., & Chung, D. (2004). Effects of chlorine and pH on efficacy of electrolyzed water for inactivating *Escherichia coli* O157: H7 and *Listeria monocytogenes*. *International Journal of Food Microbiology, 91*(1), 13-18.

Pirovani, M., Guemes, D., & Piagnetini, A. (2001). Predictive models for available chlorine depletion and total microbial count reduction during washing of fresh-cut spinach. *Journal of Food Science, 66*(6), 860-864.

Pirovani, M., Piagentini, A., Güemes, D., & Arkwright, S. (2004). Reduction of chlorine concentration and microbial load during washing-disinfection of shredded lettuce. *International Journal of Food Science & Technology*, 39(3), 341-347.

Rahman, M. B., Driscoll, T., Cowie, C., & Armstrong, B. K. (2010). Disinfection by-products in drinking water and colorectal cancer: a meta-analysis. *International Journal of Epidemiology, 39*(3), 733-745.

Rahman, S., Ding, T., & Oh, D.-H. (2010). Inactivation effect of newly developed low concentration electrolyzed water and other sanitizers against microorganisms on spinach. *Food Control, 21*(10), 1383-1387.

Rodgers, S. L., Cash, J. N., Siddiq, M., & Ryser, E. T. (2004). A comparison of different chemical sanitizers for inactivating *Escherichia coli* O157: H7 and *Listeria monocytogenes* in solution and on apples, lettuce, strawberries, and cantaloupe. *Journal of Food Protection, 67*(4), 721-731.

Sagong, H.-G., Lee, S.-Y., Chang, P.-S., Heu, S., Ryu, S., Choi, Y.-J., & Kang, D.-H. (2011). Combined effect of ultrasound and organic acids to reduce *Escherichia coli* O157: H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on organic fresh lettuce. *International Journal of Food Microbiology, 145*(1), 287-292.

Sapers, G., Miller, R., & Mattrazzo, A. (1999). Effectiveness of sanitizing agents in inactivating *Escherichia coli* in golden delicious apples. *Journal of Food Science, 64*(4), 734-737.

Sérodes, J. B., Rodriguez, M. J., Li, H., & Bouchard, C. (2003). Occurrence of THMs and HAAs in experimental chlorinated waters of the Quebec City area (Canada). *Chemosphere*, 51(4), 253-263.

Scallan, E. (2011). Foodborne Illness Acquired in the United States—Unspecified Agents-Volume 17, Number 1—January 2011-Emerging Infectious Disease journal-CDC.

Scharff, R. L. (2012). Economic burden from health losses due to foodborne illness in the United States. *Journal of Food Protection, 75*(1), 123-131.

Seymour, I. (1999). Review of current industry practice on fruit and vegetable decontamination. Campden & Chorleywood Food Research Association.

Shim, W.-B., Je, G.-S., Kim, K., Mtenga, A. B., Lee, W.-G., Song, J.-U., Yoon, Y. (2012). Effect of irradiation on kinetic behavior of *Salmonella* Typhimurium and *Staphylococcus aureus* in lettuce and damage of bacterial cell envelope. *Radiation Physics and Chemistry, 81*(5), 566-571.

Sivapalasingam, S., Friedman, C. R., Cohen, L., & Tauxe, R. V. (2004). Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *Journal of Food Protection, 67*(10), 2342-2353.

Stopforth, J., Mai, T., Kottapalli, B., & Samadpour, M. (2008). Effect of acidified sodium chlorite, chlorine, and acidic electrolyzed water on *Escherichia coli* O157: H7, *Salmonella,* and *Listeria monocytogenes* inoculated onto leafy greens. *Journal of Food Protection, 71*(3), 625-628.

Uden, P. C., & Miller, J. W. (1983). Chlorinated acids and chloral in drinking water. *Journal (American Water Works Association)*, 524-527.

Van Haute, S., Sampers, I., Holvoet, K., & Uyttendaele, M. (2013). Physicochemical quality and chemical safety of chlorine as a reconditioning agent and wash water disinfectant for fresh-cut lettuce washing. *Applied and Environmental Microbiology, 79*(9), 2850-2861.

Van Haute, S., Tryland, I., Escudero, C., Vanneste, M., & Sampers, I. (2017). Chlorine dioxide as water disinfectant during fresh-cut iceberg lettuce washing: Disinfectant demand, disinfection efficiency, and chlorite formation. *LWT-Food Science and Technology*, *75,* 301-304.

Villanueva, C. M., Cantor, K. P., Cordier, S., Jaakkola, J. J., King, W. D., Lynch, C. F., Porru, S., & Kogevinas, M. (2004). Disinfection byproducts and bladder cancer: a pooled analysis. *Epidemiology*, *15*(3), 357-367.

Wang, H., Feng, H., Liang, W., Luo, Y., & Malyarchuk, V. (2009). Effect of surface roughness on retention and removal of *Escherichia coli* O157: H7 on surfaces of selected fruits. *Journal of Food Science, 74*(1).

Warriner, K., & Namvar, A. (2010). The tricks learnt by human enteric pathogens from phytopathogens to persist within the plant environment. *Current Opinion in Biotechnology, 21*(2), 131-136.

Waters, B. W., & Hung, Y. C. (2014). The effect of organic loads on stability of various chlorine-based sanitisers. *International Journal of Food Science & Technology, 49*(3), 867-875.

Weng, S., Luo, Y., Li, J., Zhou, B., Jacangelo, J. G., & Schwab, K. J. (2016). Assessment and speciation of chlorine demand in fresh-cut produce wash water. *Food Control, 60*, 543-551.

Wu, V. C., & Kim, B. (2007). Effect of a simple chlorine dioxide method for controlling five foodborne pathogens, yeasts and molds on blueberries. *Food Microbiology, 24*(7), 794-800.

Yaun, B. R., Sumner, S. S., Eifert, J. D., & Marcy, J. E. (2004). Inhibition of pathogens on fresh produce by ultraviolet energy. *International Journal of Food Microbiology, 90*(1), 1-8.

Yeni, F., Yavaş, S., Alpas, H., & Soyer, Y. (2016). Most common foodborne pathogens and mycotoxins on fresh produce: a review of recent outbreaks. *Critical Reviews in Food Science and Nutrition, 56*(9), 1532-1544.

Zhang, S., & Farber, J. (1996). The effects of various disinfectants against *Listeria monocytogenes on* fresh-cut vegetables. *Food Microbiology, 13*(4), 311-321.

Zhang, G., Ma, L., Phelan, V. H., & Doyle, M. P. (2009). Efficacy of antimicrobial agents in lettuce leaf processing water for control of *Escherichia coli* O157:H7. *Journal of Food Protection,* 72(7), 1392-1397.

Zhou, B., Luo, Y., Nou, X., & Millner, P. (2014). Development of an algorithm for feed-forward chlorine dosing of lettuce wash operations and correlation of chlorine profile with *Escherichia coli* O157: H7 inactivation. *Journal of Food Protection*, *77*(4), 558-566.

CHAPTER 2

PREDICTING CHLORINE DEMAND OF FRESH AND FRESH-CUT PRODUCE BASED ON PRODUCE WASH WATER PROPERTIES

Chen, X., & Y.-C. Hung (2016). *Postharvest Biology and Technology*, 120, 10-15. Reprinted here with permission of the publisher.

Abstract

This study was conducted to develop models capable of predicting chlorine demand of different fresh and fresh-cut produce wash waters. Ten simulated fruit and vegetable wash waters having different chemical oxygen demands (COD) were prepared. The chlorine demand and wash water quality parameters including pH, oxidation reduction potential (ORP), ultraviolet absorbance at 254 nm (UV254), COD, turbidity, total protein content, total phenolics content and color difference between deionized water and test samples (ΔE) were measured. The correlations between variables were determined. UV254 had the highest correlation coefficient with chlorine demand of various fresh produce wash waters ($R = 0.77$). Further analysis of chlorine demand with UV254 relation showed that two clusters exits, one for produce with high phenolics content and one for low phenolics content. The phenolics-to-protein/ΔE ratio (PPC) was created to identify in which cluster each produce wash water should be. Empirical equations for predicting chlorine demand were developed as chlorine demand = 295.23 × UV254 + 6.97, if $PPC < 0.6$; or chlorine demand = 119.77 × UV254 + 2.41, if $PPC \geq 0.6$. These two prediction equations were further verified using additional produce wash waters not used for model development. The outcomes of this study demonstrated that the predictive equations developed using water quality parameters can be used to estimate the chlorine demand of different produce wash waters.

Key Words: Fresh produce, washing, chlorine demand, UV254, predictive equations

2.1 Introduction

Outbreaks associated with the consumption of fresh produce have been widely reported over the past two decades (Olaimat and Holley 2012). The percentage of foodborne outbreaks caused by fresh produce increased from 0.7 % in the 1970s to 6 % in the 1990s (Sivapalasingam et al. 2004). This number further increased to 14.8 % from 1998 to 2007 (CSPI 2009). Compared with meat or poultry products, fresh produce is usually eaten raw or minimally processed. Thus, once fresh produce is contaminated, it is difficult to totally remove or kill those pathogens.

Postharvest washing is one of the most critical processing steps to maintain the safety and quality of fresh and fresh-cut produce (Gil et al. 2015). In most processing lines, fresh fruits and vegetables are immediately dipped into a washing tank to remove dirt, pesticide residues, debris, tissue fluids and microorganisms, as well as to rapidly cool the products (Artes et al. 2009). To further enhance the antimicrobial efficacy and to prevent cross contamination, sanitizers are usually added into the wash water (Gil et al. 2009). Chlorine-based sanitizers are commonly used in the US fresh produce industry (Lu et al. 2014). Generally, chlorine is used in residual concentrations at 50-200 mg L^{-1} with a 1-2 min dipping time. The pH of chlorine-based sanitizers is usually adjusted to 6-7.5 to maintain the high antimicrobial efficacy and to minimize the generation of toxic chlorine gas (Beuchat et al. 2004).

One concern of using chlorine is its reaction with organic matter. During the industrial washing process, organic compounds accumulate in wash water due to the recycling of wash water (Barrear et al. 2012). The presence of organic compounds can lead to a rapid depletion of hypochlorous acid (HOCl), which is the main active component of a chlorinated wash solution. Although it is reported that the microbial inactivation due to chlorine on fresh produce is limited regardless of concentration (1-2 log reductions) (Beuchat et al. 2004), sufficient residual chlorine in wash water is a key factor to prevent cross-contamination (Luo et al. 2011). Many studies therefore were conducted to evaluate the impact of organic load on residual chlorine and antimicrobial efficacy of chlorine solutions (Pirovani et al. 2001; Van Haute et al. 2013; Shen et al. 2013; Zhou et al. 2014). Different parameters, such as chemical oxygen demand (COD), turbidity, produce-to-water ratio, oxidation reduction potential (ORP) and ultraviolet absorbance measured at 254 nm (UV254), were used as organic load indicators of fresh produce wash water. However, these studies were designed mostly using only one type of fresh produce, recommendation on relation between wash water properties and organic load/chlorine demand may not be valid for other types of fresh produce wash water. In realistic situations, chemical compositions and reactivity of wash water can vary a lot due to different types of fresh produce, different degree of fresh produce maturity and environmental conditions during planting (Lehto et al. 2014). Therefore, the objectives of our study were to (1) determine the relationship between chlorine demand and produce wash water quality parameters, and (2) explore the feasibility of using wash water quality parameters to estimate chlorine demand for different types of fresh and fresh-cut produce.

2.2 Materials and Methods

2.2.1 Preparation of Simulated Produce Wash Waters

Ten different types of fresh produce (iceberg lettuce, romaine lettuce, spinach, celery, mushroom, broccoli, strawberry, grape, cantaloupe, and tomato) were selected based on their botanical classification, chemical composition, and popularity for regular consumption. Produce were purchased from local grocery stores (Griffin, GA, USA) and stored at 4^{0}C until use. Any wilted, discolored leaves or damaged produce were discarded. Iceberg lettuce, romaine lettuce, spinach, celery, mushroom, broccoli, cantaloupe and tomato were cut into small pieces with clean scissors or knives before use. Approximately 50 g of fresh or fresh-cut produce was placed in Whirl Pak® bags with an equal amount of deionized water (dH_2O). Samples were homogenized in a stomacher (Seward Stomacher®, 80 biomaster, Worthing, UK) for 120 s at 265 rpm, and filtered through filter papers (No. 4, Whatman^{TM}, Boca Raton, FL, USA). The COD of each filtered produce extract was determined, and subsequently, solution was diluted to ensure the COD of chlorinated wash water at approximately 50, 400 and 750 mg L^{-1} to simulate different organic loads in commercial fresh and fresh-cut produce wash waters. Spinach extract was adjusted to a COD of 50, 250 and 450 mg L^{-1}, and mushroom and broccoli to a COD of 50, 150, and 250 mg L^{-1}, to ensure that the chlorine demand at the highest COD would not exceed 100 mg L^{-1}.

2.2.2 Measurement of Chlorine Demand

Sodium hypochlorite (NaOCl) solution was prepared on the same day of the experiment by diluting 6 % regular bleach (RICCA Company, Arlington, TX, USA) in dH_2O to obtain NaOCl solution of 110 mg L^{-1} free chlorine. The free chlorine concentration was determined using DPD-FEAS titrimetric tests (Hach Company, Loveland, CO, USA). The pH of the solution was adjusted to 6.0 ± 0.2 using 1 N HCl and NaOH. After being stored at 4^0C for 2 h, 9 mL of NaOCl solution was mixed with 1 mL of produce wash water in a test tube for 5 min. Deionized water, instead of produce wash water, was used as the control treatment. The chlorine demand of produce wash water was calculated as the difference of residual free chlorine between control and chlorinated produce wash water.

2.2.3 Analysis of Produce Wash Water Quality

Produce wash water at different COD was diluted 10 times before measuring water quality parameters, so the organic load would be similar to the samples after mixing with chlorine in previous chlorine demand experiments. Wash water quality parameters including pH, ORP, UV254, COD, total phenolics content, total protein content, turbidity, and color difference between samples and dH_2O (ΔE) were determined. Specifically, pH and ORP were measured with a dual channel ACCUMET meter (Model # AR50, Fischer Scientific, Pittsburgh, PA, USA). UV254 was determined using a DU 520 UV-Vis spectrophotometer (Beckman Coulter Inc., Brea, CA, USA) after filtering wash water through a 0.45 µm pore-size filter (Waterman®, Baco Raton, FL, USA). COD was determined with a DR/90 colorimeter (HACH®, Loveland, CO USA) using a reactor digestion colorimetric method (Hach method 8000). Turbidity was measured using a DR/90 colorimeter (Hach method 8237) and expressed as Formazin Attenuation Unit (FAU).

Total phenolics content was determined using the Folin-Ciocalteu assay (Singleton and Rossi, 1965). Briefly, 1 mL of wash water was added to 9 mL of dH_2O in a test tube plus 1 mL of Folin-Ciocalteu's phenol reagent (Sigma-Aldrich Co., St Louis, MO, USA). After 5 min, 10 mL of 7 % Na_2CO_3 solution was added followed by diluting the mixture to 25 mL with dH_2O. After incubating for 90 min at room temperature (20 °C), solutions were read at 750 nm in a 1-cm cuvette with a DU 520 UV-Vis spectrophotometer (Beckman Coulter Inc.). Total phenolics content of wash water was reported as mg L^{-1} as gallic acid equivalents.

Total protein content was determined using the Bradford assay (Bradford, 1976). Briefly, 1 mL of wash water was mixed with 5 mL of Bradford's reagent (BioWorld, Dublin, OH, USA). After 5 min incubation, solutions were read at 595 nm in a 1-cm cuvette using a DU 520 UV-Vis spectrophotometer (Beckman Coulter Inc.). Total protein content of produce wash water was reported as mg L^{-1} as bovine serum albumen (BSA) equivalents.

The color of produce wash water was measured with a MiniScan XET^{M} colorimeter (Model# 45/0-L, Hunter Associates Laboratory, Reston, VA, USA). Briefly, 25 mL of produce wash water or dH_2O was placed in a 90 mm dia × 15 mm deep plastic petridish. A standard white tile was placed at the bottom of petridish as the background. The distance between colorimeter and surface of solution was adjusted to approximately 2 cm. Color parameters of L*, a* and b* were recorded, where L* represents color lightness, a* represents redness and b* represents yellowness. The ΔE (total color difference) between produce wash water (s) and dH_2O (w) was calculated using the following equation:

$$\Delta \mathrm{E} = \sqrt{(L_s^* - L_w^*)^2 + (a_s^* - a_w^*)^2 + (b_s^* - b_w^*)^2} \quad (1)$$

2.2.4 Chlorine Demand Prediction Equations

A key element in developing prediction equations that can be applied to various fresh produce was to identify the physiochemical parameters of wash water correlated with chlorine demand. Correlations between chlorine demand and water quality parameters were developed based on Pearson correlation coefficient and graphic inspection.

2.2.5 Model Verification

Two verification experiments were conducted on the usefulness of the chlorine demand prediction equations. The first experiment included six types of fresh produce (iceberg lettuce, romaine lettuce, spinach, mushroom, strawberry and tomato) that were the same as those used for model development, but diluted to different COD levels. The second verification test used three types of fresh produce (apple, romaine hearts, and a commercial mixed leafy vegetable salad) that were different from the ten types of fresh produce used for model development. Produce wash water was prepared and its quality parameters were measured following the same procedures as described above. Predictive accuracy of each sample and root mean squared prediction error (RMSPE) of each validation test were calculated.

2.2.6 Statistical Analysis

Two replicate samples from each type of fresh produce were used. Two to three measurements were made on each sample and the model development experiment was duplicated. Correlation and linear regression between chlorine demand and water quality parameters were analyzed using the Corr and REG procedures of SAS 9.4 (SAS Institute, Cary, NC, USA), respectively. The Student's t-test at the probability level of $P < 0.05$ was used for comparisons of means.

2.3 Results and Discussion

2.3.1 Correlation between Chlorine Demand and Wash Water Quality Parameters

The water quality parameters of each simulated fresh produce wash water at different COD are shown in Table 2-1. All produce wash waters were acidic (pH < 7) and had a measurable oxidation potential (ORP > 0 mV). For each type of fresh produce, the chlorine demand increased with increasing COD, total protein, total phenolics, UV254 and turbidity (Table 2-1). This may be the reason that these parameters were successfully used as organic load indicators to predict chlorine loss and microbial reduction in some produce washing studies reported in the literature (Pirovani et al. 2001; Van Haute et al. 2013; Shen et al. 2013; Zhou et al. 2014). However, our results showed that wash water properties varied a lot among different types of fresh produce. For example, although iceberg lettuce and romaine lettuce had similar chlorine demand at the highest organic load, romaine lettuce wash water contained three times higher protein and phenolics content than iceberg lettuce wash water. In addition, chlorine demand of different fresh produce wash water at COD 750 mg L^{-1} ranged from 12.00 mg L^{-1} (grape) to 97.38 mg L^{-1} (iceberg lettuce). Such large chlorine demand variance indicated that despite the wide use of COD as the organic load indicator for produce wash water (Zhou et al. 2014), COD alone may not correlate well to chlorine demand for all types of fresh produce reported in Table 2-1.

Correlations between wash water quality parameters and chlorine demand are presented in Table 2-2. UV254 had the highest correlation coefficient (R = 0.77), followed by total phenolics (R = 0.65) and total protein content (R = 0.64). As reported in the literature, the reactivity of HOCl with organic compounds and different functional groups from high to low is: reduced sulfur moieties > primary and secondary amines > phenols, tertiary amines » double bonds, other aromatics, carbonyls, and amides (Deborde and Gunten, 2008). Due to their high reactivity with chlorine, protein and phenolics were reported to cause significant available chlorine loss when contact with chlorine-based sanitizers (Waters and Hung, 2014). Because UV254 was also correlated with phenolics (R = 0.94) and protein content (R = 0.79) as indicated in Table 2-2, it has been widely used as an indicator to predict organic load and chlorine demand during drinking water and waste water disinfection (Fitzgerald et al. 2006). Our results further demonstrated that UV254 was correlated with chlorine demand of various fresh produce wash waters (Table 2-2). Therefore, UV254 was selected for further analysis.

2.3.2 Empirical equations for Predicting Chlorine Demand

Figure 2-1 shows the scatter plot of chlorine demand versus UV254. Although two variables were positively correlated, produce wash waters could be divided into two clusters. Cluster 1 included iceberg lettuce, mushroom, grape, celery, cantaloupe, broccoli, and tomato with a steep chlorine demand vs. UV254 correlation slope; Cluster 2 included romaine lettuce, spinach, and strawberry with a flat correlation slope. The R^2 between UV254 and chlorine demand for Cluster 1 and 2 was 0.89 and 0.97, respectively. By observing produce wash water quality parameters presented in Table 2-1, produce wash waters that belonged to Cluster 2 usually had higher phenolics content than that in Cluster 1 at a similar organic load (*P*

< 0.05). This is due to the fact that compounds with electron-rich functional groups (such as aromatic rings) absorb more light than others at the 254 nm wavelength. Therefore, at the similar chlorine demand, produce wash water with high phenolics content (Cluster 2) had higher UV254 absorbance than samples with low phenolics content (Cluster 1).

Waters and Hung (2014) reported that the chlorine demand of produce wash water is mainly contributed by phenolics and protein compounds. Since the phenolics content of produce in Cluster 2 was always higher than in Cluster 1 (Table 2-1), it is reasonable to assume that protein and phenolics compounds were the major components to react with free chlorine in Cluster 1 and 2, respectively. Under this assumption, the ratio of phenolics to protein content for phenolics-dominated produce in Cluster 2 should be higher than protein-dominated produce in Cluster 1, regardless of the organic load. However, the colorimetric assay of total protein content was interfered by the color of samples as supported by the high correlation coefficient between total protein content and ΔE ($R = 0.88$, Table 2-2). The (total protein/ΔE) therefore was a better parameter than total protein content, as measured by Bradford assay, to accurately relate chlorine demand with the active protein concentration in produce wash water. Eventually, the phenolics-to-protein/ΔE or PPC ratio was developed to classify the protein-dominated (Cluster 1) and phenolics-dominated (Cluster 2) produce in Table 2-1.

$$PPC = \frac{Total\ Phenolics}{(Total\ Protein/\Delta E)} \tag{2}$$

To determine the best threshold value of PPC, percentage correction (the percentage of samples that were distributed to each cluster) at threshold PPC from 0.1 to 1 is shown in Figure 2-2. At threshold PPC = 0.6, all produce wash waters studied had the highest percentage (85 %) of samples correctly classified, and percentage correction for both clusters was higher than 75 %. PPC values presented in Table 2-1 further demonstrated that the uncorrected classification only occurred at a low organic load of some produce. Therefore, equations to predict chlorine demand for each cluster were developed as follows:

$$\text{If PPC} < 0.6\text{, Chlorine demand} = 295.23\ \text{UV254} + 6.97 \text{ with } R^2 = 0.89 \qquad (3)$$

$$\text{If PPC} \geq 0.6\text{, Chlorine demand} = 119.77\ \text{UV254} + 2.41 \text{ with } R^2 = 0.97 \qquad (4)$$

2.3.3 Model Validation

The first validation test was conducted using the same types of produce used for model development, but at different COD levels (Table 2-3). Specifically, iceberg lettuce, mushroom, and tomato from Cluster 1 and strawberry, spinach, and romaine lettuce from Cluster 2 were used. Each fresh produce wash water was diluted to two COD levels that were different from those in the model development. For example, iceberg lettuce wash water was diluted to COD at 50, 400 and 750 mg L^{-1} for model development, while the COD levels for validation were 200 and 600 mg L^{-1}.

Measured UV254, total protein, total phenolics and ΔE are presented in Table 2-3. Except the tomato wash water at COD 200 mg L^{-1}, all the tested samples can be correctly distributed to each cluster based on their PPC value. Only 3 out of 12 samples had a predicted residual larger than l0 mg L^{-1}; iceberg lettuce at COD 600 mg L^{-1} and mushroom and spinach at COD 200 mg L^{-1}. The absolute value of percentage error of prediction for produce in Cluster 2 was usually smaller than that in Cluster 1, indicating that prediction equations had better predictive performance for Cluster 2. Besides the difference between clusters, there is no clear trend or difference due to the types of fresh produce or organic loads.

The predictive performance of equations was also validated using fresh produce which differed from those used in the model development (Table 2-4). Based on the calculated PPC value, apple and romaine hearts belonged to Cluster 1, and commercial mixed leafy salad belonged to Cluster 2. Predicted chlorine demand for these three produce wash waters was calculated based on the respective equations for their clusters. It is observed that all the samples had predicted residuals lower than 15 mg L^{-1}. Apple wash water at COD 200 mg L^{-1} had the highest prediction percentage error (96.49 %), however, the predicted residual was only 4.94 mg L^{-1}. Moreover, the RMSPE of two validation experiments was 11.3 and 8.16, respectively, which was close to the MSE for model development (8.23). Validation results indicate that predictive equations based on PPC and UV254 can predict chlorine demand of various fresh produce wash waters with different organic loads.

However, the reader should be cautioned about the limitation of current equations. The cluster selection and chlorine demand prediction equations were developed empirically based on tested samples. Therefore, it is possible that the current method to define the cluster of fresh produce may not be valid or consistent for other types of fresh produce not tested in the current study.

2.4 Conclusions

In conclusion, this study shows that UV254 had the highest correlation coefficient with chlorine demand among tested produce wash water quality parameters. Fresh produce was divided into two clusters based on their PPC values. Empirically developed equations based on UV254 had relatively good prediction accuracy on chlorine demand of various fresh produce wash waters. The results from this study provide a potential way for industry to predict appropriate amount of chlorine-based sanitizers for each type of produce they process. This will ensure sufficient amount of chlorine-based sanitizers are present during fresh and fresh-cut produce postharvest washing process to maintain safety and to avoid adding excess amount of sanitizers that leads to formation of disinfection by-products.

Acknowledgements

Funding for this study were provided by Agriculture and Food Research Initiative grant no 2014-67017-21558 from the USDA National Institute of Food and Agriculture and State and Hatch funds allocated to the University of Georgia Agricultural Experiment Station, Griffin Campus.

Table 2-1. Quality parameters of simulated fresh produce wash water at different organic loads [a].

Fresh produce	Organic load	COD (mg L^{-1})	Chlorine demand	pH	ORP (mV)	Protein (mg L^{-1})	Phenolics (mg L^{-1})	UV254	Turbidity (FAU)	ΔE	PPC [b]
Iceberg lettuce	Low	52.1	6.69	3.68	586.63	2.31	0.78	0.005	5.9	0.23	0.08
	Medium	403.0	46.19	4.69	531.86	10.90	2.35	0.084	25.4	1.22	0.26
	High	751.2	82.19	5.07	516.85	19.74	3.87	0.215	43.8	2.29	0.45
Mushroom	Low	65.1	13.13	4.87	489.68	4.39	0.85	0.041	9.6	0.34	0.11
	Medium	147.3	39.31	5.20	471.29	9.71	1.98	0.144	16.1	0.64	0.13
	High	227.3	66.50	5.32	454.25	13.57	3.03	0.227	23.9	1.22	0.27
Grape	Low	51.5	2.94	3.84	533.19	1.28	0.36	-0.003	0.6	0.38	0.39
	Medium	413.4	7.94	3.84	528.19	1.40	0.93	0.004	1.6	0.22	0.16
	High	725.0	12.63	3.77	527.88	2.08	1.37	0.012	3.1	0.15	0.11
Celery	Low	61.5	3.44	4.20	421.78	0.29	0.41	0.001	4.1	0.20	0.55
	Medium	394.4	15.56	5.01	364.13	5.23	1.44	0.049	20.0	0.61	0.16
	High	728.8	25.31	5.38	357.95	8.77	2.30	0.096	40.3	1.24	0.32
Cantaloupe	Low	48.0	4.44	4.22	542.61	0.57	0.93	0.002	0.0	0.16	0.71
	Medium	382.0	29.38	5.61	462.50	5.91	1.69	0.048	1.4	0.34	0.10
	High	727.3	55.19	6.29	428.75	10.23	2.64	0.095	6.4	0.64	0.16
Broccoli	Low	61.3	13.88	4.13	452.35	2.13	1.56	0.027	12.9	0.33	0.30
	Medium	156.6	40.19	4.57	420.85	11.93	3.30	0.095	34.3	0.67	0.18
	High	255.5	66.81	5.66	363.48	23.72	5.34	0.221	39.5	1.00	0.23
Tomato	Low	70.1	9.88	3.97	452.68	0.16	0.31	0.020	0.4	0.32	0.68
	Medium	412.3	57.38	4.31	383.51	1.50	1.83	0.169	2.0	0.27	0.53
	High	722.8	94.81	4.46	358.14	2.64	3.18	0.303	3.4	0.25	0.40

Romaine lettuce	Low	56.6	6.25	5.34	518.29	7.12	1.10	0.034	12.3	1.25	0.19
	Medium	401.4	42.06	6.20	460.20	26.79	5.88	0.330	62.9	9.80	2.18
	High	737.8	74.75	6.29	470.90	41.70	10.53	0.617	113.8	16.23	4.15
Spinach	Low	58.1	13.50	5.06	410.48	13.39	2.59	0.077	31.6	3.77	0.73
	Medium	212.4	52.81	6.15	342.63	31.81	8.92	0.350	94.0	13.63	3.82
	High	412.4	89.00	6.41	314.74	41.48	17.25	0.698	187.6	24.42	10.15
Strawberry	Low	61.6	4.56	4.06	452.88	2.08	1.20	0.030	3.0	0.35	0.20
	Medium	416.6	28.75	3.89	384.58	4.06	6.88	0.260	7.0	0.83	1.36
	High	764.8	55.31	3.87	363.35	5.39	12.59	0.488	11.3	1.00	2.29

[a] Data are presented as a mean of two samples.

[b] PPC = Total phenolics/(Total protein/ΔE).

Table 2-2. Pearson correlation of chlorine demand and water quality parameters.

	Chlorine demand (mg L^{-1})	pH	ORP (mV)	Protein (mg L^{-1})	Phenolics (mg L^{-1})	UV254	COD (mg L^{-1})	Turbidity (FAU)	ΔE
Chlorine demand	1.00	0.52	-0.43	0.64	0.65	0.77	0.59	0.55	0.48
pH		1.00	-0.34	0.78	0.46	0.52	0.23	0.68	0.64
ORP			1.00	-0.31	-0.49	-0.47	-0.25	-0.35	-0.31
Protein				1.00	0.76	0.79	0.26	0.92	0.88
Phenolics					1.00	0.94	0.40	0.81	0.81
UV254						1.00	0.48	0.79	0.80
COD							1.00	0.21	0.17
Turbidity								1.00	0.96
ΔE									1.00

Table 2-3. Prediction of chlorine demand of produce wash water used for model development but at different COD.

Fresh produce (Cluster #)	Targeted COD (mg L^{-1})	UV254	Protein (mg L^{-1})	Phenolics (mg L^{-1})	ΔE	PPC	Observed chlorine demand	Predicted chlorine demand	Predicted residual [a] (mg L^{-1})	Prediction percentage error [b]
Iceberg lettuce (1)	200	0.047	5.32	1.20	0.57	0.13	26.13	20.70	-5.43	-20.77
	600	0.142	13.68	2.57	1.20	0.23	71.88	48.97	-22.91	-31.87
Mushroom (1)	100	0.080	5.92	1.44	0.51	0.12	22.75	30.66	7.91	34.78
	200	0.192	11.84	2.37	0.80	0.16	42.75	63.65	20.90	48.90
Tomato (1)	200	0.101	0.36	1.29	0.20	0.71	28.00	36.86	8.86	31.65
	600	0.290	3.18	2.91	0.31	0.28	83.38	92.51	9.14	10.96
Spinach (2)	200	0.251	33.11	7.62	12.6	2.90	46.50	32.50	-14.00	-30.10
	600	0.743	52.39	22.18	30.1	12.7	92.88	91.40	-1.47	-1.59
Romaine lettuce (2)	200	0.137	18.82	2.86	4.69	0.71	17.13	18.76	1.63	9.54
	600	0.424	29.64	7.52	13.1	3.33	48.25	53.13	4.88	10.12
Strawberry (2)	200	0.097	0.17	2.91	0.39	6.73	16.25	14.03	-2.22	-13.67
	600	0.312	2.93	8.35	0.55	1.57	49.13	39.81	-9.32	-18.96

[a] Predicted residual = Predicted chlorine demand – Observed chlorine demand.

[b] Prediction percentage error = (Prediction error)/(Observed chlorine demand) × 100.

Table 2-4. Prediction of chlorine demand of produce wash water using different produce from model development.

Fresh produce (Cluster #)	Targeted COD (mg L^{-1})	UV254	Protein (mg L^{-1})	Phenolics (mg L^{-1})	ΔE	PPC	Observed chlorine demand	Predicted chlorine demand	Predicted residual (mg L^{-1})	Prediction percentage error
Apple (1)	200	0.011	1.14	1.29	0.17	0.20	5.13	10.07	4.94	96.49
	600	0.039	3.18	3.01	0.59	0.56	13.50	18.34	4.84	35.82
Romaine hearts (1)	200	0.089	7.58	1.64	0.50	0.11	24.13	33.10	8.97	37.19
	600	0.256	19.24	3.65	1.47	0.28	75.75	82.40	6.65	8.78
Mixed leafy (2)	100	0.170	22.32	4.14	5.72	1.06	25.13	22.71	-2.41	-9.61
	300	0.495	39.58	11.25	15.51	4.40	76.50	61.64	-14.86	-19.43

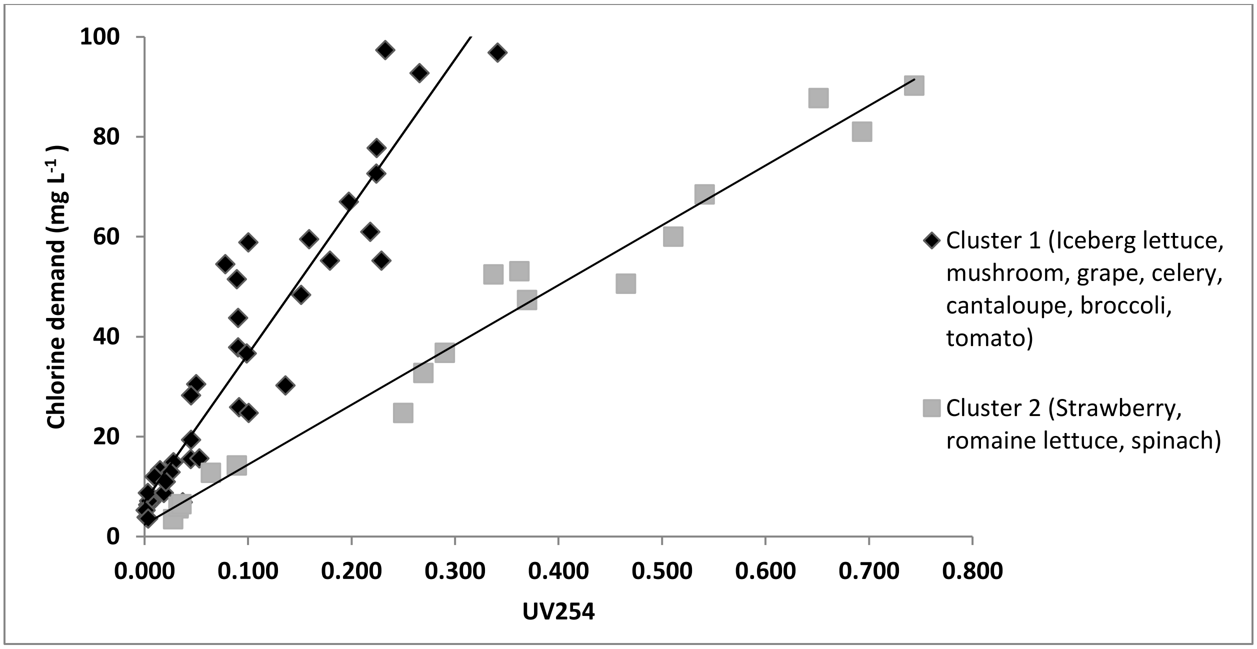

Figure 2-1. Scatter plot of chlorine demand of produce wash water at different UV254.

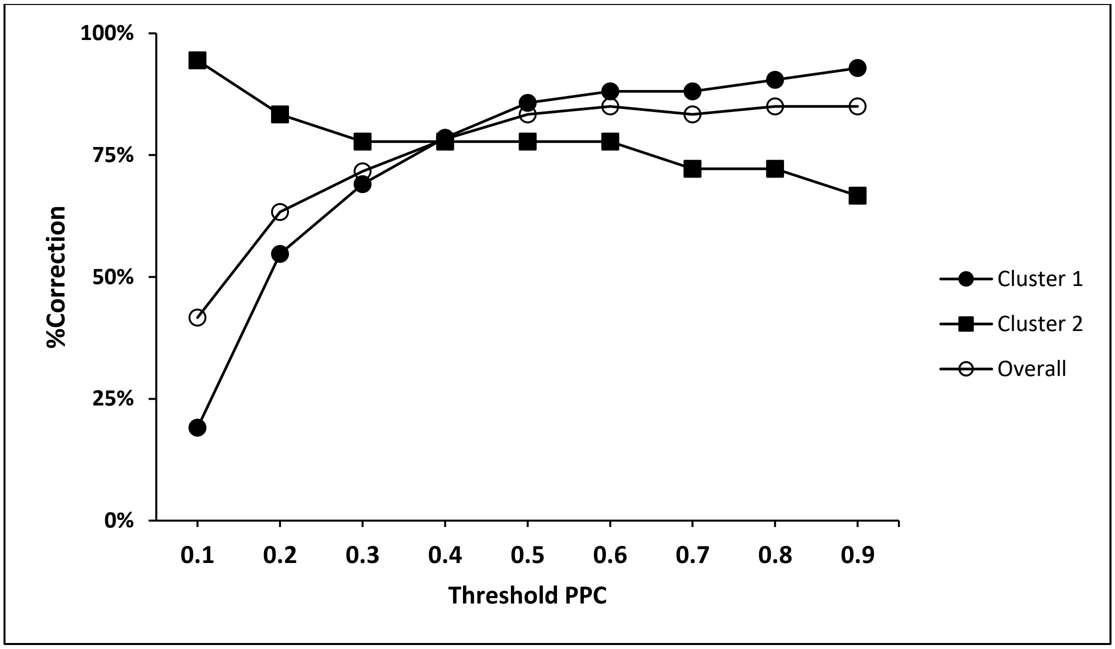

Figure 2-2. Percentage of correct classification of produce wash water to two clusters based on different threshold PPC value.

References

Artes, F., Gomez, P., Aguayo, E., Escalona, V., & Artes-Hernandez, F. (2009). Sustainable sanitation techniques for keeping quality and safety of fresh-cut plant commodities. *Postharvest Biology and Technology, 51*(3), 287-296.

Barrera, M. J., Blenkinsop, R., & Warriner, K. (2012). The effect of different processing parameters on the efficacy of commercial post-harvest washing of minimally processed spinach and shredded lettuce. *Food Control*, *25*(2), 745-751.

Beuchat, L. R., Adler, B. B., & Lang, M. M. (2004). Efficacy of chlorine and a peroxyacetic acid sanitizer in killing *Listeria monocytogenes* on iceberg and romaine lettuce using simulated commercial processing conditions. *Journal of Food Protection*, *67*(6), 1238-1242.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*, 248-254.

CSPI (Center for Science in the Public Interest) (2009). Outbreak alert. Analyzing foodborne outbreaks 1998 to 2007. https://cspinet.org/new/pdf/outbreakalertreport09.pdf. Accessed 23 July 2015.

Deborde, M., & von Gunten, U. (2008). Reactions of chlorine with inorganic and organic compounds during water treatment - Kinetics and mechanisms: A critical review. *Water Research*, *42*(1-2), 13-51.

Fitzgerald, F., Chow, C. W. K., & Holmes M. (2006). Disinfectant demand prediction using surrogate parameters – a tool to improve disinfection control. *Journal of*

Water Supply: Research and Technology, *55*(6), 391-400.

Gil, M. I., Gomez-Lopez, V. M., Hung, Y.-C., & Allende, A. (2015). Potential of electrolyzed water as an alternative disinfectant agent in the fresh-cut industry. *Food and Bioprocess Technology*, *8*(6), 1336-1348.

Gil, M. I., Selma, M. V., Lopez-Galvez, F., & Allende, A. (2009). Fresh-cut product sanitation and wash water disinfection: Problems and solutions. *International Journal of Food Microbiology, 134*(1-2), 37-45.

Hach Method 8000. (2014). Oxygen Demand, Chemical-Reactor Digestion Method 8000.

Hach Method 8237. (2013). Turbidity, Absorptometric Method 8237.

Lehto, M., Sipila, I., Alakukku, L., & Kymalainen, H.-R. (2014). Water consumption and wastewaters in fresh-cut vegetable production. *Agricultural and Food Science*, *23*(4), 246-256.

Lu, Y., Joerger, R., & Wu, C. (2014). Similar Reduction of *Salmonella enterica* Typhimurium on grape tomatoes and its cross-contamination in wash water by washing with natural antimicrobials as compared with chlorine treatment. *Food and Bioprocess Technology*, *7*(3), 661-670.

Luo, Y., Nou, X., Yang, Y., Alegre, I., Turner, E., Feng, H., Abadias, M., & Conway, W. (2011). Determination of free chlorine concentrations needed to prevent *Escherichia coli* O157:H7 cross-contamination during fresh-cut produce wash. *Journal of Food Protection*, *74*(3), 352-358.

Olaimat, A. N., & Holley, R. A. (2012). Factors influencing the microbial safety of fresh produce: A review. *Food Microbiology, 32*(1), 1-19.

Pirovani, M. E., Guemes, D. R., & Piagnetini, A. M. (2001). Predictive models for available chlorine depletion and total microbial count reduction during washing of fresh-cut spinach. *Journal of Food Science*, *66*(6), 860-864.

Shen, C., Luo, Y., Nou, X., Wang, Q., & Millner, P. (2013). Dynamic effects of free chlorine concentration, organic load, and exposure time on the inactivation of *Salmonella*, *Escherichia coli* O157:H7, and Non-O157 shiga toxin-producing *E. coli*. *Journal of Food Protection*, *76*(3), 386-393.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*(3), 144-158.

Sivapalasingam, S., Friedman, C. R., Cohen, L., & Tauxe, R. V. (2004). Fresh produce: A growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *Journal of Food Protection*, *67*(10), 2342-2353.

Van Haute, S., Sampers, I., Holvoet, K., & Uyttendaele, M. (2013). Physicochemical quality and chemical safety of chlorine as a reconditioning agent and wash water disinfectant for fresh-cut lettuce washing. *Applied and Environmental Microbiology*, *79*(9), 2850-2861.

Waters, B. W., & Hung, Y.-C. (2014). The effect of organic loads on stability of various chlorine-based sanitisers. *International Journal of Food Science and Technology*, *49*(3), 867-875.

Zhou, B., Luo, Y., Nou, X., & Millner, P. (2014). Development of an algorithm for feed-forward chlorine dosing of lettuce wash operations and correlation of chlorine profile with *Escherichia coli* O157:H7 inactivation. *Journal of Food Protection*, *77*(4), 558-566.

CHAPTER 3

EFFECTS OF ORGANIC LOAD, SANITIZER PH AND INITIAL CHLORINE CONCENTRATION OF CHLORINE-BASED SANITIZERS ON CHLORINE DEMAND OF FRESH PRODUCE WASH WATERS

Chen, X., & Hung, Y.-C. (2017). *Food Control*, 77, 96-101. Reprinted here with permission of the publisher.

Abstract

Chlorine-based sanitizers are widely used in fresh produce industry. However, maintaining sufficient free chlorine residual in wash solution to inactivate foodborne pathogens in the presence of organic matter is a challenge. The purpose of this study was to evaluate the effects of organic load, sanitizer pH and initial chlorine concentration on chlorine demand of fresh produce wash water. A full factorial design was used to study the chlorine demand of romaine lettuce wash water with different organic load when reacting with NaOCl solution at different pH (2.5, 4.0, 6.0, 8.0 or 9.5) and initial chlorine concentration (50, 75 or 100 mg L^{-1} free chlorine residual). Chlorine demand of lettuce wash water significantly increased ($P \leq 0.05$) with increasing organic load. The significant effect ($P \leq 0.05$) of initial chlorine concentration on chlorine demand was only detected at high organic load. Increasing the pH of NaOCl from 2.5 to 9.5 led to decrease in the chlorine demand except slight increase from 6.0 to 8.0. Equations for predicting chlorine demand of various fresh produce wash waters at different organic load for different sanitizer pH and initial chlorine concentration were developed, and verified using four types of produce (romaine lettuce, iceberg lettuce, strawberry and grape) and two types of chlorine-based sanitizers (NaOCl and electrolyzed water). Organic load, sanitizer pH, and initial chlorine concentration all affected the chlorine demand of produce wash water. Chlorine demand prediction equations developed can be used for different types of fresh produce and chlorine-based sanitizers.

Key Words: Produce, chlorine demand, prediction equation, pH, UV254

3.1 Introduction

Fresh fruits and vegetables without sufficient heat treatment may be a vehicle for foodborne illnesses. It was estimated that produce-associated foodborne outbreaks caused approximately $ 1.4 billion economic losses and over 1 million illnesses per year in the US (Batz, Hoffmann, & Morris, 2011). In addition, the Centers for Disease Control and Prevention (CDC, 2014) reported that produce was responsible for 27 % of outbreaks and 40 % of foodborne illnesses in 2014. Therefore, an effective washing process during produce postharvest handling is a critical step to ensure the safety of fresh produce (Gil, Gomez-Lopez, Hung, & Allende, 2015).

Chlorine-based sanitizers such as sodium hypochlorite (NaOCl) have been widely applied as disinfectants in fresh and fresh-cut produce postharvest washing process in the US (Luo et al., 2011). Previous study showed that the antimicrobial efficacy was largely depended on the free chlorine residual in processing water (Gil, Selma, Lopez-Galvez, & Allende, 2009). On the other hand, excess amount of free chlorine may react with organic compounds in produce wash water to generate carcinogenic halogenated disinfection by-products (DBPs), such as trihalomethanes (THMs) (Fan & Sokorai, 2015). Some countries in the European Union even prohibit the use of chlorine for washing fresh produce partially due to potential health risk associated with DBPs (Van Haute, Sampers, Holvoet, & Uyttendaele, 2013).

Tradeoffs between maintaining the disinfection efficacy and forming hazardous DBPs encourage the development and use of models to predict “chlorine demand”, which is the chlorine loss as a consequence of reacting with organic compounds (Pirovani, Guemes, & Piagnetini, 2001; Waters & Hung, 2014; Zhou, Luo, Nou, & Millner, 2014).

Our previous study developed equations to predict chlorine demand (NaOCl) of various produce wash waters based on the ultraviolet light absorbance of filtered produce wash water at 254 nm (UV254) (Chen & Hung, 2016). We found that UV254 had a higher correlation coefficient with chlorine demand than other water quality parameters such as chemical oxygen demand (COD) or turbidity. However, these equations can only be applied at a specific condition (i.e. non-buffered NaOCl solution at 100 mg L^{-1} free chlorine concentration and pH 6). In practice, buffering agents are commonly added into the processing water (Fan & Sokorai, 2015). In addition, the pH of chlorine-based sanitizers may vary from 2.5, e.g. acidic electrolyzed (EO) water, to 9.5, e.g. NaOCl with stabilizer (Huang, Hung, Hsu, Huang, & Hwang, 2008). Initial free chlorine concentration is usually in the range of 50 to 100 mg L^{-1} but may vary from one processor to another.

Therefore, the overall objective of this study was to evaluate the effects of sanitizer pH and initial chlorine concentration of NaOCl on chlorine demand of different fresh produce wash waters at different organic load. Specific objectives include the following:

i) to determine the effect of phosphate buffer on chlorine demand
ii) to determine the effects of organic load, sanitizer pH and initial chlorine concertation on chlorine demand of romaine lettuce wash water
iii) to develop prediction equations to estimate chlorine demand of various fresh produce wash waters at different organic load, sanitizer pH and initial chlorine concertation

iv) to validate developed equations using different types of fresh produce, chlorine-based sanitizers and processing conditions.

3.2 Materials and methods

3.2.1 Preparation of NaOCl solutions

NaOCl solution at different free chlorine concentration was prepared by diluting 6 % bleach (RICCA Company, Arlington, TX, USA) in deionized water (dH_2O). Free chlorine concentration was measured by a DPD-FEAS titrimetric method (Hach Company, Loveland, CO, USA). A dual channel ACCUMET pH meter (Model # AR50, Fisher Scientific, Pittsburgh, PA, USA) with appropriate electrode was used to measure the pH of NaOCl solution. The pH of NaOCl solution with or without 10 mmol L^{-1} phosphate buffers was adjusted using 1 mol L^{-1} phosphoric acid/NaOH or 1 mol L^{-1} HCl/NaOH, respectively.

3.2.2 Preparation of romaine lettuce wash water

Romaine lettuce (*Lactuca sative*) was purchased from a local grocery store in Griffin, GA, stored at 4 °C, and used within 48 h. Lettuce was cut into small pieces (3×3 cm^2) and homogenized with dH_2O in a stomacher (Seward Stomacher®, 80 biomaster, Worthing, UK) at 265 rpm for 120 s. Lettuce extract was then filtered through filter papers (No. 4, Whatman^{TM}, Boca Raton, FL, USA) and diluted to targeted organic load with dH_2O. For this study, standard chlorine demand (D_{ST}) expressed as the chlorine loss when 1 mL of produce wash water reacts with 9 mL of NaOCl solution containing 110 mg L^{-1} free chlorine at pH 6.0 for 5 min was used as the organic load indicator. The reaction time was set at 5 min, since practical produce washing time is usually several minutes, and more than 60 % of chlorine demand of romaine lettuce wash water occurred within first 5 min over a 90-min observation period (Weng et al., 2016).

3.2.3 Effect of buffering agent on chlorine demand

NaOCl solutions at 110 mg L^{-1} initial chlorine concentration were adjusted to pH 2.5, 6.0 and 9.5 with or without buffering agent, and stored at 4 °C for 2 h before use. A 1 mL of dH_2O (control) or romaine lettuce wash water at organic load of D_{ST} 35 mg L^{-1} was mixed with 9 mL of NaOCl solution with or without buffering capacity for 5 min. The pH and residual free chlorine of NaOCl solution before and after reacting with romaine lettuce wash water were measured. Chlorine demand of produce wash water was calculated as the difference of residual free chlorine between control and lettuce wash water after the treatment.

3.2.4 Determination of chlorine demand of romaine lettuce wash water at different organic load, sanitizer pH and initial chlorine concentration

A three-factor design (organic load, sanitizer pH and initial chlorine concentration) was used. Romaine lettuce wash waters at organic load of D_{ST} 20, 40 and 60 mg L^{-1} were prepared. Initial chlorine concentration of NaOCl solution was adjusted to 50, 75 and 100 mg L^{-1}. The pH of NaOCl solution was adjusted to 2.5, 4.0, 6.0, 8.0 and 9.5 using 1 mol L^{-1} HCl/NaOH. The pH and oxidation-reduction potential (ORP) of NaOCl solution were measured with a dual channel ACCUMET meter (Fisher Scientific) with appropriate probes. After 1 mL of lettuce wash water was added to 9 mL of chilled (4 °C) NaOCl solution for 5 min, chlorine demand of the respective solution was determined as described before.

A multiple regression model was used to identify the relation between response variable (D, chlorine demand of lettuce wash water) with three independent variables.

$$D = f\,(OL, pH, C) \qquad (1)$$

where OL is the organic load as the standard chlorine demand, pH is the sanitizer pH and C is the initial chlorine concentration.

3.2.5 Procedure for model validation

In order to validate developed equations, two validation experiments using various produce wash waters at low and high organic load were selected. For each experiment, four types of fresh produce including iceberg lettuce (*Lactuca sativa*), grape (*Vitis labrusca*), romaine lettuce (*Lactuca sative*) and strawberry (*Fragaria × ananassa*) were used. Iceberg lettuce and romaine lettuce were cut into 3 × 3 cm^2 pieces before use. Whole, undamaged strawberries and grapes were selected. Produce was then homogenized with dH_2O and filtered through filter papers (No. 4, Whatman^{TM}). Produce wash waters were adjusted to D_{ST} 30 and 50 mg L^{-1} for low and high organic load verification experiments, respectively.

Both NaOCl and EO water were used for validation experiments. NaOCl solution at 110 mg L^{-1} initial chlorine concentration was prepared by diluting 6 % bleach. EO water was prepared by electrolyzing 0.03 % NaCl solution using an EO water generator (Model #P30HST44T, EAU, GA, USA) and diluted to 110 mg L^{-1} initial chlorine concentration. The pH of NaOCl and EO water were adjusted to 2.5, 6.0 and 8.0 using 1 mol L^{-1} HCl/NaOH. Chlorine demand of different produce wash waters when reacting with NaOCl solution and EO water was determined after 5 min of contact time.

3.2.6 Wash water properties determination

Produce wash water at organic load of D_{ST} 30 and 50 mg L^{-1} was diluted 10 times in dH_2O before measuring water quality parameters, so the concentration of organic compounds would be similar to the samples after mixing with NaOCl solution. Water quality parameters, i.e. UV254, total phenolics content, total protein content and color were measured. UV254 was measured using a DU 520 UV-Vis spectrophotometer (Beckman Coulter Inc., Brea, CA, USA) after filtering wash water through a 0.45 µm pore-size filter (Waterman®, Boca Raton, FL, USA). Total phenolics content was measured by the Folin-Ciocalteu assay and expressed as mg L^{-1} gallic acid equivalents (Singleton & Rossi, 1965). Total protein content was measured by the Bradford assay and expressed as mg L^{-1} bovine serum albumen equivalents (Bradford, 1976).

The color of produce wash water was measured by following the methodology of Chen and Hung (2016) using a MiniScan XET^{M} colorimeter (Model# 45/0-L, Hunter Associates Laboratory, Reston, VA, USA). Briefly, 25 mL of produce wash water or dH_2O was placed in a 90 mm dia × 15 mm deep plastic petridish with a standard white tile as the background. The distance between colorimeter and sample surface was adjusted to 2 cm. The lightness (L*), red-green (a*) and yellow-blue (b*) values of each sample were recorded. The color difference (ΔE) between produce wash water (s) and dH_2O (w) was calculated as follows:

$$\Delta E = \sqrt{(L_s^* - L_w^*)^2 + (a_s^* - a_w^*)^2 + (b_s^* - b_w^*)^2} \qquad (2)$$

3.2.7 Statistical analysis

Two measurements were made on each sample, and the entire experiment was repeated in triplicate. Data was subjected to analysis using the SAS 9.4 program (SAS

Institute, Cary, NC). Student's T-test and least significant difference of means (LSD) test were used for pairwise and multiple comparisons, respectively. Regression equation was developed using the backward elimination of the REG procedure. All the tests were performed with a level of significance of 0.05.

3.3 Results and Discussion

3.3.1 Effect of buffering agent on chlorine demand

Table 3-1 shows the effect of buffering agent on the pH of NaOCl solution when reacting with romaine lettuce wash water. The only significant change ($P \leq 0.05$) of NaOCl pH before and after the reaction was detected in non-buffered treatment at pH 6.0, which was decreased from 6.10 to 5.15. Previous study showed that the pH of romaine lettuce wash water was around 6.0 (Chen & Hung, 2016). The decline of NaOCl pH can be explained by the chlorination breakpoint (Yu, 2004; Zhou, Luo, Nou, & Millner, 2014), in which the ammonia and other nitrogen-containing compounds in produce wash water is oxidized by HOCl to eventually form nitrogen gas as follow:

$$2\ NH_4^+ + 3\ HOCl \longrightarrow N_2 + 3\ H_2O + 5\ H^+ + 3\ Cl^- \qquad (3)$$

According to eqn (3), 5 mol of hydrogen ions are generated when 3 mol of HOCl (including 3 mol of hydrogen ions) are consumed. A small amount of hydrogen ions generated from this reaction would not significantly ($P > 0.05$) change the pH of NaOCl solution when hydrogen ion concentration was high (pH 2.5) or hydroxyl ion concentration was high (pH 9.5). However, the addition of hydrogen ion affected the pH of NaOCl solution at pH 6.0 when it was not buffered. Results from Table 3-1 also showed that there was no significant difference ($P > 0.05$) in pH for buffered NaOCl solution before and after reacting with the lettuce wash water at any pH, indicating that the buffering agent helped to improve the resistance of NaOCl solution to pH change.

The effect of sanitizer pH and buffering agent on the chlorine demand of lettuce wash water is also shown on Table 3-1. The chlorine demand of lettuce wash water decreased from about 46 mg L^{-1} at pH 2.5 to about 26 mg L^{-1} at pH 9.5, indicating a significant effect of sanitizer pH on chlorine demand ($P \leq 0.05$). This is an expected outcome because pH was reported to affect the reactivity and microbicidal efficacy of chlorine-based sanitizers in previous studies (Waters & Hung, 2014; Zhou, Luo, Nou, & Millner, 2014). However, there is no significant difference ($P > 0.05$) on chlorine demand between buffered and non-buffered NaOCl solutions at the same pH. Since the buffering capacity did not show an effect on the chlorine demand of produce wash water as demonstrated in Table 3-1, HCl/NaOH were selected to adjust the pH of chlorine-based sanitizers in subsequent experiments to be consistent with our previous study (Chen & Hung, 2016).

3.3.2 Effects of organic load, initial chlorine concentration and sanitizer pH on chlorine demand

The chlorine demand of romaine lettuce wash water at different organic load, initial chlorine concentration and sanitizer pH are shown in Table 3-2. Overall, all the three tested parameters significantly ($P \leq 0.05$) affected the chlorine demand of wash water samples. Specifically, the chlorine demand increased with increasing organic load. For example, at sanitizer pH 6.0 and initial chlorine concentration of 75 mg L^{-1}, the chlorine demand of lettuce wash water with organic load of D_{ST} 20 mg L^{-1} was 18.33, mg L^{-1}, which further increased to 36.92 and 52.33 mg L^{-1} at D_{ST} 40 and 60 mg L^{-1}, respectively. Meanwhile, the initial chlorine concentration effect on chlorine demand is more pronounced at high organic load. For example, at D_{ST} 60 mg L^{-1}, the higher the initial chlorine concentration the higher the chlorine demand regardless of the NaOCl pH ($P \leq 0.05$) except for pH 9.5. For organic load of D_{ST} 40 mg L^{-1}, only significant difference ($P \leq 0.05$) in the chlorine demand was between initial chlorine concentration of 50 and 100 mg L^{-1} except for pH 9.5. However, no statistical difference ($P > 0.05$) was detected of chlorine demand among different initial chlorine concentrations at organic load of D_{ST} 20 mg L^{-1} and same pH. The correlation result is consistent with previous findings that the overall kinetic of chlorination reaction is second order with a first order reaction with free chlorine concentration and another first order reaction with total organic compound concentration (Deborde & von Gunten, 2008).

In terms of the sanitizer pH effect, increasing the pH of NaOCl solution from 2.5 to 9.5 led to significant decrease ($P \leq 0.05$) in the chlorine demand of samples, except slight increase ($P > 0.05$) in the chlorine demand from pH 6.0 to 8.0 (Table 3-2). Generally, there are two kinds of chlorination reactions that can be affected by the pH of chlorinated water: oxidation-reduction (redox) reaction and electrophilic substitution reaction (Deborde & von Gunten, 2008). The redox reaction rate decreases with increasing pH due to the decreasing oxidation-reduction potential (ORP). For example, the ORP of chlorinated water samples was 1184.3 mV at pH 2.57, while it significantly ($P \leq 0.05$) decreased to 731.7 mv at pH 9.49 (Table 3-3). Therefore, the decrease of chlorine demand in general from pH 2.5 to 9.5 indicated that the redox reaction was the dominated reaction for organic compounds in produce wash water with chlorine for a wide pH range. On the other side, the electrophilic substitution reaction has higher reaction rate constant at alkaline pH (Criquet et al., 2015), which may be the reason of chlorine demand hump from pH 6.0 to 8.0. Our results were similar to previous study on natural water that Fleischacker and Randtke (1983) reported that nonpurgeable organic chlorine (NPOCl) formation in public water supplies decreased dramatically as pH increased from 3 to 11. Meanwhile, in their study the concentration of some purgeable organic chlorine (POCl) such as chloroform ($CHCl_3$), which is the product of electrophilic substitution reactions, increased at the same time. Due to the fact that the generation rate of NPOCl was higher than POCl, the overall amount of generated chlorinated organic compounds reduced with increasing pH.

3.3.3 Chlorine demand model development

Although linear and quadratic equations were commonly used in model development studies (Pirovani, Guemes, & Piagnetini, 2001; Waters & Hung, 2014; Zhou, Luo, Nou, & Millner, 2014), the chlorine demand at different sanitizer pH as reported in Table 3-2 (which decreased from pH 2.5 to 6.0, then slightly increased from 6.0 to 8.0, and decreased again from 8.0 to 9.5) indicated that the cubic term of pH should also be considered. Therefore, the linear and quadratic terms of organic load and initial chlorine concentration, and the linear, quadratic and cubic terms of sanitizer pH were included for model development. Prediction equation of chlorine demand based on the backward elimination is presented as eqn (4).

$$D = 40.92 - 30\,pH + 1.19\,D_{ST} + 0.18\,C + 5.14\,pH^2 - 0.005\,D_{ST}^2 - 0.28\,pH^3 \qquad (4)$$

where D is the chlorine demand of lettuce wash water, pH is the initial pH of NaOCl solution, D_{ST} is the standard chlorine demand as the organic load indicator and C is the initial chlorine concentration. The adjusted R^2 and root mean squared error (RMSE) of eqn (4) were 0.94 and 3.68 mg L^{-1}, respectively.

As indicated in our previous study (Chen & Hung, 2016), chlorine demand for different types of fresh produce can be classified into two clusters based on the PPC ratio.

$$\text{PPC} = \text{Phenolics} / (\text{Protein} / \Delta E) \tag{5}$$

where Phenolics is the total phenolics content, Protein is the total protein content, and ΔE is the color difference between produce wash water and dH_2O as calculated based on eqn (2). Generally, produce wash waters with low phenolics-to-protein ratio will have PPC value lower than 0.6, while produce wash waters with high phenolics-to-protein ratio will have PPC value higher than 0.6. From Chen and Hung (2016), the D_{ST} of various produce wash waters can be predicted based on the UV254 (UV) as follows:

$$\text{If PPC} < 0.6,\ D_{ST} = 6.97 + 295.23\,\text{UV} \tag{6}$$

$$\text{If PPC} \geq 0.6,\ D_{ST} = 2.41 + 119.77\,\text{UV} \tag{7}$$

Since the D_{ST} in eqn (4) (6) and (7) are the same, we can replace D_{ST} in eqn (4) by eqn (6) and (7) to obtain the following two cubic prediction equations:

For PPC value < 0.6,

$$D = 48.97 - 30\,\text{pH} + 330.74\,\text{UV} + 0.18\,C + 5.14\,\text{pH}^2 - 435.8\,\text{UV}^2 - 0.28\,\text{pH}^3 \tag{8}$$

For PPC value ≥ 0.6,

$$D = 43.76 - 30\,\text{pH} + 139.64\,\text{UV} + 0.18\,C + 5.14\,\text{pH}^2 - 71.72\,\text{UV}^2 - 0.28\,\text{pH}^3 \tag{9}$$

UV254 was well correlated with chlorine demand regardless of types of produce as reported in Chen and Hung (2016). Therefore, eqn (8) and (9) using UV254 as the organic load indicator can be used to predict chlorine demand of various produce wash waters.

3.3.4 Model validation using various produce wash waters and chlorine-based sanitizers

The first step to predict chlorine demand is to determine the PPC value of produce wash water. Water properties of various produce wash waters at D_{ST} 30 and 50 mg L^{-1} are shown in Tables 3-4 and 3-5, respectively. Based on the measured total phenolics content, total protein content and ΔE value (data not shown), the PPC value of each sample was calculated and presented in Tables 3-4 and 3-5. Out of four different types of produce selected, iceberg lettuce and grape wash water had a PPC value lower than 0.6; and romaine lettuce and strawberry wash water had a PPC value higher than 0.6. Therefore, the predicted chlorine demand of iceberg lettuce and grape wash water was calculated using prediction equation for PPC lower than 0.6, and chlorine demand of romaine lettuce and strawberry wash water was calculated using prediction equation for PPC higher than 0.6.

The predicted and observed chlorine demand of various produce wash waters at D_{ST} 30 mg L^{-1} for NaOCl and EO water are presented in Table 3-4. As expected, both predicted and observed chlorine demand dropped as sanitizer pH increased from 2.5 to 6.0, and then raised from pH 6.0 to 8.0. Meanwhile, there was no significant difference ($P > 0.05$) of observed chlorine demand between NaOCl and EO water at the same pH. This finding is consistent with the previous findings that NaOCl and EO water at the same pH had similar $HOCl/OCl^-$ ratio and reactivity (Park, Hung, Doyle, Ezeike, & Kim, 2001; Waters & Hung, 2013). In terms of the prediction accuracy, 71 % of samples had prediction percentage error smaller than ± 25 %. No clear trend of prediction percentage error as affected by the sanitizer pH or types of sanitizer was found.

Similar results were also observed from validation experiment using produce wash waters at D_{ST} 50 mg L^{-1} (Table 3-5). Each type of produce had the highest chlorine demand when reacting with sanitizers at pH 2.5, followed by pH 8.0 and 6.0. No significant difference ($P > 0.05$) on chlorine demand between NaOCl and EO water at the same pH was detected. In addition, 75 % of samples had prediction percentage error smaller than ± 25 %, and no one had percentage error larger than ± 30 %. The RMSE of validation experiments using produce wash waters at D_{ST} 30 and 50 mg L^{-1} was 9.06 and 10.76 mg L^{-1}, respectively. Validation results indicated that prediction equations had consistent prediction performance for different types of fresh produce and chlorine-based sanitizers.

One major difference between our study and previous ones is that we used UV254 to predict chlorine demand. Currently some commercial produce washing systems rely on ORP and pH to monitor chlorine residual, while the reliability of these parameters are questionable (Zhou, Luo, Nou, & Millner, 2014). Moreover, compared with the DPD-FEAS method, the measurement of UV254 does not require additional reagent or titration process, which makes it more suitable for automatic on-line measurement. Besides these, UV254 is correlated with phenolics compounds, which are the important disinfection by-product (DBP) precursors in produce wash water (Deborde & von Gunten, 2008). Through measuring UV254, organic load and DBPs formation potential can be monitored simultaneously. Therefore, chlorine demand prediction equations using UV254 as the organic load indicator has promising potential in fresh produce washing process.

This study shows that chlorine demand was affected by the processing conditions such as pH and free chlorine concentration. Chlorine demand was reported to be closely related to the formation of DBPs (Chang, Chiang, Chao, & Lin, 2006). Therefore, maintaining the near-neutral pH of chlorine-based sanitizers and reducing free chlorine concentration can decrease the chlorine demand and the DBPs formation potential of produce wash water. Future studies will be conducted to identify the optimal chlorine residual with high disinfection efficacy and minimized DBPs formation potential, and to apply developed equations into practical fresh produce washing systems.

3.4 Conclusions

In conclusion, there is no significant ($P > 0.05$) effect of buffering capacity of NaOCl solution on chlorine demand of romaine lettuce wash water. Organic load, sanitizer pH and initial chlorine concentration all significantly ($P \leq 0.05$) affected the chlorine demand of produce wash waters. Equations with cubic term of sanitizer pH were developed to predict chlorine demand of various fresh produce wash waters at different organic load for different sanitizer pH and initial chlorine concentration. No significant difference of chlorine demand was detected between NaOCl and EO water at the same pH.

Acknowledgements

This study was funded by Agriculture and Food Research Initiative grant no 2014-67017-21558 from the USDA National Institute of Food and Agriculture and State and Hatch funds allocated to the University of Georgia Agricultural Experiment Station, Griffin Campus. Special thanks go to Dr. Yihui Chen of the Fujian Agriculture and Forestry University for his research assistance and to Dr. Veerachandra K. Yemmireddy for specific comments on the preparation of this manuscript.

Table 3-1. Effects of NaOCl pH and buffering capacity on chlorine demand of romaine lettuce wash water.

Targeted NaOCl pH	Buffering agent	NaOCl pH before treatment	NaOCl pH after treatment	Chlorine demand (mg L^{-1})
2.5	Yes	A2.54±0.03c	A2.54±0.01d	45.1±0.9a
	No	A2.47±0.06c	A2.48±0.06d	46.5±0.8a
6.0	Yes	A6.05±0.07b	A5.96±0.02b	34.9±0.8b
	No	A6.10±0.01b	B5.15±0.03c	34.8±0.8b
9.5	Yes	A9.56±0.02a	A9.57±0.02a	26.4±0.4c
	No	A9.51±0.02a	A9.45±0.10a	26.8±0.3c

Means with the same capital letter in each row indicate no significant difference ($P > 0.05$)

Means with the same lower case letter in each column indicate no significant difference ($P > 0.05$)

Table 3-2. Effects of organic load, initial chlorine concentration and sanitizer pH on the chlorine demand of romaine lettuce wash water.

Organic load indicator (Standard chlorine demand, mg L^{-1})	Sanitizer pH	Initial chlorine concentration		
		50 mg L^{-1}	75 mg L^{-1}	100 mg L^{-1}
20	2.5	A24.17±1.04a	A25.92±2.93a	A26.67±2.65a
	4.0	A19.42±1.13ab	A21.25±2.14ab	A22.75±2.25ab
	6.0	A18.08±2.10b	A18.33±3.13b	A20.17±0.38bc
	8.0	A19.67±2.24ab	A20.92±3.00ab	A24.00±1.89ab
	9.5	A12.92±2.32c	A15.08±1.66b	A16.08±1.76c
40	2.5	B43.25±2.18a	AB49.33±2.77a	A51.67±2.57a
	4.0	B34.50±1.75b	A40.75±1.32b	A44.33±2.32b
	6.0	B32.75±2.29b	AB36.92±3.01b	A40.17±0.58b
	8.0	B34.67±2.45b	AB39.58±2.25b	A43.58±0.76b
	9.5	A26.25±2.22c	A29.92±2.10c	A31.00±2.05c
60	2.5	C51.67±1.38a	B64.92±0.76a	A74.17±2.13a
	4.0	C45.17±2.08b	B56.25±2.17b	A63.33±3.64b
	6.0	C43.33±2.60b	B52.33±2.08b	A58.83±1.01b
	8.0	C45.33±2.18b	B54.33±2.93b	A61.50±0.66b
	9.5	B36.92±2.10c	A42.75±1.98c	A45.83±2.08c

Means with the same capital letter in each row indicate no significant difference ($P > 0.05$)

Means for the same organic load and initial chlorine concentration with the same lower case letter in each column indicate no significant difference ($P > 0.05$)

Table 3-3. Effect of pH on the ORP of NaOCl solutions.

pH	ORP (mV)
2.57 ± 0.01E	1184.3 ± 1.9A
3.99 ± 0.06D	1095.6 ± 2.1B
6.01 ± 0.05C	976.8 ± 11.0C
8.02 ± 0.02B	834.5 ± 5.3D
9.49 ± 0.05A	731.7 ± 3.9E

Means with the same capital letter in each column indicate no significant difference ($P > 0.05$)

Table 3-4. Prediction of chlorine demand of produce wash waters at D_{ST} 30 mg L^{-1}.

					NaOCl		EO	
Produce	PPC	UV254	Sanitizer pH	Predicted chlorine demand (mg L^{-1})	Observed chlorine demand (mg L^{-1})	Prediction percentage error (%) [a]	Observed chlorine demand (mg L^{-1})	Prediction percentage error (%)
Iceberg lettuce	0.16±0.02	0.082±0.004	2.5	43.99±1.05A	46.17±2.10A	-4.71	48.33±2.77A	-8.98
			6.0	35.80±1.05A	29.75±1.56B	20.34	28.92±2.13B	23.81
			8.0	36.84±1.05A	31.17±1.26B	18.21	29.17±1.23B	26.32
Grape	0.06±0.02	0.045±0.006	2.5	33.61±1.72A	35.58±0.52A	-5.54	34.50±0.66A	-2.57
			6.0	25.42±1.72B	30.50±0.25A	-16.64	28.67±0.76A	-11.31
			8.0	26.46±1.72B	33.58±0.38A	-21.20	34.50±0.50A	-23.29
Romaine lettuce	2.38±0.48	0.266±0.022	2.5	46.54±2.21A	38.75±2.46B	20.10	36.42±3.25B	27.80
			6.0	38.35±2.21A	28.67±2.02B	33.78	27.33±1.61B	40.30
			8.0	39.39±2.21A	31.67±1.46B	24.39	28.75±2.29B	37.01
Strawberry	1.19±0.33	0.217±0.003	2.5	41.43±0.38A	32.50±1.00B	27.49	32.42±0.88B	27.82
			6.0	33.24±0.38A	29.08±0.80B	14.31	27.92±1.38B	19.08
			8.0	34.28±0.38A	29.92±0.88B	14.60	29.33±0.38B	16.88

Means with the same capital letter in each row indicate no significant difference ($P > 0.05$)

[a] Prediction percentage error = (Predicted chlorine demand - Observed chlorine demand) / Observed chlorine demand × 100.

Table 3-5. Prediction of chlorine demand of produce wash waters at D_{ST} 50 mg L^{-1}.

					NaOCl		EO	
Produce	PPC	UV254	Sanitizer pH	Predicted chlorine demand (mg L^{-1})	Observed chlorine demand (mg L^{-1})	Prediction percentage error (%)	Observed chlorine demand (mg L^{-1})	Prediction percentage error (%)
Iceberg lettuce	0.21±0.14	0.109±0.026	2.5	50.40±6.12B	63.33±1.77A	-20.42	62.42±1.77A	-19.25
			6.0	42.42±6.12A	50.50±0.66A	-16.41	50.06±0.38A	-15.71
			8.0	43.25±6.12B	52.58±1.01A	-17.74	52.67±1.13A	-17.87
Grape	0.50±0.07	0.082±0.001	2.5	43.82±0.30B	54.33±0.76A	-19.34	54.00±0.66A	-18.85
			6.0	35.63±0.30B	50.67±0.95A	-29.67	50.00±0.90A	-28.73
			8.0	36.67±0.30B	51.00±0.50A	-28.09	50.75±0.25A	-27.74
Romaine lettuce	4.21±1.25	0.585±0.084	2.5	71.32±4.94A	61.92±1.04B	15.19	62.17±1.84B	14.72
			6.0	63.13±4.94A	50.50±0.75B	25.01	50.92±1.01B	23.98
			8.0	64.17±4.94A	50.92±0.72B	26.03	51.75±0.90B	24.00
Strawberry	1.48±0.29	0.473±0.052	2.5	64.38±3.84A	56.67±2.25A	13.62	56.75±3.50A	13.45
			6.0	56.19±3.84A	50.08±0.14B	12.20	49.75±0.43B	12.95
			8.0	57.23±3.84A	50.17±0.76B	14.09	49.75±0.50B	15.04

Means with the same capital letter in each row indicate no significant difference ($P > 0.05$)

References

Batz, M. B., Hoffmann, S. A., & Morris, J. G. (2011). Ranking the risks: the 10 pathogen-food combinations with the greatest burden on public health. Emerging Pathogens Institute, University of Florida.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*(1-2), 248-254.

Chang, E., Chiang, P., Chao, S., & Lin, Y. (2006). Relationship between chlorine consumption and chlorination by-products formation for model compounds. *Chemosphere, 64*(7), 1196-1203.

Chen, X., & Hung, Y.-C. (2016). Predicting chlorine demand of fresh and fresh-cut produce based on produce wash water properties. *Postharvest Biology and Technology, 120*, 10-15.

Criquet, J., Rodriguez, E. M., Allard, S., Wellauer, S., Salhi, E., Joll, C. A., & Von Gunten, U. (2015). Reaction of bromine and chlorine with phenolic compounds and natural organic matter extracts–Electrophilic aromatic substitution and oxidation. *Water Research, 85*, 476-486.

Deborde, M., & von Gunten, U. (2008). Reactions of chlorine with inorganic and organic compounds during water treatment - Kinetics and mechanisms: A critical review. *Water Research, 42*(1-2), 13-51.

Fan, X., & Sokorai, K. J. (2015). Formation of trichloromethane in chlorinated water and fresh-cut produce and as a result of reaction with citric acid. *Postharvest Biology and Technology, 109*, 65-72.

Fleischacker, S. J., & Randtke, S. J. (1983). Formation of organic chlorine in public water supplies. *Journal (American Water Works Association)*, 132-138.

Gil, M. I., Gomez-Lopez, V. M., Hung, Y.-C., & Allende, A. (2015). Potential of electrolyzed water as an alternative disinfectant agent in the fresh-cut industry. *Food and Bioprocess Technology, 8*(6), 1336-1348.

Gil, M. I., Selma, M. V., Lopez-Galvez, F., & Allende, A. (2009). Fresh-cut product sanitation and wash water disinfection: Problems and solutions. *International Journal of Food Microbiology, 134*(1-2), 37-45.

Huang, Y.-R., Hung, Y.-C., Hsu, S.-Y., Huang, Y.-W., & Hwang, D.-F. (2008). Application of electrolyzed water in the food industry. *Food Control, 19*(4), 329-345.

Luo, Y., Nou, X., Yang, Y., Alegre, I., Turner, E., Feng, H., Abadias, M., & Conway, W. (2011). Determination of free chlorine concentrations needed to prevent *Escherichia coli* O157:H7 cross-contamination during fresh-cut produce wash. *Journal of Food Protection, 74*(3), 352-358.

Park, C. M., Hung, Y. C., Doyle, M., Ezeike, G., & Kim, C. (2001). Pathogen reduction and quality of lettuce treated with electrolyzed oxidizing and acidified chlorinated water. *Journal of Food Science, 66*(9), 1368-1372.

Pirovani, M. E., Guemes, D. R., & Piagnetini, A. M. (2001). Predictive models for available chlorine depletion and total microbial count reduction during washing of fresh-cut spinach. *Journal of Food Science, 66*(6), 860-864.

Singleton, V., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology* *and Viticulture, 16*(3), 144-158.

Van Haute, S., Sampers, I., Holvoet, K., & Uyttendaele, M. (2013). Physicochemical quality and chemical safety of chlorine as a reconditioning agent and wash water disinfectant for fresh-cut lettuce washing. *Applied and Environmental Microbiology, 79*(9), 2850-2861.

Waters, B. W., & Hung, Y.-C. (2013). Evaluation of different methods for determination of properties of chlorine-based sanitizers. *Food Control, 30*(1), 41-47.

Waters, B. W., & Hung, Y.-C. (2014). The effect of organic loads on stability of various chlorine-based sanitisers. *International Journal of Food Science and Technology, 49*(3), 867-875.

Weng, S., Luo, Y., Li, J., Zhou, B., Jacangelo, J. G., & Schwab, K. J. (2016). Assessment and speciation of chlorine demand in fresh-cut produce wash water. *Food Control, 60*, 543-551.

Yu, R.-F. (2004). Feed-forward dose control of wastewater chlorination using on-line pH and ORP titration. *Chemosphere, 56*(10), 973-980.

Zhou, B., Luo, Y., Nou, X., & Millner, P. (2014). Development of an algorithm for feed-forward chlorine dosing of lettuce wash operations and correlation of chlorine profile with *Escherichia coli* O157:H7 inactivation. *Journal of Food Protection, 77*(4), 558-566.

CHAPTER 4

DEVELOPMENT OF A CHLORINE DOSING STRATEGY FOR FRESH PRODUCE WASHING PROCESS TO ENHANCE MICROBIAL FOOD SAFETY

Chen X, and Hung YC. 2017. Submitted to *Journal of Food Science*

Abstract

The antimicrobial efficacy of sodium hypochlorite (NaOCl) for fresh produce washing process could be compromised by its reaction with organic matter. Excess amount of NaOCl can result in a large amount of hazardous disinfection by-products (DBPs), whereas deficiency of NaOCl may not completely remove pathogens on produce. The purpose of this study was to develop a chlorine dosing strategy to enhance microbial safety as well as minimize the DBPs formation. Prediction equations for initial chlorine concentration needed to achieve a targeted residual chlorine in various produce wash waters were developed and validated using fresh-cut iceberg lettuce and strawberries in an automated produce washer for 5 min. Validation results show that equations successfully predicted the initial chlorine concentration needed to achieve residual chlorine at 10, 30, 60, and 90 mg L^{-1} for both lettuce and strawberry washing process, with the root mean squared error (RMSE) at 4.45 mg L^{-1}. In addition, the reduction difference of *E. coli* O157:H7 on inoculated lettuce and strawberries among different residual chlorine from 10 to 90 mg L^{-1} was less than 0.6 log CFU g^{-1}. Our study demonstrates that 10 mg L^{-1} was the optimal targeted residual chlorine to ensure microbial inactivation without using excess amount of chlorine. The chlorine dosing strategy for produce washing can use prediction equations developed by this study to predict initial chlorine concentration needed to achieve a residual chlorine of 10 mg L^{-1}.

Key Words: Produce, washing, dosing strategy, prediction equation, residual chlorine

4.1 Introduction

Fresh produce without subjecting to thermal processing could be a potential vehicle for foodborne pathogens. Contamination of fresh produce with foodborne pathogens may occur during the preharvest phase in the field through contaminated seeds, water, soil and pest, and during the postharvest phase through contaminated water or cross contamination from equipment and contact surfaces (Yeni and others 2016). Every year, over 1 million foodborne illnesses in the United State are caused by fresh and fresh-cut produce, and *Escherichia coli* O157:H7 is responsible for 11,408 illnesses, 386 hospitalizations and 4 deaths annually (Batz and others 2011).

The commercial fresh-cut produce processing includes selection, cleaning, trimming, peeling, cutting, washing and packing (Ramos and others 2013). Among these steps, washing is critical to remove dirt, pesticide residues, debris, tissue fluids and foodborne pathogens on produce. To enhance produce safety, chlorine-based sanitizers such as sodium hypochlorite (NaOCl) are commonly used in the washing process (Gil and others 2009).

One concern of using NaOCl as the disinfectant is that free chlorine components (Cl_2, HOCl and OCl^-) can react with organic compounds released from produce to form hazardous disinfection by-products/DBPs (Fan and Sokorai 2015). Excess amount of NaOCl may generate a large amount of DBPs, whereas deficiency of NaOCl may not completely inactivate foodborne pathogens on produce. It is therefore important for produce industry to control the chlorine concentration in wash water.

Through estimating the chlorine demand (the amount of free chlorine that would be consumed due to organic loads during produce washing), the overdosing or underdosing problem can be avoided. Our previous study identified that the ultraviolet light absorbance of produce wash water at 254 nm (UV254) was highly correlated with chlorine demand of produce wash waters (Chen and Hung 2016). In another study, we further developed equations to estimate the chlorine demand of various produce wash waters at different organic load with different sanitizer pH and initial chlorine concentrations (Chen and Hung 2017). However, before applying these equations to predict chlorine demand, the optimal initial chlorine concentration to inactivate foodborne pathogens during produce washing should be determined. The feasibility of this disinfection strategy under a simulated food service produce washing conditions (i.e., washing produce using a produce washer) has also not been validated.

Therefore, the overall objective of this study was to develop a chorine dosing strategy for produce washing process to achieve high *E. coli* O157:H7 inactivation and minimize residual chlorine in produce wash water. Specific objectives include the following:

i) to determine the effect of initial chlorine concentration for reducing *E. coli* O157:H7 on iceberg lettuce and strawberries
ii) to develop equations to predict the initial chlorine concentration needed to reach a targeted residual chlorine for produce washing process
iii) to validate the prediction equations for washing iceberg lettuce and strawberries using an automatic produce washer
iv) to develop chlorine dosing strategy to maintain microbial safety and minimize residual chlorine in produce wash water

4.2 Materials and Methods

4.2.1 Preparation of inoculum

Five nalidixic acid-resistant strains of *E. coli* O157:H7, including 1 (beef isolate), 4 (human isolate), 5 (human isolate), 932 (human isolate) and E009 (beef isolate) were used. Each strain was subcultured in 10 mL of tryptic soy broth (TSB, BD Company, Sparks, MD, USA) with 50 mg L^{-1} of nalidixic acid at 37 °C for 24 h on an incubator shaker (New Brunswick Scientific, Edison, NJ, USA) at 150 rpm. Following incubation, *E. coli* O157:H7 cells were sedimented by centrifugation at 3000 × g in a Sigma 2-16KL centrifuge (Sigma, Germany) for 15 min. The supernatant was discarded and cell pellets were resuspended in 10 mL of phosphate buffered saline (PBS, pH 7). Equal volume of each strain suspension was combined to obtain a cocktail inoculum containing approximately 9 log CFU mL^{-1} cells. The *E. coli* O157:H7 population in the cocktail inoculum was determined by spread plating on Sorbitol MacConkey agar containing 50 µg mL^{-1} nalidixic acid (SMACN, BD Company, Sparks, MD, USA).

4.2.2 Inoculation of iceberg lettuce and strawberry

Iceberg lettuce (*Lactuca sativa*) and strawberries (*Fragaria × ananassa*) were purchased from a local grocery store in Griffin, GA, stored at 4 °C, and used within 24 h. The outer 2 or 3 leaves of iceberg lettuce were discarded; other leaves were cut into pieces with weight of 20 ± 2 g using clean scissors. A 200 µL of *E. coli* O157:H7 cocktail inoculum was deposited on the abaxial side of each leaf using a pipette, and uniformly spread using a small piece of non-inoculated leaf to avoid physical damage on leaves during spreading. The final inoculation level was approximately 10^7 CFU per gram of lettuce. For strawberries, uniform-sized strawberries (20 g ± 5 g) with no visual damage were selected. Each strawberry was spot inoculated with 100 µL of *E. coli* O157:H7 cocktail in form of small drops (~10 drops). After inoculation, lettuce and strawberries were air-dried for 45 min in a laminar flow hood to allow for the attachment of pathogens, and then stored at 4 °C overnight.

4.2.3 Preparation of NaOCl solutions

NaOCl solutions containing various levels of free chlorine concentration were prepared by diluting 6 % bleach (RICCA Company, Arlington, TX, USA) in deionized water (dH_2O), and pH was adjusted to 6.0 ± 0.1 using phosphate acid. Free chlorine concentration was determined by a DPD-FEAS titrimetric method (Hach Company, Loveland, CO, USA). A dual channel ACCUMET pH meter (Model # AR50, Fisher Scientific, Pittsburgh, PA, USA) with appropriate electrode was used to measure the pH of NaOCl solutions.

4.2.4 Effect of initial chlorine concentration on microbial inactivation efficacy

To evaluate the effect of chlorine concentration for *E. coli* O157:H7 inactivation, NaOCl solutions containing free chlorine of 25, 50, 75 and 100 mg L^{-1} at pH 6.0 were prepared as described above, and stored at 4 °C before use. The inoculated lettuce was cut into small pieces (3×3 cm^2) using a commercial lettuce cutter (Model # 55650, Nemco Food Equipment, Hicksville, OH, USA) before washing. For each washing treatment, 100 ± 10 g of fresh-cut lettuce were washed in 4 L of chilled NaOCl solution or dH_2O in an automated produce washer (Afari and others 2015) at a washing speed of 65 rpm for 5 min. After washing, 20 ± 2 g of fresh-cut lettuce were placed into a 1.5 L of Whirl-Pak bag containing 200 mL of neutralizing buffer (NB, BD Company, Sparks, MD, USA) for microbial analysis. For strawberries, 400 ± 10 g of strawberries (20 pieces) were washed in 4 L of chilled NaOCl solution or dH_2O in the automated produce washer with washing speed of 65 rpm for 5 min. At the end of the designated washing time, 100 ± 10 g of strawberries (5 pieces) were placed into a 1.5 L of Whirl-Pak bag containing 150 mL of NB broth.

For microbial analysis, iceberg lettuce in NB broth was stomached (Stomacher® 400 Circulator, Seward Ltd, Worthing, UK) for 1 min at the speed of 230 rpm. Strawberries in NB broth were hand-massaged for 1 min from the outside of the Whirl-Pak bags. Solution samples were then serially diluted with PBS and plated onto SMACN agars to enumerate. After incubation at 37 °C for 24 h, *E. coli* O157:H7 populations were counted and reported as log CFU per gram of produce.

4.2.5 Development of prediction equations for initial chlorine concentration needed to achieve a targeted residual chlorine in the wash water

Prediction equations developed by Chen and Hung (2017) were used to predict chlorine demand of produce wash water. Initial chlorine concentration needed to achieve a targeted residual chlorine level can be calculated as the sum of targeted residual chlorine plus the predicted chlorine demand.

4.2.6 Verification of prediction equations

Iceberg lettuce and strawberries were selected to validate prediction equations. For each produce, half of the samples were randomly selected for wash water quality measurements. Remaining produce samples were inoculated with *E. coli* O157:H7 as described above, and then used for microbial inactivation. Both inoculated and non-inoculated produce were stored at 4 °C overnight.

For wash water quality measurements, 100 ± 10 g of non-inoculated fresh-cut lettuce were washed in 4 L of chilled dH_2O in the automated produce washer at a washing speed of 65 rpm for 5 min. For strawberries, 800 ± 10 g of non-inoculated strawberries (40 pieces) were washed in 4 L of chilled dH_2O in order to create a high organic load. After washing, produce wash waters were collected to measure wash water properties.

Water quality parameters of iceberg lettuce and strawberry wash waters including UV254, total phenolics, total protein and CIE L*a*b* color were determined. UV254 was measured using a DU 520 UV-Vis spectrophotometer (Beckman Coulter Inc., Brea, CA, USA) after filtering wash water through a 0.45 µm pore-size filter (Waterman®, Boca Raton, FL, USA) as described in Chen and Hung (2016). Total phenolics was determined by the Folin-Ciocalteu assay and expressed as mg L^{-1} gallic acid equivalents (Singleton and Rossi 1965). Total protein was determined by the Bradford assay and expressed as mg L^{-1} bovine serum albumen equivalents (Bradford 1976). The CIE L*, a*, b* color parameters of produce wash water were determined following the procedure of Chen and Hung (2016) using a MiniScan XET^{M} colorimeter (Model# 45/0-L, Hunter Associates Laboratory, Reston, VA, USA). The color difference (ΔE) between produce wash water (s) and dH_2O (w) was calculated as follows:

$$\Delta E = \sqrt{(L_s^* - L_w^*)^2 + (a_s^* - a_w^*)^2 + (b_s^* - b_w^*)^2} \tag{1}$$

where L* is lightness, a* is red-green color, and b* is yellow-blue color.

Water property results were used to calculate the initial chlorine concentration to achieve targeted residual chlorine of 10, 30, 60, and 90 mg L^{-1} using prediction equations previously developed. NaOCl solutions at pH 6.0 and different targeted initial chlorine concentration were prepared, stored at 4 °C for 2 h before use.

For microbial inactivation, 100 ± 10 g of inoculated fresh-cut lettuce or 800 ± 10 g of inoculated strawberries (40 pieces) were washed in 4 L of NaOCl solution for 5 min. Washed lettuce and strawberries were mixed with NB broth in Whirl-Pak bags for microbial analysis as described above. The free chlorine content of NaOCl solutions before and after washing was measured using the DPD-FEAS titrimetric method.

4.2.7 Statistical analysis

Experiments were replicated twice. Each replication consisted of 2 sample for each treatment, and two measurements were taken for each sample. Data were subjected to analysis using the SAS 9.4 program (SAS Institute, Cary, NC). Least significant difference of means (LSD) test were used for multiple comparisons. Differences were considered significant at $P \leq 0.05$.

4.3 Results and Discussion

4.3.1 Effect of initial chlorine concentration for inactivating E. coli O157:H7 on iceberg lettuce and strawberries

Table 4-1 shows the surviving population of *E. coli* O157:H7 on fresh-cut iceberg lettuce after washing with NaOCl solution or dH_2O in the automatic produce washer for 5 min. The recovery of *E. coli* from dH_2O treatment was 6.63 log CFU/g, which was significantly higher than the recovery from NaOCl treatments ($P \leq 0.05$). For NaOCl treatments, increasing initial chlorine concentration from 25 to 100 mg L^{-1} only slightly decreased ($P \leq 0.05$) the recovery of *E. coli* O157:H7 on iceberg lettuce from 6.25 to 6.04 log CFU/g. This result is in agreement with previous work that chlorine addition up to

100 mg L^{-1} did not appreciably decrease the total microbial populations on the fresh-cut iceberg lettuce surface (Delaquis and others 2004).

The effect of initial chlorine concentration for reducing the population of *E. coli* O157:H7 on strawberries is shown in Table 4-2. In general, NaOCl treatments resulted in about 2 log reductions on strawberries, which was almost twice as much as the log reductions on iceberg lettuce at the same initial chlorine concentration. This finding is also consistent with the previous finding that longer treatment time was required to achieve a 1-log reduction of *E. coli* O157:H7 on shredded lettuce than strawberries (Rodgers and others 2004). Similar to iceberg lettuce, the recovery of *E. coli* O157:H7 on strawberries from NaOCl treatments were significantly lower ($P \leq 0.05$) than the recovery from dH_2O treatment. No significant difference ($P > 0.05$) of *E. coli* O157:H7 recovery was detected at different initial chlorine concentration, although treatments at 25 and 100 mg L^{-1} free chlorine had the highest and lowest *E. coli* O157:H7 recovery after washing, respectively.

Results in Tables 4-1 and 4-2 show that washing fresh-cut iceberg lettuce and strawberries in 25 mg L^{-1} free chlorine for 5 min significantly reduced ($P \leq 0.05$) the *E. coli* O157:H7 population by 0.88 and 1.99 log CFU g^{-1}, respectively. Further increase on the initial chlorine concentration up to 100 mg L^{-1} only slightly increased the reduction of *E. coli* O157:H7. Fan and Sokorai (2015) reported that there was 21 µg L^{-1} of trichloromethane formation in simulated iceberg lettuce wash water for every 100 mg L^{-1} increase in free chlorine concentration. Considering the recirculation of wash water during commercial produce washing process, the trichloromethane levels in produce wash water with high residual free chlorine can be easily over the authorized limit (80 µg

L^{-1}) fixed by the US Environmental Protection Agency (EPA) for drinking water (EPA, 2009). Therefore, a low level of free chlorine for fresh produce washing process can minimize DBPs formation potential without compromising the microbial inactivation efficacy of NaOCl treatment.

4.3.2 Development of equations to predict initial chlorine concentration for produce washing

In practice, knowing the residual chlorine in wash solution after one washing cycle is important to determine the dosing strategy. As indicated in our previous study (Chen and Hung 2017), through measuring the water quality parameters such as UV254, chlorine demand of various produce wash waters can be estimated. Specifically, various types of fresh produce can be classified into two clusters based on the PPC ratio:

$$\text{PPC} = \text{Phenolics} / (\text{Protein} / \Delta E) \tag{2}$$

where Phenolics is the total phenolics, Protein is the total protein, and ΔE is the total CIE color difference as calculated based on eqn (1). Generally, produce wash waters with low phenolics-to-protein ratio will have PPC value lower than 0.6, while produce wash waters with high phenolics-to-protein ratio will have PPC value higher than 0.6 (Chen and Hung 2016).

The chlorine demand of produce wash waters for each cluster as reported in Chen and Hung (2017) can be calculated as follow:

For PPC value < 0.6,

$$D = 48.97 - 30\ \text{pH} + 330.74\ \text{UV} + 0.18\ C + 5.14\ \text{pH}^2 - 435.8\ \text{UV}^2 - 0.28\ \text{pH}^3 \tag{3}$$

For PPC value ≥ 0.6,

$$D = 43.76 - 30\ \text{pH} + 139.64\ \text{UV} + 0.18\ C + 5.14\ \text{pH}^2 - 71.72\ \text{UV}^2 - 0.28\ \text{pH}^3 \tag{4}$$

where D is the chlorine demand, pH is the initial pH of NaOCl solution, UV is the UV254 of produce wash water, and C is the initial chlorine concentration.

The residual chlorine (R) is defined as the difference between initial chlorine concentration and chlorine demand as follow:

$$R = C - D \tag{5}$$

By combining eqn (5) with eqn (3) and (4), initial chlorine concentration needed to achieve a particular residual chlorine level can be predicted as:

If the PPC < 0.6,

$$C = (R + 48.97 - 30\ pH + 330.74\ UV + 5.14\ pH^2 - 435.8\ UV^2 - 0.28\ pH^3)/0.82 \tag{6}$$

If the PPC ≥ 0.6,

$$C = (R + 43.76 - 30\ pH + 139.64\ UV + 5.14\ pH^2 - 71.72\ UV^2 - 0.28\ pH^3)/0.82 \tag{7}$$

4.3.3 Verification of initial chlorine concentration prediction equations

Iceberg lettuce and strawberries were used to validate the prediction equations for initial chlorine concentration. In general, the NaOCl pH is commonly adjusted to ~6.0 to enhance the antimicrobial efficacy and minimize the equipment corrosion (Pangloli and Hung 2013; Zaid and others 2008). In addition, results in our previous study indicated that NaOCl at pH 6.0 had the lowest chlorine demand than NaOCl at pH 2.5, 4.0 or 8.0 (Chen and Hung 2017). Therefore, the NaOCl pH for this validation study was set at 6.0.

Water properties of iceberg lettuce and strawberry wash water are presented in Table 4-3. Based on measured total phenolics, total protein and ΔE value (data not shown), the PPC value of each sample was calculated using eqn (2). As shown in Table 4-3, iceberg lettuce wash waters had a PPC value lower than 0.6, and strawberry wash waters had a PPC value higher than 0.6, which were consistent with reported in previous studies (Chen and Hung 2016, 2017). In addition, due to the natural variation of produce between different batches, the UV254 readings of produce wash water between two replicated was very different. To validate the prediction performance of equations at different organic load, we decided not to combine the results of two replications of each produce.

The predicted initial chlorine concentration and prediction accuracy are shown in Table 4-3 as well. The targeted residual chlorine after washing process was set at 10, 30, 60 and 90 mg L^{-1}, which was then used to calculate predicted initial chlorine concentration using eqn (6) and (7). Due to the variation of UV254 between samples, the predicted initial chlorine concentration for each washing treatment to reach targeted residual chlorine was different (Table 4-3). Based on the predicted initial chlorine concentration, chlorine solutions were prepared for the washing studies, and the actual initial chlorine concentration for each wash solution was determined and also reported in Table 4-3. Although NaOCl solutions were prepared based on predicted initial chlorine concentration, there is always a small error between targeted initial chlorine concentration and actual initial chlorine concentration. Observed residual chlorine was determined after washing treatment and reported in Table 4-3. Results show that chlorine demand (difference between initial chlorine concentration and residual chlorine) increased with increasing targeted residual chlorine. For example, predicted initial chlorine concentration for iceberg lettuce wash water at 2nd replication to reach 10 mg L^{-1} targeted residual was 12.25 mg L^{-1} (8 mg L^{-1} in chlorine demand), while it increased to 101.75 mg L^{-1} (17 mg L^{-1} in chlorine demand) in order to reach 90 mg L^{-1} targeted residual chlorine. This trend is consistent with previous study (Chen and Hung 2017) that chlorine demand of produce wash waters increased with increasing initial chlorine concentration.

The prediction error and prediction percentage error were calculated as difference between predicted residual chlorine and observed residual chlorine, and the ratio of prediction error over the observed residual chlorine, respectively. The root mean squared error (RMSE) of validation data was 4.45 mg L^{-1}. In addition, 87.5 % of samples had prediction error smaller than ± 6 mg L^{-1}, and 75 % of samples had prediction percentage error smaller than ± 15 %. Prediction accuracy was also not affected by the type of produce or initial chlorine concentration.

Table 4-4 shows the *E. coli* O157:H7 inactivation on iceberg lettuce and strawberries of treatments at different targeted residual chlorine. For iceberg lettuce, *E. coli* O157:H7 reduction at 10 mg L^{-1} targeted residual chlorine was 0.77 to 1.04 log CFU g^{-1}, and reduction only slightly increased to 1.19 to 1.24 log CFU g^{-1} at 90 mg L^{-1} targeted residual chlorine. The reduction on strawberries (1.37 to 1.95 log CFU g^{-1}) was generally higher than that on iceberg lettuce (0.77 to 1.24 log CFU g^{-1}), but the reduction difference between 10 and 90 mg L^{-1} was very little (< 0.5 log CFU g^{-1}). Lower reduction on *E. coli* O157:H7 for strawberries was achieved in the verification studies (Table 4-4) than the microbial inactivation studies (Table 4-2). This may be due to the higher produce-to-water ratio in the verification studies (800 g of strawberries in 4 L of NaOCl) than microbial inactivation studies (400 g of strawberries in 4 L of NaOCl). Results presented in Table 4-4 confirmed that 10 mg L^{-1} residual chlorine was equally effective as other higher residual chlorine levels (30, 60, 90 mg L^{-1}) on reduction of the *E. coli* O157:H7 during iceberg lettuce and strawberry washing treatment.

4.3.4 Chlorine dosing strategy for fresh produce washing

When organic compounds were present in produce washing solution, microbial inactivation efficacy was depending on the residual chlorine concentration (Shen and others 2013). On the other side, chlorine demand was closely associated with the formation of DBPs (Chang and others 2006). Therefore, an effective dosing strategy may be set at ensuring high microbial inactivation efficacy and lowing DBPs formation potential. This can be achieved by adding the initial chlorine concentration in the wash solution to achieve the residual chlorine low enough but still can maintain effective inactivation of foodborne pathogens on fresh produce and prevent cross-contamination during produce washing. Previous study shows that electrolyzed oxidizing (EO) water with 2 mg L^{-1} residual chlorine completely inactivated *E. coli* O157:H7 and *Listeria monocytogenes* in pure culture at pH 2.6 to 7.0 (Park and others 2004). However, higher chlorine concentration is always needed to reach a similar reduction on food samples than in pure culture. Another study reported that 10 mg L^{-1} was the minimal residual free chlorine to eliminate *E. coli* O157:H7 in lettuce wash water and prevent the cross-contamination between lettuce (Luo and others 2011). The results in our study also show that maintaining 10 mg L^{-1} residual chlorine in wash solution significantly ($P \leq 0.05$) reduced *E. coli* O157:H7 on iceberg lettuce and strawberries. Therefore, 10 mg L^{-1} can be set as the targeted residual chlorine for produce washing process.

In summary, the first step to develop the dosing strategy is to determine the properties of fresh produce to be processed. Through measuring the total phenolics, total protein and ΔE of produce wash water, the PPC cluster (PPC > 0.6 or < 0.6) of tested fresh produce can be determined. Previous studies show that the PPC cluster of each type of produce is very consistent, so the test to determine PPC cluster of each produce only needs to be done at the first time to process that produce (Chen and Hung 2016, 2017). The next step is to determine the targeted residual chlorine and sanitizer pH for produce washing process. Although 10 mg L^{-1} residual chlorine and pH 6.0 is recommended based on findings of this and previous studies, prediction equations developed from this study can be used for a wide range of targeted residual chlorine (10 to 90 mg L^{-1}) and sanitizer pH (2.5 to 9.5). The last step of developing a dosing strategy is to determine the UV254 of produce wash water. As reported in the verification studies, UV254 can be affected significantly by the produce-to-water ratio and source of the produce (Table 4-3). Initial chlorine concentration to maintain microbial safety and minimize the potential of production of DBPs can be calculated based on the prediction equations proposed in the current study.

4.4 Conclusions

In conclusion, prediction equations that capable to predict the initial chlorine concentration needed to reach a targeted residual chlorine for produce washing process were developed and validated.

NaOCl with residual chlorine of 10 mg L^{-1} was equally effective as other higher residual chlorine levels (30, 60, 90 mg L^{-1}) for inactivating *E. coli* O157:H7 during produce washing process. Suggested chlorine dosing strategy is to determine the PPC cluster type of fresh produce first, followed by determining the targeted residual chlorine and sanitizer pH. The last step is to determine the UV254 of produce wash water. Based on the PPC, residual chlorine, pH and UV254, the initial chlorine concentration to maintain microbial food safety and minimize disinfection by-products formation potential can be determined.

Acknowledgements

This study was funded by Agriculture and Food Research Initiative grant no 2014-67017-21558 from the USDA National Institute of Food and Agriculture and State and Hatch funds allocated to the University of Georgia Agricultural Experiment Station, Griffin Campus.

Table 4-1. Effects of initial chlorine concentration for inactivating *E. coli* O157:H7 on iceberg lettuce.

Treatment [a]	Chlorine concentration (mg L^{-1})	pH	Recovery (log CFU g^{-1}) [b]	Reduction (log CFU g^{-1})	Additional reduction than dH_2O (log CFU g^{-1})
Control			7.13 ± 0.06A		
dH_2O			6.63 ± 0.09B	0.50	
NaOCl - 25	23.25 ± 0.35	5.97 ± 0.04	6.25 ± 0.04C	0.88	0.38
NaOCl - 50	47.63 ± 0.88	5.97 ± 0.01	6.13 ± 0.06D	1.00	0.50
NaOCl - 75	71.38 ± 0.53	6.05 ± 0.06	6.09 ± 0.03DE	1.04	0.54
NaOCl - 100	96.88 ± 3.36	5.99 ± 0.08	6.04 ± 0.03E	1.09	0.59

[a] NaOCl - 25, NaOCl - 50, NaOCl - 75 and NaOCl - 100 are NaOCl solution at pH 6.0 and initial chlorine concentration at 25, 50, 75 and 100 mg L^{-1}, respectively.

[b] Means with the same lower case letter in each column indicate no significant difference ($P > 0.05$).

Table 4-2. Effects of initial chlorine concentration for inactivating *E. coli* O157:H7 on strawberries.

Treatment	Chlorine concentration (mg L^{-1})	pH	Recovery (log CFU g^{-1})	Reduction (log CFU g^{-1})	Additional reduction than dH_2O (log CFU g^{-1})
Control			6.04 ± 0.16A		
dH_2O			4.95 ± 0.12B	1.09	
NaOCl - 25	21.38 ± 0.53	6.02 ± 0.02	4.05 ± 0.10C	1.99	0.90
NaOCl - 50	47.50 ± 0.35	6.02 ± 0.01	3.93 ± 0.11C	2.11	1.02
NaOCl - 75	75.13 ± 2.30	6.05 ± 0.02	3.99 ± 0.23C	2.05	0.96
NaOCl - 100	96.25 ± 0.00	6.01 ± 0.08	3.87 ± 0.11C	2.17	1.08

Table 4-3. Residual chlorine prediction performance during wash iceberg lettuce and strawberries.

Produce	Rep	PPC [a]	UV254	Targeted residual chlorine (mg L^{-1})	Predicted initial chlorine concentration (mg L^{-1})	Actual initial chlorine concentration (mg L^{-1})	pH	Predicted residual chlorine (mg L^{-1})	Observed residual chlorine (mg L^{-1})	Chlorine demand (mg L^{-1})	Prediction error [b] (mg L^{-1})	Prediction percentage error [c] (%)
Iceberg lettuce	1	0.07	0.039	10	19.04	20.25	5.90	10.98	11.25	9.00	-0.27	-2.38
				30	43.45	47.50	5.91	33.31	37.75	9.75	-4.44	-11.75
				60	80.07	81.75	5.93	61.37	56.50	25.25	4.87	8.62
				90	116.62	122.5	5.91	94.81	101.25	21.25	-6.44	-6.36
	2	0.08	0.022	10	12.73	12.25	6.00	9.61	4.25	8.00	5.36	126.01
				30	30.07	35.00	5.97	28.30	22.50	12.50	5.80	25.79
				60	73.76	69.75	6.03	56.71	53.50	16.25	3.21	6.00
				90	110.34	101.75	6.03	82.95	84.75	17.00	-1.80	-2.12
Strawberry	1	1.06	0.080	10	10.94	11.25	6.00	10.26	10.25	1.00	0.01	0.07
				30	35.38	34.50	6.03	29.28	23.75	10.75	5.53	23.28
				60	71.95	73.75	6.02	61.48	55.50	18.25	5.98	10.77
				90	108.36	116.50	5.92	96.68	99.75	16.75	-3.07	-3.08
	2	0.68	0.091	10	12.71	13.00	5.99	10.24	7.50	5.50	2.74	36.55
				30	37.08	39.50	5.98	31.99	33.50	6.00	-1.51	-4.52
				60	73.75	72.50	6.03	58.97	65.50	7.00	-6.53	-9.96
				90	110.35	111.00	6.04	90.53	85.25	25.75	5.28	6.19

[a] PPC = Total phenolics/(Total protein/ΔE).

[b] Prediction error = Predicted residual chlorine – Observed residual chlorine.

[c] Prediction percentage error = (Prediction error)/(Observed residual chlorine) × 100.

Table 4-4. *E. coli* O157:H7 reductions of validation study on iceberg lettuce and strawberries.

Treatment [a]	Iceberg lettuce				Strawberry			
	Rep 1		Rep 2		Rep 1		Rep 2	
	Log recovery (log CFU/g)	Log reduction (log CFU/g)	Log recovery (log CFU/g)	Log reduction (log CFU/g)	Log recovery (log CFU/g)	Log reduction (log CFU/g)	Log recovery (log CFU/g)	Log reduction (log CFU/g)
Control	6.75		7.03		5.73		5.52	
DI water	6.26	0.49	6.64	0.39	4.50	1.23	4.57	0.96
Residual - 10	5.71	1.04	6.26	0.77	4.32	1.40	4.09	1.44
Residual - 30	5.76	0.99	6.10	0.93	4.34	1.39	4.16	1.37
Residual - 60	5.52	1.22	6.07	0.96	4.12	1.60	4.05	1.48
Residual - 90	5.51	1.24	5.84	1.19	3.78	1.95	3.89	1.63

[a] Residual - 10, Residual - 30, Residual - 60 and Residual - 90 are NaOCl solution at pH 6.0 and targeted residual chlorine concentration at 10, 30, 60 and 90 mg L^{-1}, respectively.

References

Afari GK, Hung YC, King CH. 2015. Efficacy of neutral pH electrolyzed water in reducing *Escherichia coli* O157: H7 and *Salmonella* Typhimurium DT 104 on fresh produce items using an automated washer at simulated food service conditions. J Food Sci 80(8):M1815-M22.

Batz MB, Hoffmann SA, Morris JG. 2011. Ranking the risks: the 10 pathogen-food combinations with the greatest burden on public health. Emerging Pathogens Institute, University of Florida.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72(1-2):248-54.

Chang E, Chiang P, Chao S, Lin Y. 2006. Relationship between chlorine consumption and chlorination by-products formation for model compounds. Chemosphere 64(7):1196-203.

Chen X, Hung Y-C. 2016. Predicting chlorine demand of fresh and fresh-cut produce based on produce wash water properties. Postharvest Biol Technol 120:10-5.

Chen X, Hung Y-C. 2017. Effects of organic load, sanitizer pH and initial chlorine concentration of chlorine-based sanitizers on chlorine demand of fresh produce wash waters. Food Contr 77:96-101.

Delaquis PJ, Fukumoto LR, Toivonen PM, Cliff MA. 2004. Implications of wash water chlorination and temperature for the microbiological and sensory properties of fresh-cut iceberg lettuce. Postharvest Biol Technol 31(1):81-91.

EPA U. 2009. National primary drinking water regulations. Total Coliforms Fecal Coliforms *E Coli* 54.

Fan X, Sokorai KJ. 2015. Formation of trichloromethane in chlorinated water and fresh-cut produce and as a result of reaction with citric acid. Postharvest Biol Technol 109:65-72.

Gil MI, Selma MV, Lopez-Galvez F, Allende A. 2009. Fresh-cut product sanitation and wash water disinfection: Problems and solutions. International Journal of Food Microbiol 134(1-2):37-45.

Luo Y, Nou X, Yang Y, Alegre I, Turner E, Feng H, Abadias M, Conway W. 2011. Determination of free chlorine concentrations needed to prevent *Escherichia coli* O157:H7 cross-contamination during fresh-cut produce wash. J Food Protect 74(3):352-8.

Pangloli P, Hung Y-C. 2013. Reducing microbiological safety risk on blueberries through innovative washing technologies. Food Contr 32(2):621-5.

Park H, Hung Y-C, Chung D. 2004. Effects of chlorine and pH on efficacy of electrolyzed water for inactivating *Escherichia coli* O157: H7 and *Listeria monocytogenes*. Int J Food Microbiol 91(1):13-8.

Pirovani M, Guemes D, Piagnetini A. 2001. Predictive models for available chlorine depletion and total microbial count reduction during washing of fresh-cut spinach. J Food Sci 66(6):860-4.

Ramos B, Miller F, Brandão TR, Teixeira P, Silva CL. 2013. Fresh fruits and vegetables—an overview on applied methodologies to improve its quality and safety. Innov Food Sci Emerg Technol 20:1-15.

Rodgers SL, Cash JN, Siddiq M, Ryser ET. 2004. A comparison of different chemical sanitizers for inactivating *Escherichia coli* O157: H7 and *Listeria monocytogenes* in solution and on apples, lettuce, strawberries, and cantaloupe. J Food Protect 67(4):721-31.

Shen C, Luo Y, Nou X, Wang Q, Millner P. 2013. Dynamic effects of free chlorine concentration, organic load, and exposure time on the inactivation of *Salmonella*, *Escherichia coli* O157: H7, and non-O157 Shiga toxin–producing *E. coli*. J Food Protect 76(3):386-93.

Singleton V, Rossi JA. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 16(3):144-58.

Yeni F, Yavaş S, Alpas H, Soyer Y. 2016. Most common foodborne pathogens and mycotoxins on fresh produce: a review of recent outbreaks. Crit Rev Food Sci Nutr 56(9):1532-44.

Zaid B, Saidi D, Benzaid A, Hadji S. 2008. Effects of pH and chloride concentration on pitting corrosion of AA6061 aluminum alloy. Corros Sci 50(7):1841-7.

CHAPTER 5

SUMMARY AND CONCLUSIONS

This research provides detailed information about produce wash water properties and their correlations with chlorine demand. Wash water quality parameters of ten different types of fresh and fresh-cut produce were determined. UV254 had the highest correlation coefficient with chlorine demand of various produce wash waters among tested water quality parameters. In addition, various types of fresh produce can be divided into two clusters based on their PPC values. Chlorine demand of produce wash water was highly correlated with UV254 for different types of produce in the same cluster.

Besides the type of fresh produce, chlorine demand was also shown to be affected by washing conditions. Chlorine demand of romaine lettuce wash water increased with increasing initial chlorine concentration and organic load. Increasing the pH of NaOCl from 2.5 to 9.5 led to decrease in the chlorine demand except slight increase from 6.0 to 8.0. Equations to predict chlorine demand for different types of fresh produce, chlorine-based sanitizers, UV254, initial chlorine concentration and sanitizer pH were developed.

Chlorine demand prediction equations were then further modified to estimate initial chlorine concentration needed to reach a targeted residual chlorine for produce washing process. Based on these equations and previous findings, an effective disinfection strategy to enhance microbial safety and minimize DBPs formation potential for fresh produce postharvest washing was developed. The findings reported here will help the produce industry to improve their current chlorine-based sanitation processes, as well as be useful for regulatory agencies to develop future guidelines and polices for fresh produce postharvest washing process.