Jalapeño peppers are usually consumed raw and fresh with minimal processing. They contain antioxidants and bioactive compounds which are of human health benefits. There have been an increasing number of reports of foodborne outbreaks attributed to inaccessibility of sanitizers to *Salmonella*, *E.coli* and *Listeria monocytogenes* when embedded in fruit and vegetable surfaces. Furthermore, increasing sanitizer strength to compensate has been reported to impair the food quality attributes of the pepper fruit.

The objectives of the first part of this project were 1) to investigate the efficacy of combining electrolyzed oxidizing (EO) water and ultraviolet (UV) light on the microbiological quality (both the surface and internalized) of fresh Jalapeño peppers, and 2) to examine the effect this combination on the shelf life and appearance of the treated Jalapeno pepper. The effects of varying contact times (10, 20 and 30 minutes) all...
through 10 days of storage study were documented. *Serratia marcesens* was used as the marker organism and as a surrogate for *Salmonella*. *S. marcesens* and yeasts showed a significant reduction (p=0.0044 and 0.0134 respectively) among all the treatments and control while the aerobic plate count (APC) and coliforms counts showed no significant response to treatments (p = 0.2568 and 0.3996 respectively). In addition, there was no significant difference in responses of the pepper to various contact times with EO water and storage times, though evident effects which varied among treatments were observed. Compared to control, all EO and UV treatments reduced contamination and had no negative impact on the color, texture and the final mass of pepper. Overall, peppers treated with and UV and EO water for 30 minutes gave the best microbial inhibition.

The second part of the project focused on investigating the effect of gas exchange of Jalapeño pepper with nitrogen, oxygen and air on the uptake of EO water. The efficacy of EO water uptake in reducing surface and internalized *S. marcesens* and other native microflora was documented. In addition the post treatment effect of these treatment on the color, mass and texture of peppers was studied during 10 days of storage. Uptake of treatment solution was highest in oxygen exchanged peppers. Oxygen exchanged peppers gave the highest reduction in microbial count, yet maintaining their firmness and color.

Overall, the results of this project suggest that EO water and UV reduced microbial contamination as well as preserved the quality of Jalapeño peppers. Also, gas exchanged enhanced uptake of treatment solution and significant reduction of both internalized and surface microbial contaminants without imparting the freshness of the pepper. The findings from this project provide important groundwork for exploring the potentials of combining UV light’s antimicrobial effect with EO water for sanitization purposes. Moreover, enhancement of the uptake of treatment solution into fruits by gas exchange can be applied to reduce microorganisms lodged within the tissues of fruits and vegetable without risk of altering its physical attributes.
Efficacy of Electrolyzed Oxidizing (EO) Water Combined with UV-C light or Gas Exchange on Sanitization of Jalapeño Peppers using *Serratia marcescens* as Surrogate for *Salmonella*

by

Olufunmilayo R. Ekundayo

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Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

____________________________________________________________________

Olufunmilayo R. Ekundayo, Author
ACKNOWLEDGEMENTS

A journey that started out with hesitant, tentative steps and filled with wonderful memories has finally come to an end. I could not have accomplished this without the help of many people who dedicated their time, materials and effort to its successful completion.

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I cannot find words to express my gratitude to my confidante, brother and friend of many years, Oyebode Famubode, for cheering me all the way even when times were hard and I almost gave up. Thanks for your prayers, patience, endurance and love. A heartfelt thank you to my family for their selfless and unconditional love. Their prayerful, mental, emotional and financial support gave me reasons to go on.

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DEDICATION

To my late parents, Oguntoye and Adebola Ekundayo, who loved me to death. They told me I could touch the skies and reach the stars. Though I have a long way to go, I can proudly say I just got one of the stars. Their ideals and morals are not lost on me and it is to their loving memory that I dedicate this thesis.
Efficacy of Electrolyzed Oxidizing (EO) Water Combined with UV-C light or Gas Exchange on Sanitization of Jalapeño Pepper using *Serratia marcescens* as Surrogate for *Salmonella*

1. **INTRODUCTION**

Jalapeño pepper, a type of fruit prized for its warm burning sensation is considered nutritious, healthy, and low in saturated fat, sodium and cholesterol. It contains antioxidants and other bioactive compounds, which have been reported by various studies to reduce risk of death due to cardiovascular diseases, tumor development and cancer (Nomura and others 2008; Agudo and others 2007; Liu and others 2000).

Various preharvest factors (e.g., indigenous microbial population in the soil environment, and postharvest factors (e.g., handling, container sanitation and food processing procedures) contributes to microbiological contamination of fresh produce such as peppers (Beuchat 2002; Ryu 1997). International trading and worldwide transport as well as agronomic advancements such as the use of improperly composted organic manure instead of chemical fertilizer, use of untreated sewage water to irrigate or runoff water and animal feces has may be contributing factors to contamination of produce (Ryu 1997). In addition, ecological factors such as the environment, pH of plant tissue, and the protective cuticle on plant surfaces may influence pathogen survival/persistence.

Internalization of microorganisms also occurs within plant tissue via hydathodes in the leaf margin and stomata under high intensity of light (Gomes and others 2009; Underwood and others 2007). Storage temperature is a post harvest condition that predisposes the fruits and vegetables to internalization and may be due to increase in microbial activity (Kroupitski and others 2009). Some other studies have shown that the edible part of fruit or vegetable can be contaminated without direct exposure to irrigation water suggesting that surface sanitation may not be sufficient in eliminating microorganisms (Solomon and others 2002).
Internalization can also occur as a result of mechanical injury to plant tissue, as shown by the studies on tomato plants surface inoculated from the early stage of flowering still contained *Salmonella* (Guo and others 2001). *Salmonella* was also found to have infiltrated into the tissues, stems and cotyledon of hydroponically grown tomatoes (Guo and others 2002).

Insect predation has also been reported to aid internalization (Beuchat 2002). Investigations have also shown that vacuum cooling increases the internalization of *E. coli* into lettuce tissue when compared with a control treatment (Li and others 2008). Plant root junctions which are exudate release sites, have been observed to be sites of active internalization as a result of openings through which microorganisms can enter into the tissue of plants (Jablasone and others 2005).

In the wake of the *Salmonella* Saintpaul outbreak of 2008, in which Jalapeno Serrano peppers were implicated, 1442 people were sickened, 286 people hospitalized and 2 people died (CDC 2008). Conventional methods of surface sanitization include the use of chlorine dioxide gas (Kim and others 2008), trisodium phosphate (Liao and Cooke 2001), aqueous ClO₂, ozonated water, thyme oil (Singh and others 2002a) to mention a few. Efficacies of routine protocols of sanitations have been limited by these internalized microorganisms.

Electrolyzed oxidizing (EO) water, due to its ease of production, application, handling and modification, has made it more desirable method of sanitation than other chlorine-based or acid based sanitizers. It is produced by electrolysis of a dilute solution of NaCl and passing an electric current through an ion exchange membrane (Al-Zenki and Al-Omariah 2006). It also has a relatively low cost of production. The antimicrobial activity is attributed to its high oxidation reduction potential, low pH and high residual chlorine which can be easily modified to reduce toxicity to fresh tissues of fruits and vegetable (Kim and others 2000a; Kim and others 2000b). Its wide application varies from optometry (Shimmura and others 2000) to poultry industry as sanitizers (Park and others 2002). It has been documented to be effective in the inactivation of pathogen associated with fruits and vegetables. *E. coli, Salmonella spp, Listeria spp, Bacillus sp,*
*Pseudomonas* and fungi among others (Park and others 2004). According to the Environmental Protection agency (EPA), the use of EO water is a more desirable method of sanitation because of its safe application and potential of being less hazardous and more environmentally safe, as it does not form carcinogenic residues like other chlorine based sanitizers (EPA 2010).

In the meantime, ultra violet light C also has shown antimicrobial potency against various organisms. It has been used to disinfect fresh fruits and vegetable such as zucchini squash (Erkan and others 2001), strawberry, (Allende and others 2007), watermelon (Artés-Hernández and others 2010), broccoli (Costa and others 2006), baby spinach (Escalona and others 2010) and pepper (Vicente and others 2005), all of which have varying levels of effectiveness.

The principle of gas exchange of fruits and vegetables is applied so as to create vacuum either via temperature or pressure (non thermal) differential. This vacuum is relieved by the influx of molecules such as microorganism and gas, through the fruit’s respiratory pore. (Bartz and Showalter 1981). This method was used in the brining of cherry pepper (Daeschel and others 1990), cucumbers (Daeschel and others 1985) and sanitization of alfalfa seed (Stan and Daeschel 2003).

The objectives of this study are 1) to investigate the efficacy of combining EO water with UV in reducing microbial population and its effect on the shelf life of Jalapeño pepper and 2) to document the effect of gas exchange on uptake of treatment solution and subsequent effect on the microbial and physicochemical properties of Jalapeño pepper. *Serratia marcesens* was used a surrogate organism because of the ease of identification by producing a unique pigment (prodigiosin) which ranges from light pink to orange. It is also nonpathogenic except in nosocomial infections in immunocompromised individuals (Hejazi and Falkiner 1997).

Chapter 2 details the background reports on various health benefits of Jalapeno pepper, concerns about its contamination, outbreaks, associated pathogen and methods of sanitation. Chapter 3 documents the efficacy of using EO water in combination with UV-C light for sanitation of fresh Jalapeño peppers and their effect on the internalized
microorganism and overall quality, in comparison with using EO water only. A shelf life study was also carried out by analyzing for the microbial population, texture, color and mass of the peppers for 10 days of storage. Chapter 4 presents the effects of gas exchange of pepper with nitrogen and oxygen gas on the rate of uptake of treatment solution and its subsequent effect on both the surface and internalized microorganism. It also reports the effect of these treatments on the color, texture and the final weight of pepper over the course of 10 storage days.
2. LITERATURE REVIEW

2.1. Jalapeno peppers

2.1.1. General Properties

Jalapeño pepper is a type of fruit prized for its warm burning sensation when eaten. It is named after Jalapa, the capital city of the state of Velacruz. It contains capsaicin, the compound which produces a burning sensation (Rowland and others 1983). Jalapeño peppers are rated between 2500 to 10000 scoville units in heat which is considered to be moderately hot (Borges 2001). The burning heat sensation is primarily due to capsaicin and other capsinoid related compounds (Reilly and Crouch 2001).

Jalapeños are considered nutritious, healthy, and are low in saturated fat, sodium and cholesterol. 100 g of Jalapeno peppers contains about 30 calories, and is approximately composed of 92g of water, 1.35g of protein, 6g of carbohydrate, 2.8g of fiber and 3.36g sugars. They are also a significant source of vitamins A (retinol), C (ascorbic acid), K (phylloquinone), B6 (pyridoxine), B9 (folic acid), B3 (Niacin) and B1(thiamin), iron, magnesium, phosphorus, copper and manganese. A 100g serving of jalapeno peppers contain about 16% and 74% of the daily requirement of vitamin A and C respectively (USDA). They can be eaten raw either in the green stage or red (fully ripen), baked, fried, pickled, smoked or roasted.

2.1.2. Health benefits

Jalapeño peppers are a rich source of antioxidants, which provide protective roles of preventing cellular damage commonly associated with aging and other diseases. They scavenge free radicals of oxygen that may attack body cells. Free radicals, because of their high reactivity, react with low density proteins and lipids, leading to cell death and loss of function (Perucka and Materska 2007). A report from a Spanish cohort study observed that fruit and vegetable consumption helped reduce general mortality by mitigating the action of free radicals. (Agudo and others 2007)
Several observational studies have shown that fresh fruits and vegetables help reduce cancer risk. Specifically, fruit and vegetable consumption helps prevent esophageal, laryngeal, and pharyngeal cancer (Nomura and others 2008; Terry and others 2001). An inverse relationship between fruit and vegetable consumption was shown for colorectal cancer in men (Nomura and others 2008). According to (Liu and others 2000), greater intake of fruits and vegetables reduces the risk of death due to cardiovascular disease, the leading cause of death in the United States (CDC 2009b), though this association may be dependent on lifestyle choices such as smoking. There has been an increase in consumption of fresh fruits and vegetables and this is likely due to increased awareness of the healthy benefits associated with it (van Duijnhoven and others 2009).

Peppers, like other fruits vegetables, are very beneficial for human health since they contain lots of bioactive compounds such as antioxidants which have been associated with lowering the risk of cancer. They contain dietary fiber, which studies have shown contributes to a lower colorectal cancer risk in men (Nomura and others 2007). They also contain folic acid which if deficient in the diet could induce gene expression alterations, carcinogenesis, and tumor development (Wan Du and Fang 2010).

2.1.3. Safety, economic, and health concerns

Despite all these benefits mentioned above, fruits and vegetables have increasingly been implicated as major sources of food borne outbreaks (FDA 2001). According to the Center of Disease Control (CDC), despite being underreported, fresh produce accounts for 12.3% of all outbreaks and about 22.7% of all food borne disease between 1990 and 2007 (Anonymous 2010). This, according to the U.S. Food and Drug Administration (FDA) may be due to an increase in consumption because of the awareness of the health benefits of consuming fresh fruits and vegetables. This is evident in the 32% increase in per capita consumption from 1982 to 1997 (FDA 2001). As suggested also by CDC, it may be due to an increase in surveillance, thereby an increase in the number of identified outbreaks that were once either unreported or underreported (Polyxeni 2005). This has also been attributed to the increasing popularity of eating food that is not prepared at home (FDA 2001).
Fresh produce have also been increasingly implicated in food borne outbreaks because of changes in the methods of processing, harvesting, agronomic practices, packaging, and food consumption patterns (Beuchat 2002). With facilities trending towards spanning new geographic areas while using more minimal processing of technologies has increased the risk of microbial contamination. The demand for fruits and vegetable especially from Mexico has increased due to the domestic demands exceeding the domestic supply. For instance, fresh produce imported from Mexico grew from 2.78 billion pounds in 2005 to 3.16 billion pounds in 2007 (USDA 2010). This has prompted the need for larger amounts of fresh produces being shipped, which has consequently increased the food safety and defense risk along the supply chain.

The anatomical structure of fruits and vegetables varies tremendously and is believed to be an important parameter in determining whether contamination can occur and persist. Peppers have a waxy cuticular surface which primarily functions as a permeability barrier against moisture and gas loss. The epicuticular wax covering the outer surface also gives the ability to repel water, forming beads. Other functions include scattering short wave radiation, preventing attachment of microorganisms, and preventing adherence of developing organs of the plant (Jeffree 2006). However, it has been shown that the pepper as a whole is not completely impermeable. Using dyes and gas, Daeschel and others (1990) revealed that the primary entry for gases and liquids is the stem area. The calyx structure (stem) was noted to have numerous gas exchange pores known as stomata (Daeschel and others 1990).

2.1.4. Outbreaks associated with contaminated produce

The last two decades (1990 – 2005) have not been without numerous incidences of food borne outbreaks with fresh produce accounting for 639 outbreaks and 31,496 associated illness, i.e. 13% of all outbreaks and 21% of associated illness. The most prevalent microbial contaminant was Norovirus (40%), followed by Salmonella (18%), E.coli(8%), Clostridium botulinum (6%) and Hepatitis A virus (4%) of all outbreaks between 1990 and 2005 (DeWaal and Bhuiya 2007).
Several outbreaks of *Salmonella* have been reported; many of them are associated with fresh and processed animal products. During the winter of 1995-1996, Oregon and British Columbia reported outbreaks of Salmonellosis with 133 cases reporting diarrhea as a result of consuming alfalfa sprouts contaminated with *Salmonella enterica serovar Newport*. Also in the fall of the same year (1995), a multistate outbreak with 1213 cases was reported (Van Beneden and others 1999). In June 2003, *Salmonella* Saintpaul was isolated in a California outbreak with mangoes and tomatoes as the unconfirmed vehicles, 17 people became ill. Four years earlier (1999), 17 people were sickened from Salmonellosis after eating clover sprouts (Anonymous 2007b). In October 2006, a multistate outbreak occurred in Australia. Trace back revealed that patients had consumed cantaloupe, with 36 cases associated with *Salmonella* Saintpaul isolated from patient feces (Munnoch and others 2009).

According to the European Center for Disease Control (ECDC), *Salmonella* is most commonly resistant to antimicrobials such as sulphonamides, nalidixic acid, ampicillin and tetracycline. Some were even multidrug resistant (Anonymous 2007a). *Salmonella* Saintpaul was one of the top 15 most frequently reported serotypes of *Salmonella* between 2007 and 2008. About 66% of those affected were in the age range of 15 to 64 years; individuals 5 years or less accounted for 24% (Anonymous 2008). An outbreak of 463 cases of human Salmonellosis was reported in Germany alone between 2001 and 2009 (Beutlich and others 2010). In this study, many of the strains isolated were found to be antimicrobial resistant. The ECDC also reported that 10,300 cases of Salmonellosis were reported in 18 countries in 2008. Of these cases, 9,790 were analyzed and compared to the corresponding quarter of the previous year (2007), which reported 9,226 cases. This showed an obvious increase in incidence. *Salmonella* Saintpaul accounted for about 0.6% of these cases (Anonymous 2008).

In May of 2005, *Salmonella* Saintpaul and *S. typhimurium* were isolated from a U.S. outbreak that lasted throughout July of 2005. Patients commonly reported drinking orange juice prior to sickness. It was reported as the first outbreak associated with orange juice since the inception and implementation of juice HACCP (Hazard Analysis
& Critical Control Points). According to the CDC, although 157 cases were reported across 23 states, it is estimated that 6000 additional persons may have been infected but not reported. The initial source of contamination was never identified nor was the means through which the orange juice became contaminated, confirmed.

On the other hand, peppers have not been a common vehicle for food borne outbreaks. However, one of the largest outbreaks of botulism in the U.S. occurred in Michigan (1977) in which 59 people were sickened as a result of consuming home canned jalapeño peppers in a Mexican restaurant (MacDonald and others 1986). Worthy of mention was the Salmonella Saintpaul outbreak of 2006, involving 43 states, the District of Columbia and Canada, leaving 1442 people ill, 286 people hospitalized and 2 people dead. Salmonella Saintpaul with same genetic finger print was isolated in all of these cases (CDC 2008). A stakeholder-sponsored investigation as reported by the Produce Safety Project (PSP) revealed the source of contamination initially as tomatoes, but could not conclusively link them to it. However, jalapeño and Serrano peppers grown and imported from Mexico were eventually confirmed as the source. As a result of the initial implication of tomatoes as the culprit, the tomato sector of the vegetable industry was severely affected, costing the produce industry of Florida and Georgia more than $100 million and $14 million, respectively ((PSP) 2008).

2.2. Bacterial pathogens associated with fresh fruits and vegetables

2.2.1. Salmonella

Salmonella is a rod-shaped Gram negative, non-spore forming organism. Salmonella was first isolated by Salmon and Smith in 1889 from swine as a bacillus responsible for hog cholera. It is made up of two species, S.enterica and S. bongori. S.enterica consists of 6 subspecies. This group is referred to as Salmonella Subspecies I, one of which is S. enterica subsp. enterica (Popoff and others 2003). Salmonella Saintpaul (S. enterica subsp. enterica serovar Saintpaul) is one of several serovars within the subspecies. Salmonella has been reported to be the leading cause of bacterial food borne illness, according to the Center for Science in Public Interest (CSPI 2007). Salmonella can
usually be isolated from poultry which is considered the largest reservoir (Irwin and others 1994).

In the 1990s, *Salmonella* began to increasingly become associated with fresh produce in food borne outbreaks. Every year *Salmonella* has been estimated to cause 1.4 million illnesses and 400 deaths in the US (Jain and others 2009). According to the CDC, it was the leading cause of food borne outbreaks in the US between 1998 and 2007 with nuts, fruits and vegetables accounting for the largest number of single commodity outbreaks. In schools, *Salmonella* was reported to be the leading cause of food borne outbreaks accounting for 36% of incidences with known etiology, with fruits and vegetables being the most frequent carriers after poultry (Daniels and others 2002)

2.2.2. *Escherichia coli*

2.2.2.1. General properties of *Escherichia coli*

*Escherichia coli* is a Gram-negative, non-spore forming, rod-shaped bacterium. It is motile by means of peritrichous flagella. It may also be non-motile, capsulated or microcapsulated. It is a facultative anaerobe, which grows at an optimal temperature of 37°C. Most strains of *E.coli* are natural inhabitants of the human and animal gastrointestinal tract; they are usually harmless and serve a protective function of preventing the growth of harmful bacteria. It belongs to the Enterobacteriaceae family which includes other genera like *Salmonella, Shigella, and Yersinia* (Feng and Weagant 2002). *E. coli* has been reported to be capable of surviving in adverse conditions such as low temperature (Digirolamo and others 1970), high acidity and low pH (Lin and others 1996).

*Escherichia coli* was first described in 1885 by an Austrian pediatrician, Theodor Escherich, as a harmless commensal microorganism. It was first identified as a pathogen in 1971, when an outbreak involving 400 cases in 14 states of the US was reported. The outbreak involved imported cheese contaminated with Enteroinvasive *E.coli* (Marier and others 1973). Despite this, it was still considered to be of low virulence until the 1980s when many strains were isolated as the causative agents of
severe gastrointestinal infections (Jay and others 2005b). A scheme proposed in the 1940s classified *E. coli* on the basis of its lipopolysaccharide or somatic antigen (O), its flagella (H) and its polysaccharide antigen (K) (Myron 1987). Based on this, about 174 O antigens and 53 H antigen serogroups have been identified both constituting the O: H system of classification (Bhunia 2008).

Diarrheagenic *E. coli* are pathogenic *E. coli* strains that have potential to cause diarrhea in humans and can be classified into 6 major pathotypes, based on virulence properties, mechanisms of pathogenicity, and clinical symptoms. They are enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), diffusely adherent *E. coli* (DAEC), and enteroaggregative *E. coli* (EAEC) (Fratamico and others 1997). Enteroaggregative *E. coli* (EAEC) produces heat stable enterotoxins while ETEC produces both heat labile enterotoxins (similar to toxins produced by *Vibrio cholera* (Ctx) in terms of size, sequence, antigenicity and function) and heat stable toxins that are resistant to boiling for 30 minutes (Feng and Weagant 2009). Enterotoxigenic *E. coli* is a causative agent in traveler’s diarrhea, a symptomatic watery diarrhea, mostly with no fever. In terms of its invasiveness, low infectious dose and unknown human reservoir, EIEC resembles *Shigella*. Mostly associated with EPEC is the profusely watery diarrhea. It is a leading cause of infantile diarrhea in developing countries, despite the fact that it does not produce an enterotoxin. It possesses a plasmid which has adherence factors that enable it to attach to human tissue, causing destruction of the microvilli of the intestinal mucosa and eventually causing dysentery (Jay and others 2005a). It requires an infectious dose of about $10^6$ organisms. Little is known about the DAEC groups but they are also capable of causing diarrhea (Feng and Weagant 2009).

Of all these pathotypes, EHEC is the most significant group based on the severity of illness (Doyle 2001). It produces shiga-like toxins which have 2 prototypes: Stx1 and Stx 2. It is also capable of attaching to cells with the aid of a plasmid encoded fimbrae. The commonly known example of this group is *E.coli* O157:H7. It can be uniquely distinguished from all other *E. coli* serotypes by its inability to ferment D-sorbitol
This strain of *E.coli* was first isolated in two major outbreaks in 1982. At least 47 people from Oregon and Michigan were reported as ill, characterized by severe abdominal pain, watery diarrhea which worsened to bloody diarrhea. Feverish symptoms were rare and symptoms persisted for 3 – 7 days. The strain of *Escherichia coli* isolated did not match with previously identified ones. It was, however, linked to a previously isolated *E.coli* serotype identified in meat in 1975 which caused hemorrhagic colitis (Riley and others 1983).

Hemolytic Uremic Syndrome (HUS) is a complication and a progressive illness from hemorrhagic colitis. Symptoms include severe abdominal cramps, bloody (in some cases non-bloody) diarrhea, vomiting, and fever (Noris and Remuzzi 2005). *Escherichia coli* O157: H7 has a low infectious dose of 10 to 100 cells and therefore presents a serious public health concern. It can be detected using commercially available ELISA kits, or by detection of toxins (Stx 1 and 2) using a cytotoxicity assay (Feng and Weagant 2002).

**2.2.2.2. Food borne disease associated with *E.coli***

Prior to 1982, *E. coli* O15:H7 was not recognized as a food associated pathogen of public health concern. According to the CDC (2009), *E. coli* has been estimated to be responsible for 70,000 infections and illnesses and over 50 deaths every year; this number is still considered inaccurate as many incidences are never reported (Mead and others 1999). Most food borne outbreaks were usually associated with consumption of raw or undercooked meat (Abdul-Raouf and others 1993) and unpasteurized or poorly processed milk and milk products (Wells and others 1991). This is not surprising since the intestines of cattle and other animals are considered to be the largest reservoirs of *E. coli* O157:H7 (CDC 2009a). Fresh produce used to be considered free from such contamination until several outbreaks began to be reported of *E. coli*-tainted fresh fruits and vegetables.

Fresh pressed unpasteurized or poorly processed apple cider was reported to have sickened more than 20 people in Massachusetts because of *E.coli* O157:H7 (Besser and others 1993). Other vegetables that have been tainted with this bacterium are lettuce
(Ackers and others 1998), alfalfa sprouts from contaminated seeds (Breuer and others 2001) and white radish sprouts in Japan (Michino and others 1999). Other vegetables identified as sources include the *E. coli* O157H7 outbreak of October of 2006, involving fresh packaged spinach. About 199 people were sickened and 3 people died as a result of complications from Hemolytic Uremic Syndrome (HUS)(CDC 2006).

### 2.2.3. Listeria Monocytogenes

#### 2.2.3.1. General Properties

*Listeria monocytogenes* is a rod-shaped, Gram-positive, non-spore forming and non-acid fast organism. It grows under anaerobic and aerobic conditions, but has been found to grow best in a microaerophilic environment. It grows optimally at pH 6-8 and over a temperature range of 1-45°C. It is ubiquitous in nature and can be isolated even in adverse conditions of low pH and high salt concentration (Jay and others 2005a).

*Listeria monocytogenes* was first isolated by E.G.D. Murray from the blood of laboratory rabbits and at the time was known as *Bacterium monocytogenes* (Hof 2003). *Listeria monocytogenes* used to belong to the Corynebacteriaceae family but it is now found to be more related to and is grouped with the *Clostridium, Bacillus, Lactobacillus* and *Streptococcus* families (Jay and others 2005b). Other *Listeria spp* are *L.innocua*, *L. seeligeri*, *L. welshimeri*, *L. ivanovii*, and *L. grayi* (Rocourt 1999; Swaminathan 2001).

#### 2.2.3.2. Food borne disease associated with *Listeria monocytogenes*

According to the CDC, *Listeria monocytogenes* is also responsible for one of the most important food borne illnesses - listeriosis - due to its high mortality rate. Contaminated foods are considered the primary source of *Listeria* in people who contract listeriosis (Farber and Peterkin 1991). Listeriosis most often occurs in patients with a suppressed or compromised immune system. Predisposing factors include pregnancy, old age, alcoholism, and individuals undergoing immune suppressive therapies. Symptoms of Listeriosis include fever, muscle ache, nausea, and diarrhea, and complications may
lead to infection of the nervous system causing stiff neck, convulsion and loss of balance (CDC 2009c).

According to the CDC (2009c), Listeriosis has a case fatality of about 20% with approximately 2500 cases and 500 deaths every year in the US. *Listeria monocytogenes* has been isolated from many outbreaks such as the one in 1983 involving milk. About 49 people were sickened, leaving 14 people dead. In Switzerland from 1983-1987, 122 cases of Listeriosis were reported after consuming cheese, which led to 34 deaths. A similar outbreak associated with soft cheese occurred in 1985 in California, which led to about 142 cases and 48 deaths (Farber and Peterkin 1991). Cases of Listeriosis have however not been limited to meat and animal products; *L. monocytogenes* has been isolated from fresh produce such as cabbage (including a coleslaw case in 1981 in Canada) (Schlech III 1983), mixed salad (Little and others 2007), strawberries and mushrooms (Johannessen and others 2002).

Due to its high severity, case fatality and mortality, a zero tolerance regulation for *Listeria monocytogenes* is currently put in place by Food Safety and Inspection Service (FSIS). No detectable limit is hence permitted otherwise such foods are recalled (Anonymous 2000). It can be detected and isolated using the Christine-Atkins-Munich-Peterson (CAMP) test. It is a useful presumptive test. Other methods include the GeneTrak *Listeria monocytogenes* test, the Probelia *Listeria monocytogenes* test kit, and the VIDAS *Listeria monocytogenes* test kit (Hitchins 2002).

### 2.2.4. *Serratia Marcescens* as a surrogate food borne pathogen

*Serratia marcescens* is a rod-shaped Gram-negative bacterium. It is a facultative anaerobe which grow at a temperature range of 5-40°C. It produces a red pigmentation called prodiogiosin which disappears sometimes in older colonies. *Serratia marcescens* is widely distributed in environment and naturally found in wet places such as soil, water and plant surfaces (Hejazi and Falkiner 1997). ‘*Serratia*’ was coined in 1819 by a Venetian pharmacist, Bartolomeo Bizio, and named after an Italian Physicist, Serafino Serrati. He observed that some of the pigments faded away with time and this gave meaning to its name ‘marcescens’ (fading away). The name was changed twice in 1848
to *Monas prodigiosus* and later to *Bacillus prodigiosus*. Later in 1861, the name was changed back to *Serratia marcescens* (Sehdev and Donnenberg 1999).

*Serratia marcescens* belongs to the Enterobacteriaceae family along with *Salmonella spp*. According to the Bergey’s Manual of Identification, they are both lactose fermenters, unable to split indole, tryptophan (negative indole test) and urease. They are closely related except when tested for production of hydrogen sulfide gas, when *Salmonella* tests positive and *Serratia* is negative. It is also identified by its characteristic pigment and this obviates the need for further biochemical tests (University of Idaho Accessed June 2010).

Prodiogiosin, the pigment produced by *S. marcescens*, has been reported to be dependent on age of culture and NaCl concentration, which varies among strains (Hejazi and Falkiner 1997). It was considered a saprophytic microorganism and hence used as a marker for its unique pigment, but is now considered as an opportunistic and sometimes pathogenic mainly in immunocompromised individuals. However, it has not been implicated in food borne illness. *S. marcescens* has been used as a marker organism various studies because of its characteristic red pigment. Krisch and others (2008) used it in comparison with *Bacillus cereus* and *E.coli* to study the inhibitory effects of low pH and high anthocyanin. It was reported that *S. marcescens* showed the most (Krisch and others 2008).

### 2.3. Contamination and internalization

Contamination of fresh produce such as peppers occurs as a result of preharvest factors such as indigenous microbial population in the soil environment, and the level of fertilization of the soil which ultimately influences the microbial load. Post harvest factors include handling, container sanitation and food processing procedures (Ryu and Jee-Hoon 1997; Beuchat 2002). There has been an increasing trend in worldwide travel that may be responsible for the appearance of pathogens to and from various countries (Altekruse and others 1997). International trading and travel may expose people to pathogens that they have never been in contact with (Beuchat 2002). Agronomic advancements may have also been responsible such as the use of improperly composted
organic manure instead of chemical fertilizer, use of untreated sewage water to irrigate or runoff water and animal feces (Ryu 1997). Extrinsic ecological factors such as the environment may influence survival of microorganisms while intrinsic factors including the pH of plant tissue and the protective cuticle on plant surfaces may influence pathogen survival/persistence.

Internalization of microorganisms within plant tissue has been observed in various fruits and vegetables. It could occur either through a passive process where microorganisms rely on natural openings or accidental wounds to gain entry, or via an active process whereby they degrade tissue to gain entry to the plants. Microorganisms gain entry through the wounds, hydathodes, stomata, root and stem lenticels, and leaf margins (Bartz 2005). This was also confirmed by (Gomes and others 2009) stating that hydathodes in the leaf margin in the process of opening up to relieve plants of internal pressure from water could allow entry of microorganisms. The stomata was observed (Huang 1986) to open under high intensity light, enhancing internalization while the rate of internalization was insignificant in the dark (Underwood and others 2007).

Movement of Salmonella can also be induced towards plant stomatal openings by light intensity (Kroupitski and others 2009). Assmann and others (2003), noted that drought may induce the closing of stomata as observed in Arabidiopsis (Bolin 1989) as cited by Bartz (2005) also noted that excess water which leads to severe tissue congestion and a drop in oxygen level may cause minerals and metabolites to leak, thereby compromising the plant defense mechanism and supporting growth of plant pathogens and other microorganisms.

Elevated storage temperature is a post harvest condition that predisposes the fruits and vegetables to internalization and may be due to an increase in microbial activity (Kroupitski and others 2009). According to this study, sugar production, photosynthesis, motility, and chemotaxis of microorganisms are required for internalization to occur. It has been argued that motility should not be a requirement (Bartz 2005).

Studies have shown that edible portions of plants can be contaminated without direct exposure to the pathogen present in the irrigation water. This suggests that surface
sanitization is not adequate for eliminating microbial pathogens (Solomon and others 2002).

Experiments have shown that manure application process can also serve as a source of contamination with food borne pathogens. Solomon and others (2002) showed that irrigation water containing *E. coli* may result in contamination of the phyllosphere (edible portion of the plant) by transport through the root system (Solomon and others 2002).

Internalization can also occur as a result of mechanical injury to plant tissue. Studies have shown that tomato plants surface inoculated from the early stage of flowering still contained *Salmonella* (Guo and others 2001). In hydroponically grown tomatoes, *Salmonella* was also found to have infiltrated into the tissues, stems and cotyledon of tomatoes from the nutrient solution (Guo and others 2002).

Insect predation has also been noted as an important factor that aids internalization (Beuchat 2002). It has been shown that necrotic lesions shield pathogens from stress. These lesions formed from infection by *Xanthomonas* were seen to improve the survival of *E. coli* O157 H7 even though it could not be proven that its presence had a direct effect (Aruscavage and others 2008).

Internalization is also enhanced by the persistence of pathogens in soil. Mahbub and others (2004) demonstrated that pathogens such as enterohemorrhagic *E. coli* survived more than 7 months in the soil under harsh conditions of fall/winter weather. This finding has been reported to be of significance because continued application of contaminated irrigation water for less than 7 months increases chances of these pathogens’ survival and their ability to move vertically through the root system into the phyllosphere (Mahbub and others 2004).

Vacuum cooling is a process whereby fresh produce are uniformly cooled from a harvest temperature of about 28°C to about a 1 - 7°C (varies among produce) storage temperature within a short time, creating a vacuum in the intercellular air spaces of the plant material. This is done to improve the shelf life of produce (Anonymous 2010).
However, it has been reported to qualitatively and quantitatively increase internalization of *E. coli* into lettuce tissue when compared with a control treatment (Li and others 2008). Plant root junctions which are exudate release sites have been observed to be sites of active internalization as a result of openings through which microorganisms can enter into the tissue of plants (Jablasone and others 2005).

### 2.4. Disinfection and sanitization of fresh produce

Internalized microorganisms are protected from sanitizers and other stressful conditions in the free environment. However, a plant host response to invasion by microorganisms may compromise their long term survival. Since they are not plant pathogens (i.e. causal organisms) they fail to establish a presence that would have otherwise induced a rapid host response or elicited a defense mechanism such as stomata closing or secretion of toxins (Bartz 2005). Free living nematodes also help in protecting microorganisms. These parasites feed on water suspension which may contain microorganisms such as *Salmonella*. Once ingested, they survive in the guts of parasites and are protected from antimicrobial activities of sanitizers. These pathogens are then released when parasites defecate (Caldwell and others 2003).

Various methods have been adopted in surface sanitization of fruits and vegetables. The efficacy of chlorine, acidified sodium chlorite and peroxyacetic acid on *Salmonella* inoculated on the stem scar, smooth surface and puncture wounds of bell peppers and cucumber was investigated by Yuk and others (2006). This study revealed that aqueous sanitizers were efficient except in puncture wounds, with chlorine being the least efficient while a gaseous sanitizer (ClO$_2$) was most efficient (Yuk and others 2006). Surfactants have also been reported to enhance the activity of surface sanitizing. Hassan et al (2003) hypothesized that microorganisms attach to the surface of a plant based on its hydrophobicity (Hassan and Frank 2003). Surfactants help lower surface tension as a result of the expanding force of adsorption (Schramm and others 2003). This has been found to be effective on the surface of lettuce but not on cut surfaces which are hydrophilic in nature (Hassan and Frank 2003).
Trisodium phosphate (TSP) is also used as a sanitizer. Liao (2001) applied it to fresh cut green peppers at different concentrations and pH levels. A hundred-fold reduction in microbial load was observed. However, it was suggested that principal sites of attachment for pathogens were the wounded or cut surfaces which entrap and shield them from TSP treatment.

Singh and others (2002a), using different methods of inoculation (dip, sprinkle and drop) compared different sanitizers; deionized water, aqueous ClO₂, ozonated water and thyme oil on lettuce inoculated with E. coli O157 H7. Results showed that methods of inoculation may also impact effectiveness (Singh and others 2002a). Sequential treatment using all of these sanitizers was also found to be effective when applied to baby carrots (Singh and others 2002b).

2.4.1. Chlorine dioxide

The aqueous form of chlorine dioxide has been applied for use in sanitizing fresh produce. (Kim and others 2008) used chlorine dioxide at 50ppm to sanitize shredded iceberg lettuce. It was found to be effective against E. coli O157 H7, Salmonella typhimurium and Listeria monocytogenes. When combined with ultrasonication, it was found to be effective against E. coli on apples and lettuce (Huang and others 2006). In another study, using chlorine dioxide gas against these three pathogens, (Sun-Young and others 2004) found out that environmental factors such as humidity, temperature, and light may influence the effectiveness of this gas. (Mahmoud and Linton 2008) also applied chlorine dioxide gas to inactivate E. coli and Salmonella enterica on lettuce and it was found to significantly reduce microbial load.

2.4.2. Electrolyzed oxidizing water

Electrolyzed water, an alternative to chlorinated water, was an idea developed in Japan (Izumi and others 2000). Electrolyzed oxidizing (EO) water is produced by electrolysis of a dilute solution of NaCl passing an electric current through an ion exchange membrane which separates the anode from the cathode (Al-Zenki and Al-Omariah
2006). It is relatively inexpensive to produce as the production requires only deionized water and sodium chloride.

One of the desirable qualities of EO water is its safe application and potential of being less hazardous and more environmentally safe. It is also effective when compared to other strong acidic sanitizers within its range of pH and activity. It is much less corrosive to skin or organic products such as fruits and vegetables (Huang and others 2008). The potency of EO water is in its high oxidation reduction potential (ORP) of about +1100mV and pH of about 2.7. It also forms hypochlorous acid, which has antimicrobial properties. At the cathodic end, the basic electrolyzed water has a pH of 10.5 - 11.5 and an ORP of -800 to 900mV with active chlorine at about 10-90 ppm (Al-Zenki and Al-Omariah 2006).

Kim and others (2000) showed that the antimicrobial properties of EO water are a result of its high oxidation potential. This was carried out by modifying its different chemical properties (Kim and others 2000b). Their studies revealed that residual chlorine can be modified to a lower concentration while maintaining its effectiveness. Increase in residual chlorine and decrease in pH have been observed to increase the bactericidal effectiveness of EO water against *E. coli*, *Listeria monocytogenes* (Park and others 2004), and *Bacillus cereus* (Kim and others 2000a). Hypochlorous acid content was reported to have helped increase the permeability of the microbial cell by oxidizing the sulfhydryl compounds of the cellular surface, causing metabolic disruption and oxidation of cellular components (Leyer and Johnson 1997).

### 2.4.2.1. Application of electrolyzed oxidized water

Electrolyzed oxidizing water has been widely used in various industries as a sanitizer. In optometry, it has been applied as treatment for corneal ulcers in guinea pigs (Shimmura and others 2000) and in the poultry industry for inactivating *Campylobacter jejuni* (a common cause of human diarrhea) on the surface of chicken (Park and others 2002). It has also been studied as an alternative for fungicides in greenhouse crops (Buck and others 2002) and ornamentals (Mueller and others 2003). In comparison with sodium hypochlorite, it showed four times more potency in the reduction of *E. coli* and
Listeria monocytogenes when inoculated in strawberries (Udompijitkul and others 2007) though it couldn’t control the microbial load of pathogens in refrigerated storage.

2.4.2.2. Relevance

The safety of chlorine use in the food industry and environment has been questioned due to its formation of trihalomethane compounds when used to sanitize organic matter. These are compounds that have been reported by the Environmental Protection Agency (EPA) to induce cancer. Therefore, its wide application has been strictly regulated and limited, creating the need for a replacement (EPA 2010).

Electrolyzed oxidizing water can be produced on site, which eliminates the needs for transportation. It can easily be modified in order to reduce the chlorine while maintaining effectiveness (Al-Haq and others 2005). However, it has been reported to have negative effects (Rico and others 2008). For instance, high concentrations of free chlorine cause browning, plasmolysis and demineralization of materials even though it is effective in reducing microbial loads. It has also been observed to be corrosive to carbon, steel, copper, and aluminum. The use of stainless steel was therefore advised (Ayebah and Hung 2005). Kiura and others (2002) studied the efficacy of low salt EO water compared to high salt EO water which has higher amounts of free chlorine and is capable of forming cytotoxic byproducts. It was found that low salt EO water is also as effective as high salt EO water against pathogens (Kiura and others 2002).

2.4.2.3. Production

Electrolyzed water is generated by passing a dilute salt solution (a commonly used salt is sodium chloride) through an electrolytic cell, which consists of a positively charged anode and a negatively charged cathode separated by a membrane. When DC voltage is supplied to these inert electrodes, two types of solutions are generated as a result of migration of negatively charged ions to the anode and positive ones to the cathode. At the anode, the electrolyzed acidic solution has a pH between 2.5 -2.9 and an OR
potential of \( \approx +1100 \text{mV} \). This is the result of chloric and hydroxyl radicals to form hypochlorite which according to Izumi (1999) is bactericidal.

At the cathodic side of the cell, electrolyzed alkaline solution with pH of about 11.2-11.6 and OR potential of \( \approx -800 \text{mV} \) has been reported to have scavenging activity, i.e. antioxidative. It is also applied in the LCD and ULSI manufacturing process as a Silicon oxide particle remover (Kim and others 2000b; Hanaoka 2001).

### 2.4.3. Ultra violet (UV) radiation

#### 2.4.3.1. Introduction

Ultra violet radiation is a non-chemical and non-thermal alternative to disinfection of produce and inactivation of microorganisms. Inactivation of microorganisms occurs as a result of a photobiocchemical degradation of specific nucleic acids and proteins. Absorbance of a photon yields an excited molecule \( (A + h\nu \rightarrow A^{++}) \) which may be converted to a thermodynamically stable product. Sufficient energy and absorption of this energy are conditions required for effective inactivation of microorganisms. Photons are minute energy packets in the electromagnetic radiation and have a wave–particle duality, i.e. they can be considered as a particle but with wave attributes such as frequency, wavelength, amplitude. (Encyclopedia Britannica 2010).

The wavelength of UV ranges from 100 – 400nm and an inversely related photon energy of between 300-2400kJ/Einstein. Ultra violet light can be subdivided into 3 parts; UVA, which is responsible for skin burns, ranges from 315nm to 415nm; UVB, which causes skin cancer as a result of its burns, ranges from 280-315nm; and UVC, 200-280nm, is the range in which the microbicidal effect is highest. It reacts irreversibly with vital constituents such as nucleic acids, i.e. RNA and DNA.

It was assumed that photons within this range are effective against biomolecules. However, photons within the UVC range with wavelengths more than 200nm but less than 320nm are the most effective. This is because below 200nm, in air or water the radiation penetrates only short distances (Sastry and others 2000).
2.4.3.2. Generation

Ultraviolet light is generated using electricity to power UV lamps filled with inert gas and mercury. This mercury is ionized, causing an electron flow, and radiation is emitted. These UV lamps are not coated with phosphorus, unlike fluorescent lamps. Medium and low pressure lamps can be used in a disinfection process. Low pressure lamps emit a maximum energy output at a wavelength of 254nm while the medium pressure lamp has a wider range from 180nm to 1370nm. Ultra violet ray dosage can be affected by two factors, the exposure time (T) and the intensity (I) which is directly affected by distance of sample to the source:

\[ D = I(T) \]

For an effective dose and successful microbial inactivation every part of the sample must be exposed to at least 400J/m\(^2\) (0.4kJ/m\(^2\)) (EPA 1999).

2.4.3.3. Application

Ultraviolet technology can be applied in two ways: conventional UV treatment where UV rays are aimed at the microorganism or target pathogens present on the surface of the sample and Hormetic UV treatment where the target is the food sample itself. It is a process whereby samples when exposed to low doses of UV light may induce a host resistance(Koutchma and others 2009; Stevens and others 1998).

This exposure to UV has been observed by various studies to increase the concentration of antioxidants such as viniferins and reservatol in grapes (Langcake and Pryce 1977), phytoalexin 6-methoxy mullein in carrots (Mercier and others 1993), and also increase anthocyanin formation in strawberries and quercetin in onions in a post flavonoid analysis (Higashio 2005). In addition, it has been shown to increase lycopene and \(\beta\)-carotene content of tomato, though a decrease in \(\beta\)-carotene was observed during storage (Liu and others 2009; Maharaj and others 1999).

Ultraviolet treatment of produce also elicits compounds that help in disease resistance. Post harvest observation of brown rot and yeast antagonistic activity was seen in
peaches. Hormetic dosages have been used in controlling harvest disease in tree fruits like tangerines (Stevens and others 1996). The treatment has also been observed to cause delays in ripening, slowing down senescence and rotting (Higashio 1999).

2.4.3.4. **Effects**

Due to generation of free radicals in products, some negative effects of UV have been pointed out. It has been observed to decompose antioxidants, vitamin A, vitamin C, and β-carotene in the presence of visible light. It has also been observed to affect foods high in unsaturated fatty acids as the free radical generation leads to rancidity (Koutchma and others 2009). This is, however, contrary to studies by (Erkan and others 2008) using strawberries exposed to UV dosage. This study found that they had higher antioxidant capacity, enzymatic activity and decay inhibition compared to the non exposed controls. This agrees also with observations made with blueberries (Wang and others 2009).

2.5. **Gas Exchange**

This is a process of exchanging the internal gas with a target gas such as nitrogen and oxygen so as to create a vacuum. Partial vacuums can be formed by pressure differential and temperature differential. The latter was observed in peppers and tomato by (Corey and Tan 1990) wherein the temperature gradients were varied and different weight changes correlating with negative differential and microbial counts were reported. Pressure differential is a non thermal treatment carried out by gas exchanging the internal gas in fruits and vegetable with oxygen and nitrogen. Fleming and others (1980) employed this method in the brining of cucumbers. They were gas exchanged with oxygen to enhance the uptake of brine solution and increase in densities were reported compared to control. This was also demonstrated by Daeschel and others (1990) in a cherry pepper brining experiment using dye, such that gas and liquid molecules gained access into the interior primarily via the stem and calyx where stomatal openings are numerous. Daeschel and others (1985) also found out that oxygen exchanged cucumbers had higher bacterial and yeast count compared to other treatments. This method has been used for sanitization of alfalfa seeds, in which
oxygen exchange and EO water treatment reportedly reduced the population of *Salmonella* without impeding rate of germination of seedling (Stan and Daeschel 2003).

### 2.6. Conclusion

Demand for fresh fruits and vegetables like peppers has increased due to increase in consumer awareness of the nutritional value and health benefit. In the process of meeting the increased domestic demand, exportation has increased significantly thus exposing consumers to different pathogens of different countries with different food laws and regulations. On the other hand, a wide range of sanitization methods have been developed, the majority of which have been successfully used to reduced microbial contamination to almost non-detectable limit. However, increase in the sanitizer strength often impaired the organoleptic qualities.

Electrolyzed oxidizing water has been widely and successfully used. The ability to modify its chemical properties makes it more versatile. Ultraviolet light on the other hand has both antimicrobial and hormetic properties. These properties have also been explored for disinfection in many studies. Some studies also documented their combination with other thermal and non-thermal process. None has however pursued possible combination of both EO and UV-C light’s antimicrobial properties in addition to UV-C’s hormetic inducing characteristics.

Moreover, gas exchange has been employed in brining and pickling of fruits and vegetable, so as to enhance even exposure of fruits to brine solution and prevention of bloater damage. This same principle can be applied also to enhance uptake of treatment solution, thus gaining access to pathogens that would have otherwise been protected by the plant surface tissues.

Peppers are highly perishable like other fruits and vegetables, therefore an experimental investigation of the effect of these treatments on the organoleptic properties and shelf life of pepper will provide better understanding and lay groundwork for future research of these combinations.
3. EFFICACY OF ELECTROLYZED OXIDIZING (EO) WATER/UV LIGHT ON THE QUALITY OF JALAPEÑO PEPPER (*Capsicum annuum*)

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3.1. Abstract

Jalapeño peppers, like many other fresh vegetables, have been implicated in several foodborne outbreaks including the one associated with *Salmonella* Saintpaul in 2008. The antimicrobial effects of electrolyzed-oxidizing (EO) water in combination with ultraviolet (UV) C light (254nm) on peppers have not been investigated. This study explored the efficacy of combining EO water and UV light in reducing microbial contamination of jalapeño peppers and its subsequent post treatment effect during a 10-day shelf life study. Retail peppers (ranging from 16-50 g in weight) were introduced with ~10⁸ CFU of *Serratia marcescens* (enteric pathogen surrogate) using the spot inoculation method. After being rinsed with deionized water and air-dried for an hour, the samples were placed into EO water (pepper: water = 1:3 (w/w) for various times (10, 20, 30 min), exposed to a 2.7kJ of UV fluence on each side, and stored for 0, 3, 7 and 10 days. Surface and total microbial count data were obtained for *S. marcescens*, other coliforms, mesophilic aerobic plate count yeasts and molds. *S. marcescens* and yeast showed a significant reduction (p=0.0044 and 0.0134, respectively) among all the treatments and control while the APC and coliforms counts showed no significant difference to treatments. Moreover, there was no significant difference in various contact times with EO water and storage times, though evident effects which varied among treatments were observed. Compared to control, all EO and UV treatments reduced contamination and had no negative impact on the color, texture and mass of pepper. Overall, peppers treated with and UV and EO water for 30 minutes gave the best inhibition. This suggested that of EO water and UV light can be an effective method of sanitizing fresh produce and an alternative to using only EO water.
3.2. Introduction

Jalapeño peppers are usually consumed raw and fresh and minimally processed. Like other fresh produce, there has been increase in the rate of consumption due to the increasing public awareness of the health benefit of a fruit and vegetable-laden diet (van Duijnhoven and others 2009). The World Health Organization (WHO) estimates that 76 million cases of foodborne disease, 325,000 hospitalization and subsequent 5,000 deaths occur each year in the United States, while the numbers are estimated to be skyrocketing in developing countries despite the lack of proper documentation (WHO 2011).

As of 2007, 1097 food borne outbreaks which resulted in 21,244 illnesses were reported to the Center for Disease Control and Prevention (CDC). *Salmonella* was the second most common etiological agent, accounting for 27% of those attributed to bacteria. This estimate does not include the numerous outbreaks that were unreported or underreported (CDC, 2010). All these outbreaks were attributed to food contamination. Fresh fruits and vegetable contamination could be as a result of pre- or postharvest handling (Beuchat, 2002). These may include the use of improperly composted organic manure, untreated sewage water for irrigation and fecal droppings (Ryu, 1997). Pathogens such as *E. coli, Salmonella, Clostridium, and Listeria* have been isolated commonly from fresh fruits and vegetables.

Various efforts have been made to minimize these contaminations and their associated food borne infections. These efforts include the use of sanitizers such as chlorine, acidified sodium chlorite, peroxyacetic acid, and trisodium phosphate (Yuk and others 2006). Chlorine has been found to be effective against *E. coli O157 H7, Salmonella typhimurium* and *Listeria monocytogen* (Kim and others 2008). Chlorine dioxide gas was also effective in reducing *E. coli and S. enterica* on lettuce (Mahmoud and Linton, 2008). However, most of these effective sanitizers are known to form residual compounds in varying quantities, such as trihalomethanes, trihaloacetic acid and chloramines compounds which are potentially carcinogenic (EPA 2010). The food industry’s quest for more effective sanitation technologies (i.e. high quality of post
treatment products, and significant decrease in contamination with little or no formation of hazardous compounds), has led to exploring the use of electrolyzed oxidizing water.

Electrolyzed oxidizing water (EO water hereafter), which has long been in use in Japan, became widely known and used due to its ease of on-site production, modification and application. It is produced from electrolysis of dilute NaCl solution when passed through an electrolytic cell. As a result of migration of ions to the electrodes, chlorine and hydroxyl radicals combine at the anode to form hypochlorite. It has been used as a sanitizer for different microorganisms on various fresh produce. Udompijitkul and others (2007) used EO water to inactivate mixtures of Listeria monocytogenes and E.coli on strawberries and reported a significant reduction in the initial population. EO water effectively inactivated E. coli and Salmonella on lettuce (Koseki and others 2004), E.coli on strawberries and broccoli (Hung and others 2010), and enhanced quality without impeding the germination of alfalfa sprouts (Stan and Daeschel, 2003).

On the other hand, application of ultraviolet (UV) light is a non-chemical and non-thermal method of sanitizing pepper. Its mode of action has been attributed to the mutation of the nucleic acids and proteins, leading to the death of the microbe or the loss of its ability to reproduce. Microbicidal ability is highest within the wavelength 200-280 nm wavelength, wherein lies the ultraviolet-C (UV-C). Ultraviolet-C can be used for sanitization purposes whereby a light source is aimed at the microorganism or target pathogens present on the surface of a sample. It may also be used to induce hormetic response in a target food as a result of exposure to low dose of UV (Tatiana Koutchma, 2009; Stevens and others 1998).

In addition, this exposure to UV, especially in low doses, has been observed by various studies to increase the concentration of antioxidants such as viniferins and reservatol in grapes (Langcake and Pryce 1977), phytoalexin 6-methoxy mullein in carrots (Mercier and others 1993), anthocyanin formation in strawberry, quercetin in onion in a post flavonoid analysis (Higashio and others 2005), and lycopene and β-carotene content of tomato, though a decrease in β-carotene was observed during storage (Liu and others 2009; Maharaj and others 1999). By slowing down microbial growth, UV exposure has
also been observed to cause delays in ripening and hence, slowing down senescence (Higashio and others 1999).

Ultraviolet light C has been widely used for sanitation of stainless steel (Sommers and others 2010). It has also been used to inactivate microorganisms on watermelon (Artés-Hernández and others 2010), baby spinach (Escalona and others 2010), pepper (Vicente and others 2005), and broccoli (Costa and others 2006). Ultraviolet light also increases shelf life by controlling the rate of growth of microorganism and alteration of metabolic processes in plant. Delay of senescence and decay, in slices of zucchini was reported by Erkan and others (2001). Its germicidal and induced effect on pears have also been reported (Li and others 2010), which helped in maintaining quality.

As discussed earlier, various studies focused on individual antimicrobial effects of UV and EO and their combination with other methods of sanitation, such as UV in combination with gaseous ozone, combination of modified atmospheric packaging with UV (Allende and others 2007), UV and gamma rays for papaya, (Cia and others 2007), EO water and carbon monoxide gas on tuna (Huang and others 2006), UVC and heat on spoilage fungi of strawberry and cherry (Marquenie and others 2002), and hot air and UVC on broccoli (Lemoine and others 2008). However, to the best of our knowledge, none of these studies has explored the potentials of combining both EO water and UV treatments and their combined effect on the wholeness of Jalapeño peppers.

Peppers have not been frequently implicated in many food borne outbreaks, but the Salmonella saint paul outbreak of 2008, revealed the high risk involved in consumption of fresh produce such as Jalapeño pepper. This outbreak involved 43 states and the District of Columbia of the United States, and Canada, leaving 1442 people ill, 286 hospitalized and 2 persons dead. Salmonella saint paul with same genetic finger print was isolated in all of these cases. Jalapeño and Serrano peppers were eventually linked to this outbreak (CDC, 2008).

Challenges arise when microorganisms associated with plant material become inaccessible to sanitizers, this process is called internalization. Internalization occurs when a microorganism integrates itself into the tissue system of a plant, seed or fruit
such that minimally processed or ready-to-eat food cannot be properly sanitized because the microorganism is spatially protected from any form of sanitization or washing. Consumption of such food leads to food borne outbreaks.

This study is concerned with documenting the efficacy of combining EO water and UV in the treatment of fresh Jalapeño peppers and their effect on the microbiological quality (both the surface and internalized), shelf life and appearance as opposed to using only EO water.

3.3. Material and methods

3.3.1. Raw material

Fresh green jalapeño peppers ranging in weight from 20 to 50g were obtained from a local grocery store (Winco Foods, Corvallis, OR). These originated from farms in either in the US or Mexico. Peppers were special ordered to arrive the day before the commencement of the experiment. They were stored at 5 °C and allowed to equilibrate to room temperature prior to use.

3.3.2. Bacterial strain and growth condition

Strains of *Serratia marcescens* 3611 were used as a surrogate test organism for *Salmonella*. *Serratia marcescens* 3611 and *Salmonella* are genetically very similar and they share the same metabolic pathway, except for the fact that *S. marcescens* a produces red pigment and does not produce hydrogen sulphide gas while *Salmonella* does. (Bergey’s manual of Determinative bacteriology year). *S.marcescens* was an ideal strain for the experiment as it is not considered to be a virulent infectious species, thereby minimizing laboratory acquired infections. Moreover, the bright red pigment enabled easy identification of colonies on culture media.

Bacterial cultures were stored frozen at -80 °C before being revived in tryptic soy broth (TSB) (Difco, Becton and Dickinson Company, Sparks, MD) at 30 °C. Three successive transfers of 0.1 ml aliquot of culture were further made into another 10 ml of TSB and allowed to grow for 24 hours. Overnight cultures were serially diluted and cell
concentrations were determined and kept at level of $10^9$ CFU/ml and plated on Violet Red Bile Agar (VRBA), MacConkey Agar (MAC), Tryptic soy Agar (TSA) and Dichloro Rose Bengal Agar (DRBA). This enumeration was carried out using spread and pour plate methods. All media used were obtained from Difco, Becton and Dickinson Company, Sparks, MD).

3.3.3. Morphology

*Serratia marcescen*, when cultivated at 30 °C in TSB broth, imparted a pinkish red color on agar (VRBA, MA, TSA), the strains produced colonies with a red pigmentation. The color may vary depending on the temperature of incubation and age of culture, but it is easily distinguished from all other colonies produced by bacteria associated with fresh peppers.

3.3.4. Preparation of EO water

The electrolyzed water was generated using a batch type JED -007 Super Water Mini-Generator (Altex Janix, Kanagawa, Japan) from electrolysis of 0.5% NaCl solution. Electrolysis time was set at 10 minutes. The electrolyzed acidic and basic water were generated from the anodic and cathodic ends, respectively. The acidic water was stored in screw capped Pyrex bottles. The pH and oxidation reduction potential of the solution was determined using a pH meter (Accumet research, AR 10, Fisher Scientific, Pittsburgh PA) coupled with a pH electrode (Symphony Electrode, Thermo Electron Corp., Waltham MA) and a dual scaled pH/ORP meter (Corning 125, Medfield, MA) coupled with a platinum redox electrode model 96-78-00 (Thermo Electron Corp., Beverly, MA).

3.3.5. Inoculation of pepper with *Serratia marcescens* 3611

Only firm and intact fresh peppers fruits were selected and used in the experiment. The peppers were then rinsed in deionized water to reduce the microbial load on the surface and allowed to dry for about one hour. Using the drop inoculation method (Singh and others 2002). 100µL culture of *Serratia marcescens* 3611($\sim10^5$CFU/ml) was then added using the micropipette (Gilson PipetteMan, France). Approximately 10-12 drops were
placed mostly around the stem of the pepper. Peppers were held at an upright position to allow for downward streaming of the culture. To allow for the absorption of the bacteria, the pepper was air dried for 60 minutes in a Class II Biosafety Hood (Fisher Hamilton Inc., Two Rivers, WI, U.S.A.).

3.3.6. Treatment of inoculated peppers with electrolyzed water /UV light and storage

Surface inoculated peppers were transferred aseptically into a glass jar and EO solution was added into the jar (pepper: water =1:3 (w/w)). The glass jar was agitated using a rotary shaker (Environ Shaker, Lab-Line, Melrose Park, IL) at 165 rpm for 10, 20 and 30 minutes. After the treatment time elapsed, the solution was decanted and peppers were rinsed with deionized water in order to minimize further activity of EO water.

The peppers were transferred into a biosafety hood and placed on a thin aluminum foil sheet which had been sterilized prior to the start of experiment. Peppers were then exposed to the UV-C light source (254nm) inside a germicidal UV-C lamp (FG30T8, 30W (UV 11.2W), 36” nominal length, Japan). Each pepper was evenly distanced from each other to prevent a shadow effect which might lead to uneven exposure. The distance between the UV light source and the pepper was approximately 110 cm (1.1m). Exposure time was set for one hour. At 30 minute intervals, peppers were rotated onto their other sides to allow for total surface area exposure. The UV fluence for each side was determined as 1.53 J/m²/sec using an Ultraviolet meter (Spectroline DM-254N, Spectronics Corp. Westbury, NY). The UV dose was calculated as:

\[ \text{UV fluence} \times \text{Exposure time} (t) \text{ (at } t = 30 \text{ minutes}). \]

Peppers treated with EO and UV but without *S. marcesens* served as positive control, while peppers inoculated with *S. marcescens* but without treatment served as negative control. The peppers were stored in sampling bags for 0, 3, 7 and 10 days at 5 ± 2 °C. The efficacy of EO water and UV and subsequent storage effects were investigated and experiments were replicated at least four times while trials were repeated no less than three times for each treatment.
3.3.7. **Microbiological analysis**

The inoculated peppers with known weights were each transferred into a sterile stomaching bag using sterile tongs. Phosphate buffer solution (PBS) (99ml) was then added and the content was homogenized using a stomacher (Stomacher 400 Circulator, Seward, London England) at 200 rpm for 60 seconds. The mixture was further diluted serially with PBS and the diluted samples were inoculated onto the above-mentioned different culture media using the spread plate method. All culture plates were incubated at 37 °C for at least 24 hours. Yeast and mold cultures were incubated at 25 °C for a minimum of 5 days.

Surface and total microbial count data were obtained for *S. marcesens* and other coliforms selectively, using the VRBA, TSA for mesophilic aerobic plate counts. Dichloro Rose Bengal (DRBC) agar (EMD Chemicals Inc., Gibbstown, NJ) was used for the recovery of yeasts and molds. *S. marcescens* was distinguished from other coliforms and other mesophilic aerobic bacteria by its characteristic bright red/pink colony color. Baseline contamination was also determined using pepper without any inoculation but rinsed with deionized water.

3.3.8. **Color analysis**

External color of the peppers was measured with the Hunter LabScan Spectrocolorimeter (Hunter Associates Laboratory Inc., Reston, VA) in CIE L*a*b* mode under CIE Standard Illuminant (D65) and observer 10°. Colors on each side of the pepper were read. The L, a* and b* values were recorded. Change in hue angle (h°) which is the basic color was calculated as: h°= 180 + (Tan⁻¹ b*/a*) in degrees while the chroma distribution, which is the saturation of color, was calculated as C = √(a*²+b*²). The experiment was replicated at least three times and the average values for L, hue and chroma were reported.

3.3.9. **Relative mass difference**

Effect of treatment on the changes in mass throughout the storage study was evaluated from the relative mass difference (%) using the equation:
\[(\text{Mi} - \text{Mf}/\text{Mi}) \times 100 \%\], where

\[
\text{Mi} = \text{initial mass}
\]

\[
\text{Mf} = \text{final mass}
\]

For both texture and color analysis, peppers without any treatment served as control while distilled water (DW) and air exchanged pepper were included as part of treatments.

### 3.3.10. Texture analysis

The peppers were also analyzed for firmness using the Texture Analyzer (TA-XT2) (Texture Technologies Corp. Scarsdale, NY) with P5 probe (5 mm diameter) and force of 1.5 N. The probe was driven through the pepper at a constant speed of 1.0 mm/s to a depth of 8 mm. The puncture test was repeated at 5 different spots on the pepper and the peak height of force required to puncture was documented. The effect of treatment on texture was analyzed all through the storage days. The experiment was replicated at least 3 times and the values are reported as mean peak force (N) with the standard deviation.

### 3.3.11. Statistical analysis

Data obtained were analyzed using the General Linear Model (GLM) model of the Statistical Analysis System (SAS) version 9.1 (SAS Institute Cary, NC., USA). Post-hoc significant differences between populations of various organisms were determined using the Least Significant Difference (LSD) comparison test with the significant level set at \(p<0.05\) for all the samples. This same method was used to analyze the effect of treatment on the color and texture of peppers.

### 3.4. Results

#### 3.4.1. Physicochemical properties of treatment solutions

Electrolyzed oxidizing (EO) water generated from 0.5% NaCl solution had an average pH of 2.71 (±0.078) and an oxidation reduction potential of 1140.63 (±4.09). Dissolved oxygen and free chlorine concentrations were 11.2 ppm and 50 ppm, respectively.
3.4.2. Effect of treatment solutions on the microbial population of peppers.

**Figure 3-1**: Microbial population of *Serratia marcescens*, other coliforms, mesophilic aerobic bacteria, yeast and molds. Vertical bars indicate 95% confidence interval of mean. Treatments with same superscripts are not significantly different according to LSD at $p = 0.05$

The effects of the various treatment methods on different microbial populations on the first day (i.e. Day 0 of treatment) are as shown in Figure 3-1. Populations of *S. marcescens* showed overall significant difference between all the treatments ($p = 0.0044$). The pairwise comparison with control at $p = 0.05$ revealed a significant difference between control and those treated with UV and EO water at 10, 20 and 30 minutes with a logarithmic reduction of 1.05, 1.34 and 4.25 CFU/g, respectively. Peppers treated with only EO water did not show any significant difference from control, while reduction of 1.23 log CFU/g was achieved for *S. marcescens* (Fig 3-1).

When compared with EO30, the samples treated with both EO water and UV light showed a greater log reduction of 2.9 log CFU/g, regardless of the length of contact times. However, an exception was the EO20UV sample. This is suggestive of increased efficacy of EO water when coupled with UV. Other coliforms were also isolated on VRBA, but the colonies were differentiated from *S. marcescens* by color. Overall, there
was no significant difference in their responses to treatment (p >0.05). However, when compared to control samples, 1.34, 1.87, 4.52 and 1.82 log CFU/g reductions were observed for EO10UV, EO20 UV, EO30UV and EO30 treatments respectively.

Even though there was no significant difference (p > 0.05), mesophilic aerobic bacteria showed the highest response to EOUV30 treatment with a 2.29 log CFU/g reduction compared to the control treatment. Next to this, was EO30 with 1.10 log CFU/g reduction after which was 1.0 log CFU/g reduction observed with the EO water at 20 minute contact time and UV treatment. The highest count of 6.7 and 6.9 log CFU/g of total aerobic bacteria was observed in NT and SM (Figure 3-1).

Yeast and mold count reduction was evident in all treatment with 1.29, 1.05, 1.74 and 1.26 log CFU/g for contact times 10, 20, and 30 minutes with UV treatments respectively (p = 0.012). Also, submerging sample in EO water for 30 minutes without UV achieved a 1.26 log reduction compared to the control. All the yeast counts for all the methods of treatment on the first day were below 1 log CFU/g (Figure 3-1).

3.4.3. Effect of EO water and UV on microbial population during refrigeration storage of peppers

![Figure 3-2 1 Population of S. marcesens recovered from pepper during storage. Vertical bars indicate 95% confidence interval of mean.](image-url)
As shown in figure 3-2, the population of *S. marcescens* on Day 3 increased in comparison to the first day of all treatments. EO30UV peppers showed the least growth increase of all the treatment methods when compared to the control. There was no decline in the population of *S. marcescens* throughout the storage study. This indicates that they are capable of long term survival without declining in population. The UV effect on storage was observed between EO30 and EO30UV to range from 2.3 log CFU/g to 0.3 log CFU/g on the last day of storage. Among all the treatments, there was not much difference in the *S. marcescens* population by the last day of storage (p=0.1009) (Figure 3-2 1). Overall, there was no difference between the UV and non UV treated pepper, indicating that UV light did not appear to have any residual antimicrobial activity during storage. In addition, there was at least a 90% reduction observed for all the treatment methods (1 log CFU/g) when compared with SM.

![Graph](image.png)

Figure 3-2 2: Population of other coliforms recovered from pepper during storage. Vertical bars indicate 95% confidence interval of mean.

Other coliforms count showed varying populations in this study. This may be due to the variability in number of internalized microorganism as this was also observed in preliminary studies carried out in the laboratory. Peppers treated with EO and UV showed a comparative reduction by 1 log CFU/g when compared to the control, while EO30UV showed the least grow (figure 3-2 2). Increase in contact time with EO water
was not significant between 10 and 20 minutes. However, it was significant after 30 minutes of contact with EO water.

**Figure 3-2 3** Population of total aerobic bacteria recovered from peppers during storage. Vertical bars indicate 95% confidence interval of mean.

Throughout the storage study, the total bacteria count was high compared to other isolated groups of microorganism. Responses of these organisms among treatment varied significantly during the storage study (p=0.0009). Except for EO30UV on the first day, most of the counts were higher than 5log CFU/g. The untreated pepper (NT) maintained an almost constant population of 6.8log CFU/g (Figure 3-2 3).
The lowest count was observed in EOUVSM30 with -0.20 log CFU/g. The count for all treatment methods was below 1 log CFU/g of yeast and mold except for control (no treatment) and SM with 1.67 and 1.54, respectively. Yeast and mold populations increased for all the treatment methods after 3 days of storage. With respect to control, there was not much difference. From then on, a decline in population was observed for all treatment methods except in EO30. After 10 days of storage, the population was observed to have declined further. In comparison with the control, there was ~ 1 log CFU/g reduction in the yeast and mold counts in all the samples of the different methods of treatment. All the UV irradiated treatments had counts that were below 1 log CFU/g while a count of 1.02 log CFU/g was recovered from EO30. There was no statistical difference between UV treated and non-UV treated samples. However, the latter had a higher count than any of the former i.e EO10UV, EO20UV and EO30UV(Figure3-2 4).
3.4.4. Effect of treatments on color and texture

Table 3- 1: Peak force\(^1\) required to puncture pepper during storage.

<table>
<thead>
<tr>
<th>Storage</th>
<th>Day 0</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>a39.50±7.89</td>
<td>30.55±4.62</td>
<td>c,d29.33±5.10</td>
<td>49.62±8.32</td>
</tr>
<tr>
<td>DW</td>
<td>a38.21±4.94</td>
<td>31.05±5.58</td>
<td>c,d29.49±3.75</td>
<td>45.78±6.31(f)</td>
</tr>
<tr>
<td>EO30</td>
<td>a39.59±4.37</td>
<td>32.61±4.97</td>
<td>e25.75±4.54</td>
<td>47.15±7.88</td>
</tr>
<tr>
<td>EO10UV</td>
<td>b34.12±4.59</td>
<td>31.28±5.49</td>
<td>d,e26.54±3.54</td>
<td>49.25±5.75</td>
</tr>
<tr>
<td>EO20UV</td>
<td>a,b37.26±6.55</td>
<td>30.31±4.33</td>
<td>c,d29.17±3.74</td>
<td>51.02±8.20(f)</td>
</tr>
<tr>
<td>EO30UV</td>
<td>a40.31±8.65</td>
<td>31.98±4.81</td>
<td>c30.24±4.65</td>
<td>48.19±6.13</td>
</tr>
</tbody>
</table>

p-values 0.042 0.669 0.0249 0.4118

\(^1\) Values are mean ± Standard deviation (n≥15). Means preceded by same superscripts are not significantly different. Means succeeded by same superscripts are significantly different.

A post-hoc pairwise comparison revealed that the peak force required to puncture peppers were significantly different in the first day of the treatment (p = 0.042). When compared to the control, however, most of the treatments were not significantly different except for EO10UV (Table 3-1). It was observed that EO30UV peppers had the highest mean value of peak force while EO10UV which had the lowest mean. A potential explanation would be that the size of peppers used for the EO10UV treatment was smaller than others. There were no significant differences in the texture of the pepper on day 3 and 10 of storage for all treatments. It was observed that the treatment showed significant difference in the effect on texture on the 7\(^{th}\) day of treatment (p=0.0249). When compared with control, only EO30 showed a significant difference with a lower peak puncture force (Table 3-1).
Relative mass difference is used to predict water loss from the pepper. On day 0 of storage, slight decrease in mass was observed in both control and EO30, while others showed an increase in their masses. By the third day (day 3) of storage, only control peppers were observed to have decreased in mass, though negligible. These observed gain in mass, may be a result of the diffusion of water molecules into the tissues of the pepper especially at the stem area. Except for DW, all other peppers for other treatments clearly reduced in mass on day 7 of storage. This trend was also observed on the third day of storage.

Overall, there were differences in trends of responses of these treated peppers to the effect of storage (p = 0.0092). However, there was no significant difference between days 0, 3, and 7 of storage. There were a lot of variations in weight loss of peppers in response to the treatment methods (p < 0.001). Control, EO10UV and EO20UV showed similarities in responses during storage. EO10UV, EO20UV and EO30UV also overlapped also in their loss of mass (Table 3-2)
Various surface sanitization treatments have reported a shriveling of the skin and weight loss of fruits after treatment and this may largely be due to loss of water. Although pepper skin is hydrophobic in nature due to its high content of wax and cutin, they are prone to water loss because they contain a hollow. High rate of water loss accelerates the process of ripening thus reducing quality and shortening shelf life (Kissinger and others 2005). In this study, water loss was minimal in all types of treatment.

![Graph showing the effect of treatments on pepper color](image)

Figure 3-3 1: Effect of the treatments on lightness (L), a (greenness), hue angle (H) and Chroma(C) of pepper on the first day of treatment. Vertical bars indicate 95% confidence interval of mean. Treatments with same superscripts are not significantly different according to LSD at p= 0.05.

Fresh produce are sensitive to treatments. Therefore the impact of these treatment on the color of pepper was documented during the observed storage days (Figure 3-3). On the first day of treatment (Fig. 3-3 1), there was no significant difference between the L, a* and hue of the pepper for all treatments (p = 0.1055, 0.3790 and 0.3183, respectively). The chroma values between treatment methods showed some differences among the E020UV, EO30UV and control (p = 0.0162).

There was no significant effect of treatment on the L, a*, chroma and hue of peppers on day 3 compared to control (p = 0.2682, 0.2113, 0.3175 and 0.2270, respectively). Though there was no statistical significance, the mean a* values of EO 30 appeared to
be higher than control. The chroma of EO30UV also was different when compared with control (Figure 3-3 2).

Figure 3-3 2: Effect of the treatments on lightness (L), a* (greenness) hue (H) and Chroma (C) of pepper on the Day 3 of treatment.

Figure 3-3 3: Effect of the treatments on lightness (L), a* (greenness), hue angle (H) and Chroma (C) of pepper on the Day 7 of treatment. Vertical bars indicate 95% confidence interval of mean. Treatments with same superscripts are not significantly different according to LSD at p>0.05.
After 7 days of storage, despite a significant difference (p=0.0072), pair wise comparison did not reveal any difference except EO30 which showed has a lower mean L value of 30.7(Figure3-3 3). There was no significant difference in the means of the a* values (p=0.1797). Even when compared to control, only EO10UV revealed a significant difference in mean. The mean hue values ranged from 114 to 119, control being the lowest and EO30, the highest. Though they were all within the hue range for green color of pepper, there was a significant difference (p=0.0008). Except EO10, none of these treatments were different from control (p=0.3132). The treatments did not have any effect on the chroma of the pepper when compared to control. The only exception was EO10UV, which had a high chroma. This however can be considered to be a positive change.

Figure 3-3 4: Effect of treatment on the L, a*, hue angle and chroma of the color of pepper on day 10 of treatment. Vertical bars indicate 95% confidence interval of mean. Treatments with same superscripts are not significantly different according to LSD at p = 0.05.

The L, a, hue and chroma of all the pepper on the 10th day of storage are as shown in Figure 3-3-4. There was no significant difference in the effects of these treatments on hue (p = 0.3132), while the L and chroma values showed statistical difference (p = 0.0072 and 0.008, respectively). A pairwise comparison and graphical representation did not reveal any difference in the mean of individual methods of treatment. This may
be due to high variances among the experimental values. Overall comparison between the treatment methods showed no significant difference in L, hue and chroma values with p values are 0.1230, 0.6044 and 0.1400 respectively.

3.5. Discussion and Conclusion

In this study, EO water was observed to be effective in reducing microbial counts of the peppers. However, when compared to the peppers treated with EO water in combination with UV-C light, the latter showed a greater efficacy in reducing the population of *S. marcescens*, other coliforms, total aerobic bacteria, yeasts and molds. This was most especially evident in the peppers exposed to EO water for 30 minutes before the UV treatment. Surprisingly, there was no significant difference between the *Serratia* count of inoculated and uninoculated peppers. This could be due to the high variances among the colony counts, which in turn could be attributed to the differences in the sources of peppers and also the inability to determine the age of peppers.

It must be noted, however, that there was not much difference between the different methods treatment on the 10th day of storage. Overall increase in microbial population during storage was observed in this study. Post-treatment survival of *E.coli* and *Listeria monocytogenes* on strawberries treated with EO water and stored at low temperature was also reported by (Udompijitkul and others 2007).

Liao and others (2010) estimated that the normal microflora of Jalapeño pepper is 3.5 log CFU/g of enteric bacteria, 5.6 log CFU/g of total mesophilic bacteria and 1.9 log CFU/g of yeasts and mold. This partly agrees with the findings in the present study of 6.75 log CFU/g coliform, 6.77 log CFU/g total bacteria count and 1.67 log cfu/g yeast and mold. Therefore, with an addition of ~10^8 CFU of *S. marcescens* to the peppers, the microbial reduction may have been underestimated.

Even though the yeast and mold population increased in treated peppers on 3, 7, and 10 days of storage, the population increase was less compared to the untreated peppers. Similar to the result obtained by Allende and others (2006), the counts were maintained at 2 or 3 log CFU/g, thus increasing the shelf life of the produce by a few days. This may be desirable not only from a microbiological standpoint but also from a sensory
standpoint, since the food products are less likely to have a negative sensory impact when the yeast or mold count is less than 5 log CFU/g (Debevere 1996).

Several treatments are available for microbiological safekeeping of fresh fruits and vegetables, but only a few do so without impacting the texture or color of the peppers. Some treatments that may involve high pressure, temperature or acidic conditions may cause the relatively impermeable cell wall barrier to become permeable as a result of pectolytic actions, which may lead to the loss and leakage of nutrients and moisture, loss of weight and softening of plant tissue. The findings in this study showed that the use of EO water and UV may be used without adversely impacting the texture or color of the peppers. With appropriate modification of treatment, high efficacy against microorganism can also be achieved.

Peppers, like many other fruits, contain antioxidants which have beneficial effect to human health. There are concerns about the effect of UV on these compounds, which can be predicted from the rotting and change of color. The result of the present study did not reveal any such defect. It may be inferred from the experiment that senescence was slowed down which unlike other treatments that may trigger it. There were some variations in the texture over storage period; this may be attributable to the variation in sizes of the pepper used in the experiment, as it was observed that larger ones required a higher puncture force. It would be interesting to examine the effect of this treatment with peppers or other model fruits of the same size. It also appears that post UV irradiation conditions to which the treated samples are exposed to might be a factor to consider as the exposure to certain conditions may subject the produce to photoreversibility (Shama, 2007). This is a situation whereby microbial DNA may undergo a repair process as a result of some photo reaction.

Although no specific range of dosage is required by FDA for sanitation of food, user discretion is advised on the UV dosage level, depending on factors such as the microbial load, nature of food and intended time of exposure (FDA, 2008). It is important to note that the UV fluence used in this study was low (2.7kJ/m²), compared doses used some other studies such as 3.7kJ/m² observed to delay senescence in tomato
(Maharaj and others 1999), 4-14kJ/m² delayed chlorophyll degradation in broccoli (Costa and others 2006), 2.15-4.30kJ/m² optimally enhanced phytochemicals and anthocyanins in blueberries (Wang and others 2009).

A dose of 7kJ/m² was also used for peppers and all symptoms of decay were prevented (Vicente and others 2005). Therefore, this study may be open to higher doses of UV, with the intention of further reducing the microbial load to the normal microbial load of typically contaminated produce, though chances of attaining this level of contamination cannot be overlooked. It can be suggested that treatment might be of higher efficacy in produce of lower microbial load contamination. Moreover, EO water can be modified to suit various biological demands. Therefore, lesser contact time of treatment solution and fruits may be explored while the UV-C fluence may be increased to improve the efficacy.

The dosages required for hormetic response have been reported in several researches to be very low. It has been reported that higher doses may cause rotting and discoloration of the fruit (Maharaj and others 1999). Other studies, however, did not observe any change in response to UV-C even with a 10-fold increase of dosage (Escalona and others 2010), which suggests that the topography, shape, product type and other factors may contribute to the differences in the mode of response to treatment.

Effective penetration of UV-C may be varied or even hindered in situation where produce is irregularly shaped, unusual varying sizes or reflecting surface (Escalona and others 2010). In this case, Jalapeño pepper had a somewhat smooth surface and this may have aided the inactivation of microorganism by UV beyond the surface. In contrast, its reflective surface might have decreased the effectiveness of UV.

Liao and others (2010) reported that majority of the microorganisms recovered were reported to have been attached to, or internalized through the calyx of the peppers, directing the treatment toward the calyx and stem region may be more effective. Udompijitkul and others (2007) also reported that the roughness or smoothness of the skin may be a contributing factor to the efficacy of sanitizers. The hydrophobic nature of the skin of peppers does not allow for effective penetration of treatment solution.
This also may reduce the efficacy of the treatment, further suggesting that the efficacy of this treatment may have been underestimated.

Peppers have a high concentration of phytochemicals and flavanoids which help scavenge free radicals in the body. There are concerns about the effect of these treatments on the beneficial compounds. These effects can be predicted by the changes in color and texture. In this study, not only was there no observed change but also a probable improvement in these physicochemical properties. Erkan and others (2008) also exposed each side of a strawberry to a dose of 2.7kJ/m² without any effect on color and texture. Further studies may be necessary to confirm the effects of these combined EO-UV treatments by assaying for antioxidant and phenolic components of the pepper. Also, it will be of utmost importance to determine if there is any adverse change in the sensory quality of the peppers, as this was also not determined in this study.

The synergy of these treatments may also be investigated by changing the sequence of treatment i.e. exposure to UV light before EO water. Marquenie and others (2002) observed on strawberry and cherry, a greater reduction on the count of *Botrytis cinerea* when heat preceded UV treatment (0.01-1.50 J/cm²) and vice versa for *Monilia fructigena*. The effect of change in order of treatment might be different compared with that of this present study.

In this research, combination of EO water and UV has shown promise in the area of microbial reduction, shelf life and quality control. The higher reduction in the population of inoculated bacteria was observed in peppers treated with combination of EO water and UV light when compared to those treated with only EO water or control which imparting the desirable properties of the pepper fruit. Combination of both techniques offers new possibilities for sanitization. However, the practicality and feasibility of implementation on an industrial scale must be addressed. Since every fruit and vegetable differs in its topography and physicochemical properties, appropriate modification of treatment can be done in order to attain individual goals. In-depth look into the synergistic use of EO water and UV’s antimicrobial, phytochemical and
photochemical activities, with respect to their impacts on the physicochemical properties of fruits, will be encouraged.

3.6. ACKNOWLEDGEMENTS

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3.7. REFERENCES


4. EFFECT OF GAS EXCHANGE ON UPTAKE OF ELECTROLYZED OXIDIZING(EO) WATER AND QUALITY OF JALAPEÑO PEPPERS 
(Capsicum annuum)

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4.1. Abstract

Various improved technologies have been developed to reduce the risk of exposing fresh fruits and vegetables to associated pathogens. However, the inability of these treatments to address the problem of internalized microorganism and the effect of post-treatment is a major source of concern to producers and consumers. This study investigated the effect of gas exchange on the uptake of treatment solutions (electrolyzed oxidizing (EO) and distilled water (DW)), and their effect on both surface and internalized microorganisms well as their subsequent post-treatment effect on color, texture and relative weight loss (%) of Jalapeno peppers. Peppers artificially contaminated with $10^8$CFU/ml of *Serratia marcesens* 3611 (surrogate for *Salmonella sp*), were gas exchanged in a 2-liter glass with nitrogen (N), oxygen (O) and air (control) for 1 hour. Treatment solutions were added at a ratio of 2:1 (solution: peppers) and volume of uptake was monitored using a graduated reservoir. Oxygen exchanged (OE) peppers had the highest volume of uptake. Peppers were stored at 6 ± 2°C and observed for changes in some of physicochemical properties for 0, 3, 7 and 10 storage days. The OE peppers showed the most significant reduction and lowest count of *S. marcesens* with 3.88 logCFU/g while those treated with DW had the highest count of 6.35 log CFU/g (p=0.0218). Other coliforms and total aerobic bacteria counts did not show any significant response to treatment (p=0.563 and p=0.366 respectively). Yeast and mold counts showed response that was not indicative of a significant difference (p=0.061). There was no negative impact on color, texture and relative mass loss when compared to control. Rather, OE peppers were observed to have firmer textures and increase as opposed to decrease in weights. There were no differences in the effect of storage on the individual responses to treatments. This result suggests that, without imparting the quality of pepper, access and reduction of internalized microorganism may be enhanced by gas exchange of peppers with oxygen and the subsequent uptake of treatment.
4.2. Introduction

Investigations have revealed the sources of contamination to a multistate outbreak of *Salmonella* saint paul in 2008 were jalapeño and Serrano peppers grown and imported from Mexico. Although there was no conclusive link, tomatoes were initially identified as a source of contamination, which led to the prolonged investigation and increased incidences of the outbreak until peppers were identified as the ultimate culprit. As a consequence, the tomato sector of the vegetable industry was severely affected, resulting in the loss of more than $100 million and $14 million in the produce industry of Florida and Georgia respectively ((PSP) 2008). Recommendation of fresh fruits and vegetables for good health and increased awareness of the numerous benefits have led to an increase in consumption of such in the U.S. This huge domestic demand has exceeded domestic supply, as evidenced by the increase in the volume of exportation. This has consequently increased the food safety and defense risk along the supply chain (USDA 2010). However, contamination of fresh produce such as peppers could result because of preharvest factors such as indigenous microbial population in the soil environment, use of improperly composted manure, untreated irrigation or sewage water or post harvest factors such as improper handling and food processing procedures ( Ryu 1997; Beuchat 2002)

Various methods have been adopted in sanitization of fruits and vegetables, many of which have been focused on effective surface sanitation. Yuk and others (2006) investigated the use of gaseous chlorine dioxide (ClO₂), chlorine, peroxyacetic acid, acidified sodium chlorite and peroxyacetic acid on *Salmonella* that was inoculated on the stem scar, smooth surface and puncture wounds of bell peppers and cucumber. They were found to be more effective in smooth surface than on punctured wound, chlorine and ClO₂ being the least and most efficient, respectively. ClO₂ has been reported to be effective in inactivating *E. coli* and *Salmonella enterica* on lettuce (Yuk and others 2006). When combined with ultrasonication, it was found to be effective against *E. coli* on apples and lettuce (Huang and others 2006). However, it has been reported that its
effectiveness in its gaseous form can be affected by humidity temperature and light (Sun-Young and others 2004).

Unlike many of the sanitizers that are commonly used, the use of electrolyzed oxidizing water can be advantageous, not only because of its relatively lower cost but also because of the ease of production, as it requires only clean water and sodium chloride. It is also environmentally safer and less hazardous as it does not form compounds that could be carcinogenic. Its potency can be attributed to its high oxidation reduction potential (ORP) and low pH, which is in turn is due high concentration of hypochlorous acid (Izumi H. 2000). The chemical properties responsible for the antimicrobial activities such as the residual chlorine, ORP and pH of EO water can also be modified to suit various needs while maintaining its potency (Kim and others 2000a; Kim and others 2000b). However, the progress of these technologies has been stalled by the inaccessibility of the treatments to internalized microorganisms.

Internalization of microorganisms within plant tissue has been observed in various fruits and vegetables. It could either be via a passive process, where microorganisms rely on natural openings or accidental wounds to gain entry, or through an active process whereby they degrade tissue to gain entry to the plants. Microorganisms gain entry through the wounds, hydathodes, stomata, root and stem lenticels, and leaf margins.

The anatomical structure of fruits and vegetables varies tremendously and is believed to be an important parameter in determining whether contamination can occur and persist. For instance, pepper has a waxy cuticular surface which is hydrophobic in nature, which primarily serves as a permeability barrier against moisture and gas loss. The outer surface is covered with epicuticular wax. This also gives the pepper the ability to repel water, forming beads (Jeffree 2006). However, it has been shown that the pepper as a whole is not fully impermeable.

The inaccessibility of internalized microorganisms may be overcome by enhancing the uptake of treatment solutions into the interior of fruits by creating a vacuum. Rapid metabolic conversion of oxygen into carbon dioxide which solubilizes rapidly, leads to a decrease in internal pressure or increase in external pressure, thereby creating a
vacuum. This vacuum is relieved by the influx of molecules such as microorganism and gas, through the fruit’s respiratory pore. The rate of uptake can be affected by the number and size of stomatal pores, time of exposure, temperature differential and viscosity of the external environment (Bartz and Showalter 1981).

Partial vacuums can be formed by pressure differential and temperature differential. The latter was observed in peppers and tomato by Corey and Tan (1990), wherein the temperature gradients were varied and different weight changes correlating with negative differential and microbial counts were reported. Pressure differential is a non-thermal treatment carried out by oxygen and nitrogen gas exchange of the internal gas space in fruits and vegetable with oxygen and nitrogen. This was demonstrated by Daeschel and others (1990) in a cherry pepper brining experiment using dye, that gas and liquid molecules gained access into the interior primarily via the stem and calyx where stomatal openings are numerous. Daeschel and others (1985) also found out that oxygen exchanged cucumbers had higher bacterial and yeast count compared to other treatments. This has method has been used for sanitization of alfalfa seeds, in which oxygen exchange and EO water treatment reportedly reduced the population of Salmonella without impeding rate of germination of seedling (Stan and Daeschel 2003).

The objectives of this study were therefore 1) to investigate the efficacy of gas exchange treatment and EO water on surface and internalized S. marcesens and other native microflora, and 2) to examine the post treatment effect of these treatment on the color, mass and texture of peppers. To the best of our knowledge there has been no documentation of such study.

4.3. Materials and Methods

4.3.1. Raw material

Fresh jalapeño peppers (ranging in weight from 20 to 50 g) were obtained from a local grocery store (Winco Foods, Corvallis, OR). These originated from farms in either the US or Mexico. Peppers were special ordered to arrive the day before the
commencement of the experiment. They were stored at 2°C and allowed to equilibrate to room temperature prior to use.

4.3.2. Bacterial strain and growth condition

A strain of *Serratia marcesens* 3611 was used in this study in place of *Salmonella* since they both have same metabolic pathway except that *S. marcesens* produces red pigment and does not produce hydrogen sulfide gas while *Salmonella* does (Bergey’s manual of Determinative bacteriology).

Bacterial cultures were kept frozen at -80 °C before being revived in tryptic soy broth (TSB) (Difco., Becton and Dickinson Company, Sparks, MD) at 30 °C. Three successive transfers of 0.1 ml aliquot of culture were further made into another 10 ml of TSB and allowed to grow for 24 hours. Overnight cultures were serially diluted and cell concentrations were determined and kept at level of $10^9$ cfu/ml for both strains on Violet Red Bile Agar, MacConkey Agar and Tryptic soy Agar. This enumeration was carried out using spread and pour plate methods. All media used were obtained from Difco., Becton and Dickinson Company, Sparks, MD.

4.3.3. Morphology

*Serratia marcesens*, when cultivated at 30 °C in TSB broth, imparted a pinkish red color on agar (VRBA, MA, TSA), the strains produced colonies with a red pigmentation. The color may vary depending on the temperature of incubation and age of culture, but it was easily distinguished from all other bacteria associated with fresh peppers.

4.3.4. Inoculation of peppers with *Serratia marcesens* 3611

Whole peppers with known weights and without any visible blemishes were washed in deionized water, and treated with NaOCl to remove the background surface contaminant. They were aseptically towel dried and inoculated mostly around the stalk of the peppers with 0.1ml of broth culture of approximately $10^8$ CFU of *S. marcesens* using the drop inoculation method. The peppers were allowed to air-dry for 3hrs in a Biosafety Class II hood.
4.3.5. Preparation Of Electrolyzed Oxidizing Water

The electrolyzed water was generated using a batch type JED-007 Super Water Mini-Generator (Altex Janix, Kanagawa, Japan) from electrolysis of 0.5 % NaCl solution. Electrolysis time was set at 10 minutes. The electrolyzed acidic and basic water were generated from the anodic and cathodic ends respectively. The acidic water was stored in screw capped Pyrex bottles. The pH and oxidation reduction potential of the solution was determined using a pH meter (Accumet research, AR 10, Fisher Scientific, Pittsburgh PA) coupled with a pH electrode (Symphony Electrode, Thermo Electron Corp., Waltham MA) and a dual scaled pH/ORP meter (Corning 125, Medfield, MA USA) coupled with a platinum redox electrode model 96-78-00 (Thermo Electron Corp., Beverly, MA).

4.3.6. Gas treatment of peppers

Two liter jars were filled with inoculated peppers. Three holes were bored into the caps of the jars; each for the inlet, outlet of gas and a graduated liquid reservoir. The gas cylinders were connected to the glass jar through the inlet tube while the outlet was sealed. The graduated reservoir was unsealed to allow for the purging of the internal gas atmosphere. Peppers were exposed to gas (nitrogen or oxygen) with the flow rate of 50 ml/min and exchanged with the internal atmosphere for one hour. The gas tube was then clamped to prevent leakage or entry of air from the external atmosphere.

4.3.7. Measurement of components of gas after exchange

Immediately after gas exchange, pepper samples were taken for each treatment to analyze for the exchanged gas concentration in the peppers. Gas sample were taken from the core of the peppers using a gas-tight 5 ml syringe with a gauge-22 hypodermic needle. The sample was injected into the bottom injector of the gas chromatograph (Shimadzu Gas Chromatograph system-GC 2014, Shimadzu Scientific Instruments, Columbia, MD) using a thermionic conductivity detector (TCD) for nitrogen and oxygen and flame ionization detector (FID) for carbon dioxide. Using a pressure of 350kPa, 120mA current was supplied at a flow of 21.14 ml/minute, and linear velocity
of 172.5 cm/sec. Hold time was set at 20 minutes and column temperature kept at 80°C. The percentages of each gas were obtained by baseline calibration with helium: air mixture at 100:0 %, 75:25 %, 50:50 %, and 25: 75% respectively. The peaks for oxygen, nitrogen and carbon dioxide were observed at 6.5, 7.5 and 9.8 minutes respectively, and individual percentages were obtained.

4.3.8. Treatment of gas exchanged peppers with electrolyzed water and Storage

Electrolyzed oxidizing water was added into the jar containing the surface inoculated and gas exchanged (peppers: water =1:2 (w/w)). The uptake of treatment solution was monitored for 3 hours at 20 °C. For microbiological analysis, using the EO water treatment solution, liquid uptake was observed for only 2 hours. This experiment was set up for nitrogen, oxygen and air (Figure 4-1). The positive control was the uninoculated peppers gas exchanged with oxygen. These peppers were also treated with distilled water (DW) but were not analyzed for microbial properties except for the oxygen exchanged peppers. The final weights of peppers were determined and stored in sampling bags at a 6±2 °C over the course of study for 10 days. Untreated peppers served as control.

Figure 4- 1: Experimental Setup for gas exchange of pepper with nitrogen, oxygen and air.
4.3.9. **Microbiological analysis**

Peppers with known weights were transferred each into a sterile stomaching bag with the aid of sterile tongs and 99 ml of phosphate buffer solution (PBS) was added and homogenized using a stomacher (Stomacher 400 Circulator, Seward, London England) at 200rpm for 60 seconds. The mixture was further diluted serially with PBS and using the spread plate method, diluted samples were inoculated onto different culture media. All culture plates were incubated at 37 °C for at least 24 hours except for yeast and mold cultures, which were incubated at 25 °C for a minimum of 5 days.

Total microbial count data were obtained for *S. marcesens* and other coliforms selectively using the VRBA, TSA for mesophilic aerobic plate counts. Dichloro Rose Bengal (DRBC) agar (EMD Chemicals Inc., Gibbstown, NJ, USA) was used for recovery of yeasts and molds. *S. marcescens* was distinguished from other coliforms and other mesophilic aerobic bacteria by its characteristic bright red/pink colony color. Baseline contamination was also determined using peppers without any inoculation but rinsed with deionized water (-Gas-EO-SM). Peppers gas exchanged with oxygen and treated with water (EO+SM+H₂O) was also analyzed to determine effect of DW on microbial count. Effect of EO water on uninoculated pepper (-Gas-SM) was determined as well as the overall contamination (-Oxy+SM) was determined.

4.3.10. **Color Analysis**

External color of the peppers was measured with the Hunter LabScan Spectrocolorimeter (Hunter Associates Laboratory Inc., Reston, VA) in CIE L*a*b* mode under CIE Standard Illuminant (D65) and observer 10°. Colors on different sides of the pepper were read. Change in hue angle (h°) which is the basic color was calculated as: $h^\circ = 180 + \left( \tan^{-1} \frac{b^*}{a^*} \right)$ in degrees where 0° is purple color; 90° is yellow color; 180° is blue-green color; and 270° is blue color, while the chroma distribution which was the saturation or intensity of color was calculated as $C = \sqrt{a^{*2} + b^{*2}}$. 
4.3.11. Relative mass difference (%)

Effect of treatment on the changes in mass throughout storage was evaluated from the relative mass difference(%) using the following equation:

$$(M_i - M_f)/M_i \times 100\%,$$

where

$M_i =$ initial mass

$M_f =$ final mass

For both texture and color analysis, peppers without any treatment served as control while distilled water (DW) and air exchanged pepper were included as part of treatments.

4.3.12. Texture analysis

The peppers were also analyzed for firmness using the Texture Analyzer (TA-XT2) (Texture Technologies Corp. Scarsdale, NY) with P5 probe (5 mm diameter) and force of 1.5 N. The probe was driven through the peppers at a constant speed of 1.0 mm/s to a depth of 8 mm. Pre- and post-test speeds were 1.5 and 10.0 mm/sec, respectively. The puncture test was repeated on at least four different locations depending on the size of pepper and the average of peak puncture force was determined. This was observed for 0, 3, 7 and 10 days of storage of peppers.

4.3.13. Statistical Analysis

Data obtained were analyzed using the General Linear Model (GLM) model of the Statistical Analysis System (SAS) version 9.1 (SAS Institute Cary, NC., USA). Post-hoc significant differences between treatments and their effects on the microbial quality of the peppers were determined using the Least Significant Difference (LSD) Test with the significant level set at $p < 0.05$ for all the samples and treatments. The difference in the effect of treatments on texture and color of the peppers was also analyzed using the same statistical analysis methods.
4.4. Results

4.4.1. Effect of nitrogen, oxygen and air exchange on internal gas composition (%) of peppers.

a)

b)
4.4.2. Effect of gas exchange on uptake of treatment solution

The rate of uptake of EO and DW are as shown in figure 4-3. Oxygen had the highest rate of uptake with a mean volume of 40.00±1.41 and 43.25±2.22 while that of air was 15.5±2.12 and 16.75±2.06. On the contrary, nitrogen exchanged peppers did not appear to have taken up the treatment solution, instead, there was an increase in the level of water in the graduated reservoir in both EO and distilled water.
Figure 4-3: Uptake of a) EO water b) DW after gas exchange. The lines represent the standard error of the means (SEM) ($n>4$).
4.4.3 Effect of treatments on the microbial population of peppers

Figure 4-4: Effect of treatment on the population of a) *Serratia marcesens* b) Other coliforms. The lines atop the bars represent the SE of the means ($n>16$). Treatments with same superscripts are not significantly different.
Figure 4-5: Effect of treatment on the population of c) Total plate count d) Yeast and molds. The lines atop the bars represent the SE of the means (n>16). Treatments with same superscripts are not significantly different.

The effect of gas exchange on the populations of *S. marcescens* is illustrated in figure 4-4a. As expected, there was no growth observed for positive control, -Gas-SM, and -Gas-EO-SM. The oxygen-exchanged peppers with EO water treatment had the lowest
count of 3.88 log CFU/g, while gas exchanged peppers with distilled water (O2+H20+SM) had the highest count of 6.35 log CFU/g. This was observed to be higher than the negative control (-Oxy+SM) which 5.5 log CFU/g. This might be as a result of enhanced internalization of microorganisms that would have otherwise remained on the surface, during the uptake of distilled water which had little or no antimicrobial property. Nitrogen also had a low count of 4.8 log CFU/g compared to air. This may be because there was neither an uptake of solution nor an enhancement of internalization of microorganism. Therefore, most of the inoculated microorganisms remained on the surface and were easily removed via surface sanitization by EO water. Overall, responses were different (p=0.0218), even though pairwise comparison revealed most of them did not appear to be significantly different (figure 4-4a).

Although other coliforms counts did not show any significant difference in response to the treatments (p=0.563), the counts in the oxygen exchanged peppers with EO water was the lowest (4.18 log CFU/g) while the air and oxygen exchanged peppers with DW showed the highest count of 6.20 and 5.98 log CFU/g (figure4-4b). This lack of significance might be due to varying populations of internalized population of microorganism. The total aerobic bacteria count also did not show significance in difference among the treatments (p=0.3663). The count was observed to be highest in the air (control) samples. As expected, the counts were generally higher for all treatments than any microbial group isolated (Figure4-4c).

Figure 4-4 d depicts the populations of yeast and mold. The yeast count in oxygen exchanged peppers treated with EO water was found to be higher than that of nitrogen or air exchanged peppers. However, the yeast count was relatively lower when compared to the count obtained from the negative control and oxygen exchanged peppers. It might be that the oxygen exchanged peppers were able to internalize more microorganisms but the due to high organic load inside the peppers, the efficacy of EO water reduced and could not achieve optimum reduction of yeast population. Although peppers inoculated with S. marcescens did not show a significant difference (p=0.061), a pairwise comparison at 95% confidence level, revealed a significant difference
between all forms of treatment except for peppers exchanged with oxygen and treated with distilled water.

**4.4.4. Effect of treatment on the color of peppers**

**a)**

![Graph showing effect of treatment on color of peppers on Day 0](image1)

**b)**

![Graph showing effect of treatment on color of peppers on Day 3](image2)

Figure 4-6: Effect of treatment on the color and of Peppers on a) 0 b) 3 days of storage. The lines atop the bars represent the SEM (n>12). Treatments with same superscripts are not significantly different.
Figure 4-7: Effect of treatment on the color and of Peppers on c) 7 d) 10 days of storage. The lines atop the bars represent the SEM (n>12). Treatments with same superscripts are not significantly different.

The L, a*, hue angle and chroma of peppers after 0, 3, 7 and 10 days of storage is depicted in figure 4-5. Although no statistical significance was indicated on the first day of storage (p=0.689), oxygen exchanged peppers appeared to be the darkest with an
average L value of 31.9, while peppers treated with distilled water (DW) was the lightest with L value of 34.16. There was no difference in the a* values of the peppers for all treatments (p=0.160) Nitrogen samples appeared to be the most colorful of the all the sample with a chroma value of 21.54 while oxygen being the least colorful at 17.76 (p=0.025) (figure 4-5a). However, this difference was not visibly evident to the naked eye.

By the third day of storage, none of the peppers appeared different from each other not only in changes perceptible to the eye but also in terms of their L, a*, hue angle and chroma, which all showed no statistical significance at p= 0.268, 0.211, 0.317 and 0.227 respectively (Figure 4-5b).

On the 7th day of storage, the means values of these characteristics did not appear to be different although the chroma showed statistical significance with p-value of 0.008 (figure 4-5c). This may be attributed to variations in the values within the treatments. No visible or significant change was observed in all peppers of the different treatments on the 10th day of storage (figure 4-5d).

Overall, the air exchanged pepper samples appeared to be darker (lower L values) than DW, oxygen and nitrogen exchanged sample (p=0.0087). In terms of the overall storage effect, day 7 peppers were significantly higher L values than day 0 and day 10 (p=0.0081). The average a* values of all peppers in all of the treatment were not different. Upon storage, day 7 and 10 peppers had lower a* values than day 0 (p=0.0153). This may indicate that senescence might have be prevented or slowed down. Although the chroma of the peppers among treatment did not vary (p=0.2169), day 3 and day 7 peppers were more colorful than day 10 with higher mean values of chroma. All peppers retained their basic color (hue) and did not differ within the different treatments (p=0.4106) or even over the length of storage (p=0.1381).
4.4.5. Effect of Treatment and Storage on Texture and mass loss of peppers

Table 4-1: Relative mass difference (%)\(^1\) of treated pepper during study.

<table>
<thead>
<tr>
<th>Storage Days</th>
<th>Treatment Effect(^3)</th>
<th>0</th>
<th>3</th>
<th>7</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.10±0.18</td>
<td>0.12±0.20</td>
<td>0.03±0.31</td>
<td>0.19±0.33</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DW</td>
<td></td>
<td>0.61±0.13</td>
<td>0.65±0.14</td>
<td>0.48±0.16</td>
<td>3.81±5.55</td>
</tr>
<tr>
<td>Air</td>
<td></td>
<td>2.69±1.11</td>
<td>2.52±1.06</td>
<td>2.26±1.26</td>
<td>1.98±2.40</td>
</tr>
<tr>
<td>Nitrogen</td>
<td></td>
<td>0.27±0.25</td>
<td>0.32±0.18</td>
<td>0.01±0.32</td>
<td>0.23±0.50</td>
</tr>
<tr>
<td>Oxygen</td>
<td>Storage Effect(^2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>E</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>e</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Values are mean ± Standard deviation (3≤n≤15). \(^2\) Columns (Storage days) with same letters are not significantly different in response to storage according to LSD at p=0.332. \(^3\) Rows with same letters are not significantly different in response to treatment according to LSD at p=<0.0001. Negative values indicate an increase in relative mass difference.

Table 4-2 Peak force (N)\(^4\) required to puncture pepper during storage.

<table>
<thead>
<tr>
<th>Storage Days</th>
<th>Treatment</th>
<th>0</th>
<th>3</th>
<th>7</th>
<th>10</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>a,b</td>
<td>f</td>
<td>h,i</td>
<td>j</td>
<td>0.037</td>
</tr>
<tr>
<td></td>
<td>DW</td>
<td>a,b</td>
<td>f</td>
<td>h,i</td>
<td>j</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Air</td>
<td>c</td>
<td>d,e</td>
<td>g,h</td>
<td>i</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>Nitrogen</td>
<td>c</td>
<td>d,e</td>
<td>g,h</td>
<td>i</td>
<td>0.221</td>
</tr>
<tr>
<td></td>
<td>Oxygen</td>
<td>a,c</td>
<td>d</td>
<td>g</td>
<td>j</td>
<td>0.037</td>
</tr>
</tbody>
</table>

\(^4\) Values are mean ± Standard deviation (3≤n≤15) Values with the same superscripts are not significantly different according to LSD at p>0.05
Post-harvest water loss heavily influences the shelf life of peppers and could trigger other factors that affect their shelf life. Evaluation of the relation weight loss revealed that on the first day there was a negligible change in weight for non-treated pepper as was expected. Nitrogen and DW showed a slight increase in weight. This may be due solely to diffusion of water molecules of the treatment solution into the tissues of the pepper through the stem region. Air and oxygen showed average relative weight gain of 2.69 and 6.17%, respectively. As expected, this may be a direct effect of the uptake of treatment solution reported in figure 4-3. All through the storage study, there was no significant difference in trend of response to storage for the different treatments \( p = 0.332 \). At the end of the 10-day study, there was an overall difference in the effect of the treatments on the relative weights of the peppers \( p < 0.0001 \). Similar trends were observed between control and nitrogen treated peppers. Though minimal, control showed the highest relative weight loss (Table 4-1).

Firmness of pepper was reported as the peak force (N) required to puncture the peppers to a depth of 8mm (Table 4-2). Immediately after treatment (day 0), air, nitrogen, followed by oxygen treated pepper required higher puncture force compared to other treated. Except for oxygen and DW, these showed significant differences compared to control \( p = 0.037 \). Therefore, this could be attributed to differences in the sizes of the peppers used with respect to their surface area (SA) and weight (W). Díaz-Pérez and others (2007) concluded that apples of smaller sizes lost firmness faster than larger ones due to a low SA: W ratio.

On both the 3\(^{rd}\) and 7\(^{th}\) days of storage, the oxygen treated peppers had the highest puncture force and significantly different from all other treatment with the exception of air \( p \)-values 0.001 and 0.024, respectively). Similar trend was also observed on the 10\(^{th}\) day of storage however, there was no significant difference \( p = 0.221 \) (Table4-1). This high puncture force may be correlated with the relative mass loss (%) observed in this study, wherein oxygen not only showed no decrease but also an increase in mass (Table 4-2). This increase was not unexpected, as the uptake of treatment solution was observed earlier in this study (fig. 4-3). This explanation may also be true for air
exchanged peppers, which was observed to have taken up some treatment solution. Its puncture force was also relative high compared to other treatments on all the storage days.

Kissinger and others (2005) reported that water loss is directly related to softening or loss of firmness, which ultimately affects the quality of pepper. This confirmed the observations made from air and oxygen treated peppers, which had higher puncture forces compared to other forms of treatments. One potential explanation may be that the influx of water may have enhanced turgor pressure of the within the tissues, thus contributing to its mechanical strength and stabilizing the conformation of the polymers (Van Buren 1979).

It is important to note that the puncture forces showed a linear trend until the 10th day of storage. It will be interesting to extend the duration of storage in future studies to examine the trend of response. Puncture force may not adequately describe firmness, although it has been used in many studies. Some studies have used interalia, hedonic scale (Díaz-Pérez and others 2007), tissue deformation (Vicente and others 2005), compression force (Marquenie and others 2002).

4.5. Discussion and conclusion

The uptake of treatment solution in oxygen exchanged peppers may be the result of a partial vacuum created as a result of oxygen depletion due to respiration of fruit tissue, which leads to production of CO₂. The solubility of CO₂ has been reported to be 80 times greater than that of O₂ at 27 °C. As CO₂ solubilized, a pressure gradient from the exterior to the interior caused an influx of molecules of EO or distilled water from the exterior (Fleming and others, 1980). The decrease in level of water in air (control) may be attributed to diffusion of molecules. On the contrary, nitrogen exchanged peppers did not appear to have taken up the treatment solution as there was an increase in the level of liquid in the graduated reservoir in both EO and distilled water. This contradicted the report by Stan and Daeschel (2003) on the uptake of EO water by alfalfa seeds, in which a liquid uptake by nitrogen exchanged alfalfa seeds was observed. Their observation was attributed to a simple diffusion process into alfalfa seeds.
This observed difference may also be a result of the differences in the anatomical structure of Jalapeño peppers, which has a cuticular outer covering that prevents diffusion of molecules and equilibration of pressure. Furthermore, nitrogen is inert and cannot be utilized or metabolized by the tissue and microorganisms that could be present in the interior of the peppers (Jamie and Saltveit 2002). Therefore, internal pressure may be exerted by the nitrogen-filled gaseous space, which may have been responsible for rise in the level of treatment solution in the graduated tube.

Although oxygen exchange enhanced the uptake of treatment and accessibility to internalized microorganism in the peppers, it may have also aided the entry of other microorganisms that would have otherwise remained on the surface (Daeschel and others 1985) found out that oxygen exchanged cucumbers had higher bacterial and yeast counts compared to other treatments. This may also account for the higher counts observed in oxygen exchanged peppers treated with DW. In addition, the count in the gas exchanged pepper treated EO water could have been much lower.

Variations in the microbial population observed in the data, especially for the total aerobic bacteria count may be attributed to the differences in the types of peppers used and the different days of purchase. It may also be that the peppers were supplied by different growers or suppliers and so had varying amounts of microflora. The appearance of fresh produce is of paramount importance as an indicator of quality and predicted level of acceptance. Overall, these various methods of treatment did not appear to have altered the physical quality of the jalapeño peppers. Light exposure sanitation treatments and temperature changes to which peppers are exposed can influence changes in the color of peppers and senescence. This may be a result of degradation of chlorophyll and increased carotenoid content. The L value may serve as an indicator for ripening and the associated decrease in the chlorophyll content (i.e. lower L value). Lower L values can also be associated with the darker pigment of the peppers.

In fresh Jalapeño peppers, chlorophyll is more abundant than carotenoid. Therefore, negative values of a* are more desirable than positive values which were observed for
all storage days to be negative. Attractiveness of color is attributed to chroma, therefore a higher chroma is a desirable quality as this indicates colorfulness (Gomez-Ladron de Guevara and others 1996). During these experimental days, color which was predicted by the chroma, a*, hue angle and L values were observed to be stable. Therefore, post treatment effect on color may be of lesser concern for prolonged storage study.

In conclusion, oxygen exchanged peppers treated with EO water effectively reduced the microbial population without affecting the color and texture of peppers when compared to the control. During the texture analysis, gas exchanged peppers appeared to be crisp; a desirable quality as opposed to being rubbery which is an indication of senescence in fruits. In event of an adoption of this method of sanitization, it is advised that holding time between gas exchange and addition of EO water be reduced to the lowest possible. This is because the internal atmospheric gas of the peppers has been observed to diffuse quickly when held for longer periods, or the gases may be metabolized rapidly by the tissues of the fruit.

4.6. Acknowledgements

Thanks to Dr Zhao for use of Spectrocolorimeter, Gas Cromatograph System and Texture analyzer. Thanks also to Joo Yeoun Jung for helping with SAS software.

4.7. References


CHAPTER 5: General Conclusions and Suggestions

The first part of this thesis research focused on investigating the efficacy of combining electrolyzed oxidizing (EO) water with UV light in comparison with using only EO water in the sanitization of Jalapeño peppers. The experiment used a marker organism; *Serratia marcescens* as a surrogate for *Salmonella*. The EO water owes its antimicrobial activity to its high concentration of chlorine, low pH and high oxidation-reduction potential. However, when EO water was combined with the DNA-damaging UV light, greater antimicrobial effect was observed.

Peppers treated in EO water for 30 minutes appeared to be the most effective treatment and did not impact the organoleptic qualities of the peppers. After the 10 days of storage, there was an observed increase in the population of microorganism, demonstrating that there was no post-treatment residual antimicrobial activity. Moreover, it was observed during the storage that there was no indication of fruit rotting, senescence and decay which are often major concerns when exposing fruits and vegetables to UV or other treatments. On the contrary, peppers treated with EO water and UV appeared to be firmer in texture compared to control or those treated with only EO water. The UV dose, compared to other treatments was relatively low, thus allowing for higher doses in situations where greater microbial reduction is required.

The aim of the second research objective was to study the effect of gas exchange on the uptake of treatment solutions. Distilled water and electrolyzed oxidizing distilled water were used as treatment solution. Peppers that were gas-exchanged with oxygen showed a higher uptake of treatment solution as a result of the presumed vacuum formed within the pepper and a higher reduction in the count of *Serratia marcescens* compared to air and nitrogen exchanged peppers. There was also significant increase in the mass of oxygen exchanged peppers attributed to the uptake of treatment solution. Overall, no significant impact on the color of peppers was observed in comparison with the control. The texture of oxygen exchanged peppers was firmer when compared to control. The results demonstrated that internalized organisms can be accessed and inactivated by gas exchange followed by uptake of a sanitizer into the fruit or vegetable.
In conclusion, this study lays groundwork for further application and modification of EO and UV treatment methods. Because low doses of UV induce hormetic responses, further studies must be done on the phytochemical effect of this method of treatment on peppers. Additional research may provide an insight not only into the effect of gas exchange on enhancement of treatment solution, but its impact on the physicochemical properties of peppers and application to other types of fruit. In order to reduce risk of foodborne illness, the importance of prevention of contamination during pre- and post-harvest handling cannot be the understated and that sanitation should not be viewed as a standalone approach to ensuring the safety of fresh produce.

The findings in these two studies are, however, subject to some limitations. The inoculum load used in this study was much higher than what is commonly observed in an actual produce contamination. Also, *Serratia marcesens*, as stated previously, was used as a marker organism because of its ease of identification and the non-pathogenicity. Though predictable, it is not known what the outcome will be, if an actual produce associated-pathogen were to be used. During the inoculation, the level of internalization was not known. Therefore, the efficacy of this treatment was difficult to estimate based on the actual count of internalized microorganisms.

In addition, peppers were sourced from a local grocery store, which were bought from different exporters and marketers. While lending an opportunity for an actual contamination condition, grocery bought peppers had various microbial conditions, and thus affected the consistency of data obtained. Furthermore, it was difficult to estimate the exact postharvest age of the pepper. For future research, it will be important to ensure that peppers are sourced from the same farm or have all undergone the same post harvest handling. The sensory quality of fresh fruits is of paramount importance for consumer acceptance. Therefore, knowledge of the impact of this method of treatment on the organoleptic quality of peppers is important.
BIBLIOGRAPHY


Anonymous. 2007b. Outbreak Surveillance Data

Reported Foodborne Disease Outbreaks and Illnesses by Etiology and Food Commodities, United States(Foodborne Outbreak Online Database (FOOD), Annual Listing of Foodborne Disease Outbreaks, United States (1998–2007)). Centre for Disease and Prevention.

Anonymous. 2010. Analysis of Produce Related Foodborne Illness Outbreaks Commissioned by the Alliance for Food and Farming


CDC Center for Disease Control and Prevention. 2006. Update on Multi-State Outbreak of E. coli O157:H7 Infections From Fresh Spinach, October 6, 2006.


CDC Center for Disease Control and Prevention. 2009b. Leading Causes of Death.


CSPI Center for Science in Public Interest. 2007. Outbreak Alert Database.


FDA Food and Drugs Administration. 2001. Chapter IV. Outbreaks Associated with Fresh and Fresh-Cut Produce. Incidence, Growth, and Survival of Pathogens in Fresh and Fresh-Cut Produce

FDA Food and Drugs Administration. 2009. Analysis and Evaluation of Preventive Control Measures for the Control and Reduction/Elimination of Microbial Hazards on Fresh and Fresh-Cut Produce.


USDA United States Department of Agriculture-. Nutritive data for fresh Jalapeno pepper.


