EFFECTS OF ULTRASONICATION IN COMBINATION WITH SELECTED SANITIZER AND SURFACTANT ON THE QUALITY AND MICROBIAL SAFETY OF PRODUCE

BY

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THESIS

Submitted in partial fulfillment of the requirements for the degree of Master of Science in Food Science and Human Nutrition with a concentration in Food Science in the Graduate College of the University of Illinois at Urbana-Champaign, 2013

Urbana, Illinois

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ABSTRACT

In the United States, outbreaks of foodborne illnesses caused by pathogenic microorganisms associated with consumption of fresh and fresh-cut produce is a recurring problem. It is estimated that 1 in every 6 US residents contracts a foodborne illness every year. There is an urgent need to develop better sanitization methods and sanitizing agents that are effective against pathogens, safe for operators, environmentally friendly, and with minimal negative impact on produce quality. This study was undertaken to address the need for the development of more effective sanitization strategies and better sanitizers. Specifically, the effects of a new sanitization strategy and a set of sanitation combinations on reduction of microorganisms on produce, as well as the potential impact of these treatments on produce quality, were examined.

To examine if lettuce will be floating or submerged in a washing solution, the water absorption and thus changes in specific gravity of whole head Iceberg lettuce at two storage (5°C and 23°C) and two washing solution (4°C and 23°C) temperatures were first investigated. The reduction of Escherichia coli O157:H7 population during a sanitization treatment as affected by produce surface area to weight ratio was examined with whole baby carrots and baby carrots cut into sticks and shreds. The decay of chlorine and peroxyacetic acid at different surface to weight ratios was evaluated. The reduction of E. coli O157:H7 on Iceberg lettuce by two washing sequences, i.e. “cutting-before-washing” and “washing-before-cutting” was compared by treating the samples with water, and combinations of ultrasound with chlorine (free chlorine concentration 20 mg L⁻¹) and Tsunami (final acid concentration 80 mg L⁻¹) was examined.

Iceberg lettuce stored at 23°C and washed at 4°C had the highest percentage increase in weight and specific gravity. Chlorine and peroxyacetic acid availabilities decreased when the surface-to-weight ratios increased, and chlorine was consumed faster than peroxyacetic acid.
Correspondingly, a lower reduction of *E. coli* O157:H7 counts on carrot samples having high surface area-to-weight ratio was found, evidencing the effect of chlorine decay on inactivation of microorganisms. In sanitizer-only washing tests, the *E. coli* count reduction for lettuce treated by “washing-before-cutting” was higher by 0.79 and 0.80 log$_{10}$ CFU/g in chlorine and peroxyacetic wash, respectively, compared to the traditional “cutting-before-washing” process. When ultrasound was used in combination with a chemical sanitizer, a further increase in the *E. coli* population reduction of 0.68 and 0.37 log$_{10}$ CFU/g (again, for chlorine and peroxyacetic acid, respectively) was achieved by the “washing-before-cutting treatment”, reaching total reductions of 2.43 and 2.24 log$_{10}$ CFU/g for the chlorine and peroxyacetic washes, respectively.

In tests to evaluate the effects of sonication in combination with 2 sanitizers (chlorine and Tsunami) and sodium dodecyl sulfate (SDS) on the quality of fresh-cut Iceberg and Romaine lettuce, lettuce samples were sonicated for 1 minute in a custom-designed ultrasonic (US) tank containing one of the following treatment solutions: tap water, chlorine (100 mg L$^{-1}$ free chlorine), Tsunami (80 mg L$^{-1}$ peroxyacetic acid), and a combination of Tsunami with 0.1% (w/v) SDS. Washed samples were bagged and sealed under modified atmosphere conditions and stored at 4 °C for up to 14 days. Changes in headspace gases, texture, color, tissue damage, visual quality, and populations of aerobic mesophiles and yeasts and molds were determined. The oxygen concentrations and CO$_2$ accumulation in Romaine lettuce were not significantly different among the treatments. In Iceberg lettuce, lower O$_2$ and higher CO$_2$ concentrations in the samples treated with Tsunami and Tsunami + SDS were recorded. After 14 days of storage, the tissue damage measured by electrolyte leakage rate (ECR), total color differences, firmness, and total aerobic plate counts were not significantly different for all the treatments in both types of lettuce samples (P>0.05). Treatments of Iceberg lettuce with sonication in combination with
Tsunami or Tsunami + SDS do not cause more quality changes compared to the chlorine treated samples. For Romaine lettuce, chlorine treated samples had a significantly higher overall quality score than those from the other treatments.
With love to my family
ACKNOWLEDGEMENTS

I would like to thank God for giving me the faith and perseverance to accomplish my objectives. With him by my side the whole process was easier.

I would like to express my sincere gratitude and admiration to my advisor, Dr. Hao Feng, for his guidance, support, and mentorship over the last three years. I would also like to thank my committee members, Dr. Michael Miller and Dr. Arne Pearlstein for their time and support, as well as all professors who in some way helped me throughout my journey. My appreciation and gratitude to my lab mates, especially Dr. Hee-Kyung Park, for her helpful suggestions, ideas and assistance throughout my research.

Finally I would like to thank my family and friends for their unconditional love and support. To my mother Teresa and my brothers Jonathan and Samuel, thank you for your love and words of encouragement. To my best friend and fiancé Luis Vargas, thank you for your thoughtful consideration, unconditional love and support that made my work easy and pleasant.
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CHAPTER 1
INTRODUCTION

Lettuce is the second most consumed fresh vegetable in the United States (USDA, 2012); its consumption is linked to a number of health benefits as a result of its high phyto-nutrient content. Lettuce is consumed in a variety of ways and is the base for many dishes due to its ease of preparation. Over the years, lettuce in the US has gone from being purchased as whole heads and prepared at home to being purchased processed and ready-to-eat in bags. Ready-to-eat lettuce offers American households and restaurants the convenience of skipping further preparation while providing the nutrients and other health benefits (Thompson & Wilson, 1999).

With the increase in demand for fresh-cut lettuce, consumers’ expectations regarding quality and shelf life have also increased. On average, bagged lettuce has a shelf life of 14-18 days (Clarkson, O'Byrne, Rothwell, & Taylor, 2003), during which consumers expect to receive a product that is clean, crisp, free of visible damage, and with a bright green color. It is worth noting that these parameters can change depending on the season and time of purchase. Many studies have reported that changes in quality of ready-to-eat bagged lettuce are visible as early as day 7 post-processing, while others have reported the ability to extend the shelf life for over 14 days. Processors are constantly in search for optimum sanitizing methods that can preserve the nutritional value and quality parameters of lettuce over a 14-day period while making it microbiologically safe (Guan et al., 2010).

On a regular basis, processors add sanitizers to the wash water used for disinfection of fresh-cut lettuce. The addition of sanitizers not only enhances the microbial quality of lettuce but also helps to maintain produce quality during storage (Brackett, 2000). Chlorine and chlorinated products are the most common sanitizers used in the food industry, their proven capability in
eliminating microorganisms, low cost, high availability, and ease of use make chlorine and chlorinated products the favored sanitizers used in the industry (Suslow, 2013; Zhou, 2010). However, over the last few years the use of chlorine has raised concerns because of its lack of stability in the presence of organic matter and the generation of chlorinated by-products having negative environmental and human health impacts (Keskinen, Burke, & Annous, 2009). Sonication, irradiation, new sanitizers, and modified atmosphere packaging, when used alone or often in combinations, are among the technologies that have emerged in recent years as potential alternatives to reduce the health and environmental impacts while facilitating an enhanced wash treatment to improve microbial reduction and enhance produce quality retention.

Another incentive for the development of new and more effective produce sanitation methods is the fact that increased consumption and production of fresh produce has been accompanied by an increase in associated human illness outbreaks. Between 1996 and 2005, leafy green consumption increased 9.0% and leafy green associated outbreaks increased 38.6% (Herman, 2008). Among the human pathogens that are reported in the outbreaks, Escherichia coli O157:H7 has been considered an important pathogen linked to consumption of lettuce. To address the “recurring outbreaks of E. coli O157:H7 associated with fresh and fresh-cut lettuce”, the US Food and Drug Administration (FDA) developed a Lettuce Safety Initiative aiming to reduce the public health risks. One objective of the Lettuce Safety Initiative is to “document observations that identify practices that potentially lead to product contamination”. Over the years, the produce industry has used a “cutting-before-washing” method to produce and sanitize fresh cut produce, including fresh-cut lettuce. This process has a number of problems including generation of organic matter, risk of pathogen internalization through cutting, and cross contamination. These known issues can turn the current sanitization processes into one
“potentially lead to product contamination.” Obviously, in the effort to explore better produce sanitation strategies, the current washing procedure needs to be scrutinized.

The overall objective of this study is to explore strategies to enhance the microbial safety of fresh and fresh-cut lettuce by examining the use of ultrasound in combination with selected sanitizers and by modifying the current head lettuce washing procedure. The specific objectives are as follows:

- To evaluate the effects of surface-area-to-mass ratio obtained by different produce preparation and washing methods on reduction of *E. coli* O157:H7 population on Iceberg lettuce and carrots, and

- To examine the effect of ultrasound in combination with selected sanitizers and surfactant on the quality of Iceberg and Romaine lettuce during 14 days of storage.
CHAPTER 2
LITERATURE REVIEW

2.1 LETTUCE

Lettuce (*Lactuca sativa* L.) is a cool-season crop grown in many countries. In the United States the major producers of lettuce are the states of Arizona and California. In 2010 the production of lettuce in the U.S. was nearly 8.7 billion pounds and production value of $2.2 billion, which indicates that lettuce is the leading vegetable crop in terms of value in the U.S (Boriss & Brunke, 2011). There are many varieties of lettuce such as Iceberg, Romaine, butterhead, and curled, all of which form the base for many dishes that are eaten raw (Gunes & Dogu, 2011). Studies have shown that consumption of leafy greens is associated with many health benefits such as the prevention of chronic and cardiovascular diseases, the top cause for death in industrialized countries (Liu, 2004). The consumption of lettuce has considerably increased over the last 10 years. On average Americans annually consume around 28 pounds of Iceberg and Romaine lettuce.

2.1.1 Minimally processed lettuce

According to Wolf (1999), prior to 1980 lettuce was sold in bulk and the major buyers were supermarkets and food service establishments. Since then, lettuce has become a branded product sold as packaged salads and a minimally processed product whose major buyers are restaurant and supermarkets. Branding lettuce as a minimally processed product has offered advantages for consumers at an individual level and large buyers such as hotels and restaurant, since it allows for skipping any further processing thus reducing cost. In order to be considered minimally
processed, lettuce must undergo a series of steps shown in Figure 2.1 such as washing, trimming and packaging (FDA, 2009). During this process lettuce can undergo changes that have a detrimental effect on the quality of the final product. Delaquis et al. (2000) and Koseki & Isobe (2006) reported that fresh-cut lettuce washed with tap water had lower quality and acceptability among consumers compared to lettuce that was washed with chlorinated or with ozonated water. Hence, the fresh produce industry is constantly in search of a sanitizer capable of reducing the incidence of pathogenic and indigenous microorganisms while maintaining the quality of lettuce for a long period of time (Guan, Huang & Fan, 2010).

**Figure 2.1** General supply chain flow for leafy greens processing.


2.2 MICROBIAL SAFETY ISSUES IN THE LEAFY GREEN INDUSTRY

With an increase in the consumption of bagged leafy greens worldwide, foodborne disease outbreaks associated with such consumption have also increased (Mathews, 2009). Although the chance of eating produce contaminated with pathogens is very low, any human pathogens on produce may be enough to cause diseases due to their low infectious dose. It is estimated that every year foodborne illnesses due to all foods, cause an estimate of 5000 deaths in the United States (Smith et al., 2009; Mandrell, 2009).

In the U.S. and Europe, the most common pathogens associated with foodborne illnesses where leafy greens have been implicated are E. coli O157:H7 and Salmonella. In the U.S the outbreaks have been linked to domestically grown produce while in European countries the outbreaks have been linked to both domestically grown as well as exported product from nearby regions (Nygård, et al., 2008). The ability of Pathogens to survive, colonize and interact with fresh and fresh-cut produce depends on several factors, such as type of produce, physiological state of the plant, vehicle host and type of pathogen (Deering et al., 2012). Some experimental trials indicate that E. coli and Salmonella can proliferate in compost and irrigation water, and can survive for long periods of time in the soil (Mootian, Wu & Matthews, 2009). Therefore, measures to enhance microbial safety of leafy produce must also consider pre-harvest operations and processes, and seek to eliminate the chance of pre-harvest contamination.

2.3 ESCHERICHIA COLI O157:H7 OUTBREAKS IN LETTUCE

Since 2005, several shiga toxin–producing E. coli O157:H7 outbreaks linked to bagged lettuce have been reported (Mathews, 2009). E. coli O157:H7 is an enterohemorrhagic bacterium with a very low infectious dose (10-100 cells). It is characterized by the production of shiga
toxins and accounts for 75% of the worldwide *E. coli* related outbreaks. According to the Center for Disease Control and Prevention (2012), individuals of all age groups are susceptible to be affected by *E. coli* spp. The most vulnerable groups are young children under the age of four, the elderly, and those with compromised immune systems.

*E. coli* O157:H7 can internalize within the tissue of produce at different points in the growing and distribution process. This has been proven in a number of studies, which identified the routes through which *E. coli* can internalize within fresh produce. According to Jablasone et al. (2005), *E. coli* O157:H7 can attach to and survive on fresh produce as early as in the seeding stage. Franz et al. (2007) and Mootian, Wu & Matthews (2009) examined the internalization of *E. coli* during the growth of lettuce; they utilized contaminated soil, contaminated fertilizer, and contaminated water as the contamination sources and concluded that lettuce exposed to *E. coli* O157:H7 at any of the growing points might become contaminated and represent a threat to human health.

*E. coli* O157:H7 can also internalize into lettuce tissues during post-harvest processing. If bacteria are present in water or food-contact surfaces, *E. coli* can infiltrate lettuce tissue through natural openings such as the stomata and lenticels, through sites of biological physical damage, or through mechanically induced open surfaces (Deering, Mauer & Pruitt, 2012; Nou & Luo, 2010). Plant leaves use a thin protective layer to against attachment of microorganisms, and if this layer is disrupted, it offers a site for penetration of bacteria (Takeuchi & Frank, 2000). In addition, during post-harvest processing of fresh produce it is very common to use water wash to remove soil and debris; likewise, it is very common for processors to recycle the water for conservation and to reduce cost. However, if the sanitizer concentration in the wash water is
below a threshold value, pathogens can survive in the wash water and can transfer to the subsequent batch of fresh-cut produce, causing cross contamination (Herdt & Feng, 2009).

2.4 SANITIZERS

Generally, fresh and fresh-cut fruits and vegetables are sanitized with an aqueous solution of a chemical sanitizer in order to reduce bacteria count and prevent microbial contamination (Fransisca, 2011). Several factors such as pH, temperature, presence of organic matter, produce surface topography, sanitizer concentration, and contact time determine the efficacy of a sanitizer in removing bacteria from protected sites in the epidermis and localized within produce tissue (Burnett & Beuchat, 2001). A higher antimicrobial concentration, combined with high temperature, long contact time and high shear rate always results in the highest reduction of microorganisms harbored on the surface of fresh and fresh-cut produce (Herdt & Feng, 2009). The desired sanitizer concentration will be determined by what is permitted by the law and economic considerations (McGlynn, 2004).

2.4.1 Chlorine

Chlorine is the most widely used chemical sanitizer in the disinfection of fruits and vegetables. Its effectiveness against a wide spectrum of microorganisms (indigenous bacteria, yeasts, fungi and spore forming microorganisms) as well as its low price and availability makes chlorine the most popular sanitizers. Generally, chlorine concentration is expressed as total chlorine or free chlorine available in the solution; the allowed concentration for disinfection of fruits and vegetables is 50 to 200 mg L\(^{-1}\) with a contact time of 1 to 2 minutes (Food and Drug Administration, 1996). The action of chlorine disrupts metabolism of bacterial cells due to the
interaction of hypochlorus acid (HClO) with the cellular membrane of the microorganisms, resulting in termination of metabolic reactions essential for microbial proliferation (Herdt & Feng, 2009; Beuchat, 1992). In terms of the concentration required for removal of microorganisms from surfaces of fresh-cut fruits and vegetables, Nou et al. (2010) reported that 70 mg L$^{-1}$ reduced indigenous microorganisms in Romaine lettuce by 1 log, whereas Park & Beuchat (1999) and Gonzales et al. (2004) found that 200 mg L$^{-1}$ of chlorine reduced *Listeria monocytogenes* and *E. coli* O157:H7 in honeydew melon and Iceberg lettuce by 1 and 1.5 log$_{10}$ CFU/g, respectively. Therefore, constant monitoring of sanitizer concentration is vital for the proper disinfection of fresh-cut produce.

Although the use of chlorine for disinfection of fresh and fresh-cut fruits and vegetables offers many advantages to food processors, its use has drawbacks as well. Chlorine is considered a highly corrosive chemical, especially in acidic conditions, which oftentimes reduces the life of tanks and stainless steel equipment used in processing facilities. Furthermore, chlorine stability is affected by several factors. An increase in temperature of the washing solution will accelerate evaporation of active species. Chlorine’s efficacy is also affected by heavy loads of organic matter in the washing solution. As mentioned before, processors tend to recycle water and resulting accumulation of organic species allows production of organochlorine compounds (including trihalomethanes) considered dangerous for the environment and human health (Nou et al., 2011; Nieuwenhuijsen, Toledano & Elliott, 2000).
2.4.2 Peroxyacetic acid

Peroxyacetic acid is a sanitizer more effective than chlorine killing microorganisms at low concentrations. The maximum concentration of peroxycetic acid used in wash water should not exceed 80mg L\textsuperscript{-1}. Some studies have shown its effectiveness at much lower concentrations against all sorts of microorganisms and others have found its greatest effectiveness at the maximum allowed concentration (Ölmez & Kretzschmar, 2009; Food and Drug Administration, 1999). For example, Neo et al. (2013) found that (70 mg L\textsuperscript{-1}) of peroxycetic acid can reduce \textit{E. coli} O157:H7 on mung bean sprouts by 2.3 logs compared to 2 logs achieved with 170 ppm of chlorine. Wang et al. (2006) observed a total reduction of \textit{E. coli} O157:H7 counts by 1.23 log\textsubscript{10} CFU/g after treating fresh-cut apples with 80 ppm of peroxycetic acid compared to reduction of 0.23 log and 0.75 log achieved with water and chlorine (88 mg L\textsuperscript{-1}), respectively. Hilgren & Salverda (2000) reported log reductions of 2.02 and 1.86 log\textsubscript{10} CFU/g of aerobic plate count, and yeast and mold counts respectively, in celery treated with (80 mg L\textsuperscript{-1}) peroxycetic acid.

The efficacy of peroxycetic acid is attributed to its high oxidizing potential; its mode of action is associated with transfer of electrons and disruption of outer cell walls causing the death of pathogenic microorganisms (Herdt & Feng, 2009). Additionally, peroxycetic acid is stable in presence of organic matter, and no pH adjustment in the solution is necessary. Unlike chlorine, no production of byproducts due to its interaction with organic matter has been reported.

Although peroxycetic acid has a significantly higher cost compared to chlorine its advantages and benefits of using peroxycetic acid outweighs the cost difference.
2.4.3 Surfactants

Surfactants are substances that have the ability to reduce the surface tension between two liquids, between a solid and a liquid or between a liquid and a gas, and are present in most household and industrial cleaning products (Broze, 1999). The role a surfactant plays in reducing microbial counts on surfaces of fruits and vegetables is attributed to detachment of cells from produce surfaces rather than inactivation, and effectiveness is linked to the ability to increase wettability of the surface rather than by improving chemical penetration (Sapers, 2009).

There are many conflicting views and inconclusive data about the use of surfactants during disinfection of fruits and vegetables. For example, some researchers have found that the combination of a chemical sanitizer with a surfactant can enhance microbial reductions of *E. coli* O157 and *Yersinia enterocolitica* in apples, lettuce and carrots by 0.5 to 1.2 log_{10} CFU/g, respectively (Sapers et al., 1999; and Escudero et al., 1999). Sagong et al. (2013) reported that no changes in quality of fresh produce treated with a surfactant were observed. In contrast, (Guan, Huang & Fan, 2010; and Raiden, Sumner, Eifert & Pierson, 2003) stated that acids in combination with surfactant have a detrimental effect on the quality of lettuce, although the acid and surfactant combination did provide a higher microbial count reduction compared to a water rinse. There is a need to further investigate the interactions between produce, sanitizer, and surfactant to better understand the potential to enhance sanitization efficacy.

2.5 MODIFIED ATMOSPHERE PACKAGING

Modified atmosphere packaging (MAP) is a technology that utilizes packaging in combination with inert gases such as nitrogen to achieve an atmosphere different than normal air. Modified atmosphere packaging is widely used in the food industry for storage and
transportation of several types of foods (Yahia, 2009). Modified atmosphere packaging offers a series of advantages for food processors such as the possibility of extending shelf life of the product by protecting it during transportation and maintaining its freshness longer. By maintaining the moisture content inside the package (Ballantyne et al., 1988; Flodin et al., 1999), it allows prediction of shelf life based on previous data (McKellar, et al., 2004). Additionally, since most MAP packages are see-through they are attractive for the consumer (Rojas-Graü et al., 2009).

The disadvantages of MAP include the high costs associated with control equipment and installation of a modified atmosphere packaging system, high cost of packaging (Ives et al., 1996), and modified atmosphere packaging of fresh produce must always be preceded by disinfection, since MAP alone cannot kill bacteria (Allende & Artes, 2003).

2.6 ULTRASOUND

The term “ultrasound” refers to sound waves with frequencies above the range that is audible to humans. The ultrasonic waves used in surface decontamination have a frequency ranging from 20 to 100 kHz, and are also known as power ultrasound. Power ultrasound has long been used as an industrial surface-cleaning tool. The application of ultrasound for fresh produce surface decontamination, however, is relatively recent. Surface decontamination with ultrasound involves the application of sound waves into a washing solution, which leads to the creation of cavitation bubbles. Cavitation refers to the formation, growth and implosion of gas- or vapor-filled tiny cavities in liquids when large pressure differences occur (Feng and Yang, 2004). Generally, power ultrasound can enhance cleaning or decontamination efficacy by four mechanisms: 1) enhanced sanitizer transport in the bulk liquid, 2) mechanical dislodging of dirt
and microbes due to water jetting, 3) impingement effect of water jets that force sanitizer into crevices, and 4) shear forces on produce surfaces produced by micro-streaming.

Ultrasound’s effectiveness in enhancing the ability of chemical sanitizers to reduce counts of microorganisms is very well documented. Lillard (1993) found that chlorine’s efficacy in reducing Salmonella typhimurium inoculated on chicken skin can be enhanced by 1.5 to 2 log_{10} CFU/g if combined with ultrasound. When ultrasound is used together with acids it offers a similar enhancement in microbial reduction. Zhou et al. (2009) found that peroxyacetic acid combined with ultrasound can reduce E. coli inoculated on surface of spinach by 2.9 log_{10} CFU/g. Ultrasonication can be combined with heat, also known as thermo-sonication to further boost the removal of pathogens. Beuchat & Scouten (2002) found that reduction of E. coli populations from alfalfa seeds using peroxyacetic acid + ultrasound + heat can be improved by 2 logs compared to treating with peroxyacetic acid and ultrasound. All of these findings suggest that ultrasound is an excellent technology that can aid chemical sanitizers in reducing pathogens and indigenous microorganisms on fresh produce to levels that are safe for human consumption.

2.7 REFERENCES


Urbana, IL, USA: University Of Illinois At Urbana-Champaign.


CHAPTER 3

THE EFFECT OF SURFACE AREA TO MASS RATIO ON REDUCTION OF E. COLI O157:H7 ON ICEBERG LETTUCE (LATUCA SATIVA L.) AND CARROTS (DAUCUS CAROTA)

3.1 INTRODUCTION

Washing is an important step in produce processing. It reduces microbial populations, and is the only step that removes soil and debris. In large-scale produce operations, sanitizers such as chlorine, ozone, and chlorine dioxide are usually added to wash water to reduce microbial load on produce surfaces. Chlorine is the most widely used antimicrobial for washing fresh fruits and vegetables, because of its low cost and effectiveness against a wide spectrum of microorganisms, including viruses, vegetative bacteria, bacterial spores, fungi, algae and protozoa (Trueman, 1971; Suslow, 2013). However, washing with chlorine has limited effects on reduction of pathogens attached to produce surfaces. As an oxidizing agent, free chlorine (hypochlorous acid) readily reacts with organic materials in the wash solution, rapidly depleting its concentration and thus reducing its efficacy in pathogen inactivation (Luo et al., 2012).

Currently, the produce industry applies a “triple-wash” system where cut fresh produce are prewashed in the primary flume/tank, followed by a sanitization wash in a flume/tank, and then a clean water rinse to remove residual sanitizer from produce surfaces. All wash/rinse steps are performed after cutting. For instance, Iceberg head lettuce is cut into slices or shreds before washing. There are a number of issues associated with this process. First, cutting, especially shredding, increases the organic load in the wash solution that can react with free chlorine causing rapid depletion (Pirovani et al., 2004; Nou & Luo, 2010). Also, cutting has the potential
to allow internalization of contamination to the interior and to wounded produce tissues (Lopez-Galvez, et al., 2010; Gleeson & O’Beirne, 2005). It is known that pathogens preferentially attach to cut produce edges (Singh et al., 2002), and once attached, they are extremely difficult to remove or inactivate by subsequent sanitizer treatments. In addition, once large amounts of organic latex are released into the washing solution, reaction with chlorine can produce harmful byproducts, including trihalomethanes (THMs) and other carcinogenic compounds (Fawell, 2000). Moreover, if the sanitizer concentration falls below a critical level, the washing can become a process to promote cross contamination (Luo, et al., 2011).

Cutting or shredding is a physical process to reduce the size of produce, providing convenience in packaging, transportation, and consumption. For the same amount of produce, cutting increases the surface area to mass ratio. In this process, produce exudates are leaked into washing solution, wounds are created, and rapid depletion of sanitizer is expected. Thus, the produce cutting process introduces a number of adverse impacts that compromise the sanitization effort. There is a need to re-evaluate this traditional fresh-cut process. In the study reported in this chapter, a new strategy in head lettuce sanitization is proposed and tested with the aim of avoiding these problems. The new method was to wash the Iceberg head lettuce (Lactuca sativa L.) before cutting it. The reduction of *Escherichia coli* O157:H7 inoculated on whole head Iceberg lettuce was examined with a pilot-scale continuous-flow ultrasonic washer with “washing-before-cutting” and traditional “cutting-before-washing” treatments. The study was part of the effort supported by a USDA Specialty Crop Research Initiative project. For this purpose, the surface area to mass ratio was proposed as a control parameter, and carrot (*Daucus carota*) was used as a model produce to examine the effect of different surface-area-to-mass ratios on reduction of inoculated bacteria. In addition, efforts were also made to examine the use
of ultrasound as an aid to enhance produce sanitation efficacy, and to evaluate the use of Tsunami-100® (peroxyacetic acid) as an alternative sanitizer to reduce *Escherichia coli* O157:H7 population on lettuce.

### 3.2 MATERIALS AND METHODS

#### 3.2.1 Pilot plant wash system

The Iceberg lettuce wash was carried out in a pilot-scale continuous-flow ultrasonic washing system. The washer (Figure 3.1, adapted from Zhou et al, 2012) consisted of a water tank capable of holding approximately 400 gal of water (W). The tank was equipped with three pairs of ultrasound transducer boxes (T1, T2, and T3). Each pair of transducer boxes was driven by an ultrasound generator (Quality Sonic Products, EZ, SOEST, Netherlands) with rated power of 2 kW at frequencies of 25, 40 and 75 KHz, respectively.

Prior to the start of each test, the wash tank was filled with chilled tap water (10 °C). Chlorine (active ingredient sodium hypochlorite, free chlorine concentration 20 mg L⁻¹) or Tsunami-100® (active ingredient peroxycetic acid, final acid concentration 80 mg L⁻¹) was added and then degassed for 10 minutes to remove dissolved oxygen and improve ultrasound efficacy.
3.2.2 Sample and chemical preparation

3.2.2.1 Bacterial strain preparation

A nalidixic acid resistant mutant of nonpathogenic *Escherichia coli* O157:H7 strain ATCC 87-23 was used in the experiments. The bacterial strain was previously prepared by repeated sub-culturing on a nutrient plate containing 50mg L$^{-1}$ of nalidixic acid (Sigma Aldrich, St. Louis, MO). Cultures of *E. coli* O157:H7 were grown in Tryptic soy broth (TSB) (Sigma Aldrich, St. Louis, MO) overnight at 37 °C, cells were harvested by centrifugation and washed twice in sterile 0.1% Peptone water (PW). The recovered *E. coli* precipitate was diluted in 6 ml of 0.1% peptone water; the final inoculation level was $2.5 \times 10^7$ CFU/mL.

3.2.2.2 Sample preparation

Iceberg lettuce (*Lactuca sativa* L.) heads were purchased from a local supermarket and immediately transported to a processing laboratory where they were stored at $6 \pm 1$°C and used
within 24 h. The outer three leaves of each head of lettuce were removed and discarded. A kitchen knife was used to slice each head of lettuce in pieces of 1 in$^2$.

Baby-cut carrots (*Daucus carota* L.) were purchased from a local supermarket and immediately transported to a processing laboratory where they were stored at 6 ± 1°C and used within 24 h. Pieces free of visible damage, free of crevices and of similar size and weight were selected for the experiments. In order to achieve different surface-area-to-mass ratio, fifty grams of carrots were cut in three different forms: whole (51.34 cm$^2$) sticks (76.74 cm$^2$) and shreds (2278.40 cm$^2$) with the use of a kitchen peeler. An illustration regarding inoculation sites and spots is provided in Figure 3.2

**Figure 3.2** Illustration of inoculation sites for Iceberg lettuce and carrots. (A) Iceberg lettuce denoting inoculation spots, (B) different cuts of carrots.
3.2.2.3 Sample inoculation

Cleaned heads of lettuce were inoculated in 10 different spots of the upper half of the head of iceberg lettuce with 200 μl of *E. coli* O157:H7 ATCC 87-23 inoculum and dried for at 22 °C for 2 hours in a laminar-flow purifier PCR enclosure with a vertical airflow of 60-80 fpm (Labconco®, Kansas City, MO, USA). After drying, the samples were cut into 1×1 inch pieces prior to or after washing for two minutes in the continuous-flow ultrasound tank in Tsunami (Peroxyacetic acid as active ingredient, final acid concentration 80 mg L⁻¹) or chlorine (final free chlorine concentration 20 mg L⁻¹) solution.

Fifty grams of carrots were spot inoculated with 2 mL of *E. coli* O157:H7 ATCC 87-23 and dried at 22 °C for 2 hours in a laminar-flow purifier PCR enclosure with a vertical airflow of 60-80 fpm (Labconco®, Kansas City, MO, USA). Three styles of cuts: uncut, sticks and shreds were performed after drying the carrots.

3.2.3 Evaluation of degradation of chemicals

In this study the decay of free chlorine and peroxyacetic acid during a disinfecting procedure of carrots cut in four types of cuts; whole, sliced, sticks and shredded using a sample- to-solution ratio of 1:12 were investigated. The chlorine washing solution (free chlorine concentration 60 mg L⁻¹) was prepared by adding a commercial sanitizer Clorox® (6.15%, sodium hypochlorite active ingredient) to distilled water. The peroxyacetic acid washing solution (final acid concentration 60 mg L⁻¹) was prepared by adding a commercial sanitizer of Tsunami-100® to distilled water. Fifty grams of carrots were submerged in 600 mL of washing solution and washed for 1 minute, during the washing procedure, the samples were agitated at 100 rpm using a Corning® bench top stirrer (CORNING, Tewksbury MA, USA). The concentrations of
the chemicals in the washing solutions were measured prior to the addition of carrots and after 1 minute of treatment. Free chlorine concentration was measured using an N, N-diethyl-p-phenylenediamine (DPD) free chlorine standard method kit from Hach Company® (Loveland, CO). The concentration of the peroxyacetic acid was measured with a titration peroxyacetic test kit provided from ECOLAB Company (St Paul, MN).

### 3.2.4 *E. Coli O157:H7* inactivation with chlorine, peroxyacetic acid wash and ultrasound

#### 3.2.4.1 Washing of lettuce

The lettuce wash was performed with two methods “washing-before-cutting” and “cutting-before-washing.” In both cases, the head lettuce was inoculated with *E. coli O157:H7* ATCC 87-23 inoculum and dried for 2 hours at 22 °C. In the traditional “cutting-before-washing” treatment, the inoculated heads of lettuce were cut into pieces of 1 in². Cuts were performed across the inoculation sites in order to bring bacteria from the outer layer to the inside pieces of Iceberg lettuce and to simulate a cross-contamination scenario. The cut samples were then washed for 2 minutes in the continuous flow ultrasonic tank (Figure 3.3) containing, water, chlorine, Tsunami-100®, chlorine + ultrasound and Tsunami-100®+ ultrasound. In “washing-before-cutting” tests, the head lettuce was dried for 2 hours at 22 °C and washed for 2 min with the sanitizer solutions mentioned above. After washing, the head was cut into 1 in² pieces. Washed samples were drained for 1 minute to remove excess water before subsequent microbial analysis.
Whole head going through ultrasound tank

Cut iceberg lettuce going through ultrasound tank

Figure 3.3 Illustration of washing procedures.
3.2.4.2 Washing of carrots

Inoculated carrots were submerged for 1 minute in a glass beaker containing 600 ml of one of the following solutions: tap water (20±1°C), sodium hypochlorite (free chlorine concentration 60 mg L⁻¹), and Tsunami-100® (final acid concentration 60 mg L⁻¹). During the washing procedure, the samples were agitated at 100 rpm using a Corning® bench top stirrer (CORNING, Tewksbury MA, USA) The sample to sanitizer solution ratio was 1:12.

3.2.4.3 Enumerating E. coli O157:H7 in lettuce and carrots

Washed lettuce and carrot samples were aseptically transferred to a sterile kitchen blender containing 0.1% peptone water supplemented with 10% sodium thiosulfate to neutralize chlorine and stop the reaction. For peroxyacetic acid, phosphate buffer saline was added to stop the reaction. Samples were macerated for two minutes followed by a two minute resting period to allow formed foam to dissolve. The filtrate was 10-fold serial diluted with 0.1% peptone water; 100 µL of the serially diluted samples were spread plated in duplicates over Tryptic soy agar (TSA) plates supplemented with 50 µg L⁻¹ nalidixic acid. The plates were incubated at 37 ºC for 24 hours and colonies were counted manually.

3.2.5 Determination of specific gravity and water absorption by whole head Iceberg lettuce

3.2.5.1 Determination of specific gravity

The specific gravity of Iceberg lettuce stored at two temperatures and washed at two different temperatures was determined using the method described by (Mohsenin, 1996) and with the following equation
3.2.5.2 Water absorption by whole-head Iceberg lettuce

The effects of lettuce temperature and temperature of the disinfecting solutions on water intake of Iceberg lettuce were evaluated. Iceberg lettuce heads were weighed and aseptically placed in a room overnight at 23°C or in a cold storage room at 5°C. Two washing solution temperatures were used, which were obtained by filling a tank with tap water at 4 and 23 °C. Each head of lettuce at either 5°C or 23°C was individually submerged one by one in the wash tank filled to its 100% capacity with water at 4 or 23 °C for one minute. The head of lettuce was transferred into a second container and drained for 10 minutes to collect water entrapped inside the lettuce. After draining, each head of lettuce rested for 30 minutes and was weighed a second time in order to determine increase in weight (%).

3.2.6 Statistical analysis

This experiment was performed as a complete randomized design (CRD); all treatments were replicated three times. *E. coli* O157:H7 count populations were subjected to log transformation before statistical analysis. Data were analyzed using a general linear model using the Statistical Analysis System version 9.1. (SAS Institute, Raleigh, NC, U.S.A.). Mean separation was determined using Tukey’s test with α= 0.05.
3.3 RESULTS AND DISCUSSION

3.3.1 Changes in Iceberg lettuce weight after washing procedure

In this experiment, we examined the changes in specific gravity and water absorption of whole-head Iceberg lettuce at two storage temperatures (5°C and 23°C) and two washing solution temperatures (4°C and 23°C). All samples pick up water from the tank in 1 min so a weight increase was observed in all four tests. From Table 3.1 it can be observed that lettuce that was stored at 23°C and washed at 4°C generated the greatest increase (%) in weight, followed by lettuce that was stored at 5°C and washed at 4°C and 23°C. For lettuce washed at 4°C, there is a significant difference in water absorption depending on whether it was stored at 23°C (P<0.05). All specific gravity values were lower than that of the washing solution and no differences among samples were observed (P>0.05).

Table 3.1 Specific gravity and water absorption of lettuce at two storing (5°C, 23 °C) and two washing (4°C, 23 °C) temperatures.

<table>
<thead>
<tr>
<th>Water temperature (°C)</th>
<th>Storage temperature (°C)</th>
<th>Specific Gravity Mean ± SE*</th>
<th>Increase In Weight (%) Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>5</td>
<td>0.86 ± 0.03 a</td>
<td>7.59 ± 0.87 b</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>0.76 ± 0.13 a</td>
<td>9.26 ± 0.45 a</td>
</tr>
<tr>
<td>23</td>
<td>5</td>
<td>0.65 ± 0.20 A</td>
<td>7.09 ± 1.74 A</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>0.69 ± 0.09 A</td>
<td>7.60 ± 1.17 A</td>
</tr>
</tbody>
</table>

a-c Treatment means within water temperature (4 °C) with different letters are different at α 0.05.
A-C Treatment means within water temperature (23 °C) with different letter are different at α 0.05.
* SE Standard Error

Since the specific gravity of water is 1, the fact that all the lettuce samples had a lower SG indicates that the lettuce would float in washing solution surface. As a result, the unsubmerged lettuce will receive no sanitization treatment, which is an undesirable situation. To address this
problem, a special holder was designed and installed in the pilot-scale washer, as can be seen in Figure 3.3. The metal bars were made of aluminum having a relatively smooth surface and placed just below the water surface. With this device, all the head lettuce will fully immersed in the washing solution to receive the same wash treatment. The water absorption is a problem deserving close attention. Since there are gaps between lettuce leaves, it is no surprise to see that all head lettuce picked up water and showed an increased weight (Cosgrove, 1993). The water absorption will carry sanitizer into the interior of the head lettuce. When the sanitizer concentration is high, it will help to sanitize the interior leaves. However, if the sanitizer concentration is below the threshold to prevent cross contamination, the water absorption may carry dirt or even microorganisms into the lettuce head, causing cross contamination. From Table 3.1, since all industrial lettuce washes are done at low temperatures, it is clear that the lettuce before washing should be kept at refrigeration temperature if one intends to minimize water absorption during a wash. Buchanan et al. (1999) examined the location of E. coli O157:H7 in intact cold (2°C) or warm (22 °C) apples after being immersed in cold (2°C) 1% peptone water, and reported less internalization of the E. coli O157:H7 was reported when cold apples were immersed in cold peptone water. Studies have provided evidence that bacteria can internalize passively through the movement of contaminated water used during irrigation and product disinfection. Therefore, it is imperative to maintain a minimal temperature differential between washing solution and produce in order to minimize absorption of water during disinfection (Deering, Mauer, & Pruitt, 2012). Washing head lettuce at room temperature (~23°C) is mainly a household practice and the water uptake has significant, regardless of whether the lettuce was “warm” or “cold” (Table 3.1).
3.3.2 Chlorine and peroxyacetic acid degradation in presence of organic matter

From Figure 3.4 we can see that availability of free chlorine and peroxyacetic acid is affected by the presence of organic substances, which were released from cut surface of carrots.

![Graph showing chlorine and peroxyacetic acid consumption caused by increase in presence of organic matter.](image)

**Figure 3.4** Chlorine and peroxyacetic acid consumption caused by increase in presence of organic matter.

a-c Treatment means within type of chemical with different letters are different at α 0.05.

The chlorine concentration decreased by 60% when shredded carrots were submerged in the chlorine solution for one minute. Similarly, a decrease in peroxyacetic acid availability was observed when carrots were shredded prior to sanitization; the decay was around 20%. Compared to peroxyacetic acid, the decay of chlorine was more evident and significantly higher (P<0.05). These results are in agreement with the studies done by Hajenian & Butler (1980) and Hilgren & Salverda (2000), where chlorine and peroxyacetic acid availability was affected by the presence of organic matter; both sets of investigators concluded that peroxyacetic acid was more stable than chlorine. In a study done by Luo et al. (2011), it was determined that a concentration equal to 10 mg L⁻¹ of free chlorine is necessary to inactivate *E. coli* O157:H7 on fresh-cut
produce. We can see from Figure 3.4 that monitoring the free chlorine and peroxyacetic acid concentration and timely replenish the sanitizer during sanitization of fresh-cut produce is essential to ensure an effective sanitation process.

### 3.3.3 Reduction of *Escherichia coli* O157:H7 on carrots affected by the surface area to mass ratio

The *E. coli* O157:H7 population reduction on carrots with different surface-area-to-mass ratios is shown in Figure 3.5. The whole, stick, and shredded baby carrot samples had a surface area to mass ratio of (1.02, 6.24, 45.56 cm²/g) respectively. With an increase in the surface-area-to-mass ratio, the microbial reduction on carrot samples washed with 60 mg L⁻¹ chlorine decreased. The logarithmic reduction of *E. coli* O157:H7 on whole baby carrots was 1.44 log₁₀ CFU/g, significantly higher than the 0.83 and 0.72 log₁₀ CFU/g achieved with the two types of cut carrots. The lower microbial reduction for carrots with higher surface-area-to-mass ratios may be caused by a number of factors. First, the cut surfaces of carrots with damaged cells may provide sites for the bacteria to attach or internalize into the cut surfaces (Deering, Mauer & Pruitt, 2012). The organic matter leaked from the cut surfaces into wash solution will cause depletion of free chlorine, and thus reduce the sanitization efficacy (Alegria, et al., 2009). Furthermore, agitation becomes less effective with large numbers of carrot pieces present in the beaker and, as a result, the flow over each piece of carrot and thus the surface shear force that helped to dislodge the *E. coli* cells was lower for cut pieces compared to the whole baby carrots. Indeed, a reduction of free chlorine concentration was observed when increasing the surface area to mass ratio, as shown in Figure 3.4. Similar results have been reported in washing of other vegetables. Nou & Luo (2010) used a chlorinated solution to wash Romaine lettuce before
cutting and reported a higher logarithmic reduction of *E. coli* O157:H7 than in a cut-before wash treatment. In green pepper treatment with an aqueous chlorinated wash solution, a greater reduction in *L. monocytogenes* counts was achieved from uncut surfaces (Han et al., 2001).

**Figure 3.5** *E. coli* O157:H7 reduction from carrots cut in different ways. 

a-c Treatment means within type of cut and chemical with different letters are different at α 0.05.
3.3.4 Reduction of *Escherichia coli* O157:H7 inoculated in surface of Iceberg lettuce that has been cut before or after sanitization

In this experiment we evaluated the efficacy of selected sanitizers on reduction of *E. coli* O157:H7 counts from the surface of Iceberg lettuce (Figure 3.6). It can be seen that by washing lettuce following the traditional guidelines used in the leafy green industry, including cutting followed by washing in a chemical solution such as chlorine and peroxyacetic acid, we can reduce *E. coli* O157:H7 by 0.96 and 1.07 log\(_{10}\) CFU/g respectively. By shifting the order in which disinfection was done, washing the whole head first followed by cutting, we can improve the reduction of *E. coli* O157:H7 counts by 0.79 and 0.80 log\(_{10}\) CFU/g, respectively. Furthermore, when ultrasound was introduced we can boost the reduction by 0.68 and 0.37 log\(_{10}\) CFU/g, reaching a total reduction of 2.43 and 2.24 log\(_{10}\) CFU/g for the chlorine and peroxyacetic acid wash, respectively. Since the cut lettuce had a much larger surface-area-to-mass ratio compared to the whole head wash, the 0.79 and 0.80 more log reduction of *E. coli* for the whole head wash over that of the “cutting-before-washing” treatment should be attributed to the three factors outlined in the previous section. Besides the leakage of organic matter, the cut lettuce surfaces also provided shelter to protect the bacterial cells, and the effectiveness of sanitizers to inactivate microorganisms depends on its availability to access the cells (Takeuchi & Frank, 2000). Therefore, means to prevent the creation of wounds and the possible microbial internalization should be considered a critical step in ensuring the safety of fresh produce (Allwood et al., 2004). In addition, the increased sanitizer efficacy with the new washing procedure can be attributed to the fact that it enabled a more uniform disinfection because there were no cut pieces that would cause nutrient leakage into the washing solution to reduce chlorine and peroxyacetic acid availability (Nou et al., 2011).
Figure 3.6 E. coli O157:H7 reduction from Iceberg lettuce that was cut prior or post sanitization. a-c Treatment means within type of cut and chemical treatment with different letters are different at $\alpha 0.05$.

3.4 CONCLUSIONS

A difference between the temperature of produce and washing solution can lead to absorption of water by the produce samples to be sanitized. More absorption was found when lettuce was stored at temperatures around 23 °C and the washing solution was 4°C, suggesting that the temperature difference between lettuce and wash water must be kept small to prevent absorption of water that can facilitate pathogen internalization. As the surface-area-to-mass ratio increased...
during sanitation, the depletion of available chlorine and peroxyacetic acid increased and less reduction of *E. coli* O157:H7 form the surface of carrots and Iceberg lettuce was recorded. Therefore, produce should be first washed before cutting to enhance the sanitization efficacy and minimize microbial contamination.

### 3.5 REFERENCES


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CHAPTER 4

QUALITY OF ICEBERG (*Lactuca sativa* L.) AND ROMAINE (*Lactuca sativa* L. var. *longifolial*) LETTUCE TREATED BY COMBINATIONS OF SANITIZER, SURFACTANT, AND ULTRASOUND

4.1 INTRODUCTION

Consumption of lettuce in the U.S. has increased over the last decade due to new trends in diet that emphasize the importance and popularity of vegetable salads, the convenience offered by fresh-cut products, and increases in salad bar patronage and meals eaten outside the home (USDA, 2002; Buck, Walcott & Beuchat, 2003). This increase in lettuce consumption has led to annual U.S. production of nearly 8.7 billion pounds of lettuce in 2010, while in the same year 7.2 million pounds were imported from Mexico and Canada to meet demand (Boriss & Brunke, 2011). Increased production and consumption of lettuce has drawn significant public interest to the potential for foodborne illness associated with lettuce and other leafy green vegetables. During the period 2010-2012, three multi-state outbreaks of Shiga toxin-producing *Escherichia coli* O157:H7 and *Escherichia coli* O145 associated with consumption of lettuce were reported (CDC-Centers for Disease Control and Prevention, 2012). These high profile foodborne illness outbreaks highlight the importance of further improving the microbial safety of fresh produce.

Currently, the produce industry processes lettuce by cutting it into bite-size pieces, washing the cut lettuce with chlorinated water, followed by rinsing, dewatering or drying, and packaging. However, washing produce with chlorine in industrial-scale operations, for instance at a throughout of 45 kg/min, has been reported to reduce the survival count of *E. coli* O157:H7 by no more than one log cycle (Luo et al., 2012). In addition, chlorine is consumed when organic
matter is present, leading to an increase in turbidity of the wash water (O'Beirne & Zagory, 2009; Luo, et al., 2012). The presence of organic matter in wash water can also enhance formation of chloroform (CHCl₃), haloacetic acids or other trihalomethanes (THM), all of which are known to be harmful to human health (Artés et al., 2009). Efforts have thus been made to find alternative and/or more effective sanitization agents/methods to enhance reduction of microbial populations.

Treatments that create an acidified environment in a washing system through the use of organic acids such as lactic, citric, peroxyacetic, and levulinic acids, or their salts, have been reported as an alternative to the traditional chlorine wash (Oms-Oliu et al., 2010). In tests performed in a beaker, 1.74 log cfu g⁻¹ reduction of *E. coli* O157:H7 on lettuce washed with 2% lactic acid for 5 min was achieved (Sagong, et al., 2011). Another study reported more than a 6 log cfu g⁻¹ reduction of *E. coli* O157:H7 population on lettuce when treated with 3% levulinic acid in combination with the surfactant sodium dodecyl sulfate (1%, SDS) for 1 min (Zhao et al., 2009). The use of a surfactant aims to allow the (dissolved) sanitizer to penetrate small cracks and crevices on the complex topography of lettuce. The combination of a chemical wash with a physical process, such as sonication, has also been tested for enhancing the efficacy of a sanitizer wash (Zhou et al., 2009; Zhou et al., 2012).

Lettuce, unlike other fresh produce, lacks an external protective tissue, and processes like cutting expose its tissues to air, leading to a series of chemical reactions that cause damage and make the plant material vulnerable to dehydration. Several studies have shown that many sanitizing agents, such as chlorine, organic acids, ozone and some surfactants are excellent antimicrobials, especially for planktonic microorganisms. However, many of these compounds have a detrimental effect on the quality of leafy produce when used beyond certain critical concentrations, leading to quality degradation through browning, tissue damage, color changes,
water segregation, and overall poor appearance (Garcia et al., 2003). For instance, Fan et al. (2010) reported that treatment with 0.5% to 3% levulinic acid plus 0.05% SDS rendered fresh-cut Iceberg lettuce sensorially unacceptable beyond seven days due to development of sogginess and tissue damage. In general, for the development of any sanitizer or sanitization method, the effect of the treatment on produce quality is a primary consideration. The only meaningful microbial count reductions are those that are achieved for treatment times and sanitizer concentrations below the threshold for unacceptable quality changes during storage long enough to be consistent with retail sale. For this reason, this study was undertaken to examine the effects of sonication in combination with two sanitizers (chlorine and Tsunami 100®) and a surfactant (sodium dodecyl sulfate) on the quality of fresh-cut Iceberg and Romaine lettuce during 14-day refrigerated storage.

4.2 MATERIALS AND METHODS

4.2.1 Ultrasound wash system

This study was carried out in a custom-made ultrasonic washing tank. The tank was made of welded aluminum sheet, with a capacity of 115 L. Two ultrasound (US) transducer blocks (each operating at 25 kHz, and with 2 kW nominal power), with sound emitting planes facing each other, were vertically placed in the tank against two walls. Prior to the start of each test the wash tank was filled with chilled tap water (10°C) to which was added chlorine (active ingredient sodium hypochlorite), Tsunami 100® (active ingredient peroxyacetic acid), or Tsunami 100®+ sodium dodecyl sulfate (SDS). To minimize “blockage” (Zhou et al. 2012) and allow ultrasonic waves to reach each piece of the cut lettuce, a plastic holder (Fig. 1) measuring 12” × 6” × 5” (L × W × H) with mesh size of 0.48” × 0.48” was used to hold lettuce samples. The
walls of the holder were made of stretchable molded polyethylene mesh (McMaster-Carr, Elmhurst, IL, USA) and the holder can hold up to 450 g of cut lettuce. The holder was submerged in the tank during treatment.

![Ultrasound Washing Tank](image)

**Figure 4.1** Illustration of lab-scale ultrasonic washer.

### 4.2.2 Sample preparation and treatment procedure

#### 4.2.2.1 Preparation of lettuce pieces

Iceberg (*Lactuca sativa* L.) and Romaine (*Lactuca sativa* L. var. *longifolial*) lettuce were purchased at a local supermarket and immediately transported to the laboratory, where they were stored at 6 ± 1°C and used within 24 h of purchase. The three outermost leaves of each head of lettuce were removed and discarded. A kitchen knife was used to cut lettuce into pieces of 1 in². The lettuce pieces were randomized at the beginning of the experiment and divided into batches of 300 g for treatment.
4.2.2.2 Treatment procedure

Three hundred grams of fresh-cut lettuce were submerged in the water tank containing one of the following solutions: tap water (control), sodium hypochlorite (final free chlorine concentration 100 mg l⁻¹), Tsunami 100° (peroxyacetic acid as active ingredient, final acid concentration 80 mg l⁻¹), and Tsunami100° in combination with 0.1 % (w/v) SDS. For each washing solution, samples were run for one minute with and without ultrasound, except for the tap-water control. After the one-minute treatment the samples were rinsed with tap water for 1 min and de-watered with a manual salad spinner (OXO, New York, NY, USA). One hundred grams of each de-watered sample were placed in polypropylene plastic film bags (OTR 7,000 cc/m²/day and CO₂ 21,000 cc/m²/day) (PD-961 EZ, Cryovac, Duncan, SC). The lettuce bags were vacuumed and flushed with N₂ using an Audionvac 101/151 packaging machine (Audion Elektro, Hogeweyselaan, Netherlands) and stored at 4 ± 1°C until further analysis. Nine bagged samples were set aside for sampling, with three each taken at days 0, 7 and 14 to perform triplicate quality analyses, including electrolyte leakage rate, texture, color, sensory evaluation, headspace O₂ and CO₂ content, total aerobic plate count, and yeasts and molds.

Figure 4. 2 Illustration of polyethylene-mesh cube used for sanitization of lettuce in ultrasonic washer.
4.2.3 Analysis of quality parameters

4.2.3.1 Analysis of headspace $\text{O}_2$ and $\text{CO}_2$ in packages

Headspace gas in the packages was analyzed at days 0, 7 and 14 of storage. To measure the content of $\text{O}_2$ and $\text{CO}_2$ inside the packages, gas from the headspace was withdrawn through a needle using a built-in pump into a portable dual headspace analyzer (model 650, Mocon Inc. Minneapolis, MN, U.S.A.).

4.2.3.2 Visual quality

Visual quality was assessed immediately after headspace analysis of packages by a 5-member panel using the same parameters as Guan et al (2010). Overall visual quality was rated on a 9 to 1 scale: 9 = excellent, essentially free from defects; 7 = good, minor defects, not objectionable; 5= fair, slightly to moderately objectionable defects, lower limit of sales appeal; 3 = poor, excessive defects, limit of salability; 1 = extremely poor, not usable. Cut edge tissue browning, surface browning, and soginess/watery were rated on a scale of 5 to 1: 5 = severe; 4= moderately severe; 3 = moderate; 2 = slight; 1 = none.

4.2.3.3 Texture measurement

The firmness of fresh-cut lettuce leaves was measured using a TA-XT2i Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, U.S.A.) and a Kramer Shear press with five blades (TA-91). Twenty-five grams of sample were positioned in the press holder and the five-blade plunger was moved down at a velocity of 2 mm/s to 1 cm below the bottom of the holder. The maximum cut force (MCF) was recorded using the Texture Expert Software (version
1.22, Texture Technology Corp., Scarsdale, NY, USA). Two aliquots were taken for measurements from each bag, resulting in a total of six measurements for each combination of treatment and sampling day.

4.2.3.4 Electrolyte leakage analysis

Electrolyte leakage from fresh-cut lettuce was measured immediately after a treatment and during storage to determine the rate of tissue deterioration. Five grams of lettuce leaves were submerged in 100 mL of deionized water in a beaker and incubated for 1 min at 23°C. During incubation the samples were agitated using a New Brunswick incubator with built-in shaker (Model I-24, New Brunswick Scientific, Enfield, CT, USA) at a speed of 100 rpm. Electrical conductivity (µS/cm) of the bathing solution was measured at 1 min (C1) and 60 min (C60) using a conductivity meter (Accumet Basic AB30, Fisher Scientific Co., Pittsburgh, PA, USA). The samples were then autoclaved (121°C) for 25 min, and total conductivity (CT) was measured after cooling. The electrolyte leakage rate was calculated using the following equation (Zhou B., 2010).

\[
ECR = \left( \frac{C^{60} - C^{1}}{CT} \right) \times 100
\]

(4.1)

Where ECR=Electrolyte leakage rate.

4.2.3.5 Color measurement

For color measurement, five pieces of cut lettuce were withdrawn from each packed bag and analyzed using a Minolta Chroma Meter CR-300 (Minolta Corp., Osaka, Japan). Hunter’s color values (L, a, b) were measured at 3 locations of each piece of lettuce for a total of 45
readings for each treatment/sampling day. Total Color Difference (TCD) was determined using the following equation (Heimdal et al., 1995).

\[ \Delta E_{ab}^* = \sqrt{(L_1^* - L_0^*)^2 + (a_1^* - a_0^*)^2 + (b_1^* - b_0^*)^2} \]  \hspace{1cm} (4.2)

where \( L_0^* \ a_0^* \ b_0^* \) are Hunter’s color values from a reference and \( L_1^* \ a_1^* \ b_1^* \) are Hunter’s color values from the treated samples.

4.2.4 Microbiological analysis

Ten grams of treated lettuce were homogenized in 90 mL of 0.1% sterile peptone water (pH 7.4) in a lab stomacher (model 400, Seward Medical, London, UK) and agitated for 2 min at 260 rpm. Homogenates were serially diluted in peptone water, and logarithmically plated (100 µL in duplicate). The total aerobic plate count (TPC) was determined by plating the samples on Tryptic Soy Agar (TSA, Difco Lab, Detroit, MI, USA) and incubated at 37 °C for 48 hours. Yeasts and molds were determined by plating the samples in acidified Potato Dextrose Agar, pH adjusted with tartaric acid (PDA, Difco Lab, Detroit, MI, USA) and incubated at 25° C for 5 days.

4.2.5 Statistical analysis

In this completely randomized experimental design (CRD); all treatments were replicated three times and analyzed at each sampling time. Aerobic plate count populations were subjected to log transformation before statistical analysis. Data were analyzed with Statistical Analysis System version 9.1 (SAS Institute, Raleigh, NC, U.S.A) using a general linear model. Mean separation was determined using Tukey’s test with \( \alpha=0.05 \).
4.3 RESULTS AND DISCUSSION

4.3.1 Changes in headspace composition

Changes of the O$_2$ and CO$_2$ concentrations inside the bagged lettuce stored at 4°C are shown in Table 4.1. An increase in O$_2$ concentration can be observed in the control and three treated samples for both Iceberg and Romaine lettuce. At day 0, both lettuce types exhibited low oxygen levels, between 3.07 to 3.58% because of the N$_2$ flush and vacuum packing. From day 0 to day 7, the oxygen concentration increased rapidly, reaching high levels of 8.86 to 9.20% in Romaine lettuce and 11.20 to 12.52% in Iceberg lettuce. Afterwards, the oxygen concentration continued to increase but at a lower rate, and at day 14, the final oxygen content was between 12.55 to 14.48% for Romaine and 11.75 to 13.40% for Iceberg lettuce. It is known that fresh-cut produce has a relatively high respiration rate and therefore packaging films with high oxygen transmission rate (OTR) are normally used for this type of product (Toivonen et al., 2009). The polypropylene film used in this study had a high OTR of 7,000 cc/m$^2$/day. This allowed rapid transport of O$_2$ by diffusion from the surroundings into the packages at the beginning of the storage period. The O$_2$ inflow was driven by the partial pressure difference of O$_2$ across the packaging film, as shown by the concentration differences, i.e., 20.95% in the air and 3.07-3.58% at day 0 in the packages. The O$_2$ consumption by lettuce in the bags was much less than the O$_2$ diffusion rate into the bags, and as a result, a rapid increase in O$_2$ concentration in the first 7 days of storage was observed. From day 7 to day 14, the partial pressure difference of O$_2$ across the film was much less than on day 0. However, since the amount of lettuce in each bag was small (100 g) and the size of the package was relatively large (12.25 × 8.5 inch, L × W), the amount of O$_2$ that diffused in was still greater than that consumed by the lettuce, leading to a continued increase in O$_2$ concentration in the bags. This result was in agreement with the tests by
Guan et al. (2010) with PDF961 films having an OTR of 7,000 cc/m$^2$/day. Guan et al. washed Iceberg lettuce with combinations of levulinic acid and SDS and reported a rapid increase of O$_2$ concentration in the bags during the first 7 days of storage. From day 7 to day 14, they observed a decrease in O$_2$ concentration. This latter result differed from our observations, and could be due to differences response in sanitization and lettuce species used in this study and the lettuce used by Guan et al.

Accumulation of CO$_2$, and thus the rate of respiration in the packages, was increased in the first 7 days storage. For instance, in the Romaine lettuce packages, the CO$_2$ levels reached 1.65 to 2.02% on the seventh day. This might be attributed to the respiration activity of cut and treated lettuce. After the seventh day, however, the CO$_2$ content in Romaine and Iceberg lettuce samples remained nearly unchanged or decreased slightly. During storage, the differences in CO$_2$ concentration between lettuce samples treated with sonication in combination with chlorine, Tsunami, or Tsunami + SDS were not significantly different for Iceberg lettuce at day 14. Kim et al. (2005) observed similar respiratory behavior after packaging Romaine lettuce, where the CO$_2$ production rate increased at the beginning of storage and later decreased gradually towards the end of the storage period. It is expected that a relatively high level of O$_2$, accompanied by relatively low levels of CO$_2$ between days 7 and 14, will create an environment unfavorable for maintaining the quality of cut lettuce.
Table 4.1 Changes in headspace content of Iceberg and Romaine lettuce during storage.

<table>
<thead>
<tr>
<th>Lettuce type</th>
<th>Treatment</th>
<th>O₂ content (%)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0 Mean ± SE</td>
<td>Day 7 Mean ± SE</td>
<td>Day 14 Mean ± SE</td>
<td></td>
</tr>
<tr>
<td>Romaine</td>
<td>Water</td>
<td>3.50 ± 0.20 a(x)</td>
<td>11.20 ± 0.34 a(y)</td>
<td>14.48 ± 2.32 a(z)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chlorine + ultrasound</td>
<td>3.34 ± 0.20 a(x)</td>
<td>12.00 ± 0.13 a(y)</td>
<td>13.42 ± 0.98 a(z)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tsunami + ultrasound</td>
<td>3.07 ± 0.82 a(x)</td>
<td>11.48 ± 0.51 a(y)</td>
<td>12.55 ± 0.63 a(z)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tsunami+SDS+ultrasound</td>
<td>3.15 ± 1.30 a(x)</td>
<td>12.52 ± 2.20 a(y)</td>
<td>13.97 ± 0.67 a(z)</td>
<td></td>
</tr>
<tr>
<td>Iceberg</td>
<td>Water</td>
<td>3.57 ± 0.20 a(x)</td>
<td>8.86 ± 0.43 b(y)</td>
<td>12.88 ± 0.27 ab(z)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chlorine + ultrasound</td>
<td>3.34 ± 0.20 a(x)</td>
<td>9.73 ± 0.51 a(y)</td>
<td>13.40 ± 0.74 a(z)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tsunami + ultrasound</td>
<td>3.08 ± 0.81 a(x)</td>
<td>9.20 ± 0.25 ab(y)</td>
<td>12.10 ± 0.69 bc(z)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tsunami+SDS+ultrasound</td>
<td>3.50 ± 0.10 a(x)</td>
<td>8.75 ± 0.35 b(y)</td>
<td>11.75 ± 0.79 c(z)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lettuce type</th>
<th>Treatment</th>
<th>CO₂ content (%)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0 Mean ± SE</td>
<td>Day 7 Mean ± SE</td>
<td>Day 14 Mean ± SE</td>
<td></td>
</tr>
<tr>
<td>Romaine</td>
<td>Water</td>
<td>0.60 ± 0.06 bc(x)</td>
<td>1.85 ± 0.12 a(y)</td>
<td>1.70 ± 0.23 bc(z)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chlorine + ultrasound</td>
<td>0.70 ± 0.01 a(x)</td>
<td>1.65 ± 0.05 a(y)</td>
<td>1.63 ± 0.16 c(z)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tsunami + ultrasound</td>
<td>0.53 ± 0.05 c(x)</td>
<td>2.02 ± 0.28 a(y)</td>
<td>2.23 ± 0.14 a(z)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tsunami+SDS+ultrasound</td>
<td>0.62 ± 0.04 b(x)</td>
<td>1.95 ± 0.45 a(y)</td>
<td>2.00 ± 0.28 ab(z)</td>
<td></td>
</tr>
<tr>
<td>Iceberg</td>
<td>Water</td>
<td>0.60 ± 0.06 bc(x)</td>
<td>1.70 ± 0.17 a(y)</td>
<td>1.63 ± 0.16 b(y)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chlorine + ultrasound</td>
<td>0.70 ± 0.01 a(x)</td>
<td>1.58 ± 0.08 a(y)</td>
<td>1.60 ± 0.13 b(y)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tsunami + ultrasound</td>
<td>0.53 ± 0.04 c(x)</td>
<td>1.65 ± 0.12 a(y)</td>
<td>1.90 ± 0.06 a(y)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tsunami+SDS+ultrasound</td>
<td>0.65 ± 0.12 b(x)</td>
<td>1.67 ± 0.18 a(y)</td>
<td>1.92 ± 0.19 a(y)</td>
<td></td>
</tr>
</tbody>
</table>

a-c Treatment means within time (columns) with different letters are different at α 0.05.

x-y Treatment means within treatments (rows) with different letters are different at α 0.05.

*SE Standard Error.

4.3.2 Electrolyte leakage rate

Changes in electrolyte leakage rate (ECR) in Iceberg and Romaine lettuce as a function of storage time are presented in Figure 4.3. Romaine and Iceberg lettuce treated with Tsunami 100® + sonication had the highest ECR leakage rates on day 0, at 1.70 and 1.79, respectively (P<0.05). A decrease in electrolyte leakage rate was observed by the end of day 7 of storage. Both lettuce samples treated with water had significantly lower (P<0.05) electrolyte leakage rates than that of other treatments, an indication of less tissue damage. The decreased ECR observed...
on day 7 can be attributed to tissue recovery and electrolyte reabsorption by the plant material as a defense mechanism (Fan, 2012). Samples taken on day 14 showed an increased ECR, without significant differences among the treatments of Romaine and Iceberg lettuce. This increase in ECR can be attributed to permanent tissue damage and accumulation of CO₂ from respiration (Wang, 2004). Similar trends were reported by Luo et al. (2004), and Kim et al. (2005) who bagged minimally processed cilantro and lettuce and reported a decrease in electrolyte leakage rate during first few days of storage followed by an increase in packages sampled towards the end of 14 days of storage.

![Figure 4.3](image)

**Figure 4.3** Electrolyte leakage of Romaine and Iceberg lettuce during storage.  
(a) Romaine lettuce electrolyte leakage rate, (b) Iceberg lettuce electrolyte leakage rate.  

a-c Treatment means within day of storage point with different letters are different at α 0.05.
4.3.3 Firmness

The effects of processing conditions (cutting, treatment, and modified atmosphere packaging) on the changes of Iceberg and Romaine lettuce texture during storage are shown in Figure 4.4. At day 0, all samples were compared against an untreated raw sample. For Iceberg lettuce, the MCF values for the treated samples were smaller and significantly different from the untreated sample (P<0.05), indicating a loss of turgor in the treated samples. After day 7, an increase in MCF values was observed, which might be caused by self-repair and production of phenolic compounds as a defense mechanism (Wang, 2004; Qi et al., 2011). Nonetheless, on days 7 and 14, the MCF values for all four treatments were still lower than that of the untreated sample. On the other hand, the response of Romaine lettuce was quite different. Except for the Tsunami 100® + SDS + US treatment, the day 0 MCF values for treated Romaine were significantly higher than for the untreated sample, and the MCF values were higher on days 7 and 14 than on day 0. A similar trend was reported by Manolopoulou et al. (2010), who after cutting and washing lettuce with chlorinated water and, reported a (statistically insignificant, P > 0.05) increase in textural properties during a 15 day storage at 5°C.
Figure 4.4 Firmness of Romaine and Iceberg lettuce throughout 14 days of storage. (a) Romaine lettuce maximum cut force during storage, (b) Iceberg lettuce maximum cut force during storage. a-c Treatment means within days of storage with different letters are different at $\alpha 0.05$. 

## Days of storage
0 7 14
Romaine lettuce Maximum cut force (Newtons)
0
200
400
600
Untreated sample
Water
Chlorine + ultrasound
Tsunami + ultrasound
Tsunami + SDS + ultrasound

## Days of storage
0 7 14
Iceberg lettuce Maximum cut force (Newtons)
0
200
400
600
Untreated sample
Water
Chlorine + ultrasound
Tsunami + ultrasound
Tsunami + SDS + ultrasound

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days of Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated sample</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td></td>
</tr>
<tr>
<td>Chlorine + ultrasound</td>
<td></td>
</tr>
<tr>
<td>Tsunami + ultrasound</td>
<td></td>
</tr>
<tr>
<td>Tsunami + SDS + ultrasound</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.4 Firmness of Romaine and Iceberg lettuce throughout 14 days of storage. (a) Romaine lettuce maximum cut force during storage, (b) Iceberg lettuce maximum cut force during storage. a-c Treatment means within days of storage with different letters are different at $\alpha 0.05$. 

## Days of storage
0 7 14
Iceberg lettuce Maximum cut force (Newtons)
0
200
400
600
Untreated sample
Water
Chlorine + ultrasound
Tsunami + ultrasound
Tsunami + SDS + ultrasound

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days of Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated sample</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td></td>
</tr>
<tr>
<td>Chlorine + ultrasound</td>
<td></td>
</tr>
<tr>
<td>Tsunami + ultrasound</td>
<td></td>
</tr>
<tr>
<td>Tsunami + SDS + ultrasound</td>
<td></td>
</tr>
</tbody>
</table>

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4.3.4 Sensory evaluation

The mean ratings of visual quality parameters such as overall quality (OQ), surface browning (SB) and sogginess of Iceberg and Romaine lettuce stored at 0, 7 and 14 days are shown in Figure 4.5. Progressive quality degradation as shown by decreasing OQ values was observed during storage for all the treatments. On the day 0, there were no significant differences in the hedonic rating of OQ, SB and sogginess in both lettuce types for all the treatments (P>0.05).

On day 7, the highest hedonic rating for overall quality received by Iceberg lettuce was 5.27 (treated with Tsunami 100® + SDS + US), while the corresponding value for Romaine lettuce was 5.93 (treated with chlorine + US). The corresponding low values of the surface browning hedonic rating were 1.60 and 1.93 for Iceberg and Romaine, respectively. The samples were still appealing to panelists (P<0.05). After 14 days of storage, there were no significant differences in Iceberg lettuce hedonic rating for overall quality and surface browning (P>0.05). On day 14, the Romaine lettuce treated with chlorine received the highest OQ rating (4.68 ± 1.70) and the lowest surface browning (2.33 ± 0.72) hedonic rating (P<0.05). However, none of the treatments were rated as appealing to panelists by the end of 14 days of storage, regardless of the relatively high rating in overall quality of samples treated with chlorine. These results are comparable with the work of Rodgers et al. (2004), who stated that chlorinated products helped to preserve the overall quality of fresh-cut lettuce. The results are consistent with those of McWaters et al. (2002), who in a combined wash with sanitizers’ hydrogen peroxide and an organic acid wash also reported adverse effects of treatment on the sensory quality of lettuce, as well as decreasing sensory ratings during storage. We found no significant differences in sogginess during storage for Iceberg or Romaine lettuce (P > 0.05). Allende et al. (2004)
observed that modified atmosphere packaging (MAP) of lettuce did not improve the quality of the product, but did delay decay during storage.

Figure 4.5 Sensory evaluation parameters of Iceberg and Romaine lettuce during 14 days of storage. (a) Iceberg lettuce overall quality score during storage, (b) Romaine lettuce overall quality score during storage, (c) Iceberg lettuce surface browning score during storage, (d) Romaine lettuce surface browning score during storage, (e) Iceberg lettuce sogginess score during storage, (f) Romaine lettuce sogginess score during storage.

a-c Treatment means within sanitizer with different letters are different at $\alpha 0.05$
4.3.5 **Total color difference**

The total color changes in the Romaine lettuce during storage and among the treatments were all not very different (Table 4.2). The color changes observed for Iceberg lettuce were similar, except for the chlorine + US treatment at day 7. From this we infer that different chemical and ultrasound treatments had little effect on color change during 14 days of storage at 4°C for lettuce that was cut and then washed. We note that the color readings all have relatively large standard errors, which we attribute, in part, to the heterogeneous composition of different tissues in cut-lettuce samples, as discussed by Baur et al. (2004).

### Table 4.2 Total color difference (TCD) of Romaine and Iceberg lettuce through 14 days of storage.

<table>
<thead>
<tr>
<th>Lettuce type</th>
<th>Treatments</th>
<th>Day</th>
<th>TCD day 0 Mean ±SE*</th>
<th>TCD day 7 Mean ±SE</th>
<th>TCD day 14 Mean ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Romaine</td>
<td>Water</td>
<td>NA</td>
<td>13.88 ± 4.60 a(x)</td>
<td>7.843 ± 5.87 a(y)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chlorine + ultrasound</td>
<td>13.48 ± 5.28 a(x)</td>
<td>12.47 ± 4.40 a(x)</td>
<td>12.04 ± 6.25 a(x)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tsunami + ultrasound</td>
<td>14.00 ± 5.88 a(x)</td>
<td>14.49 ± 5.57 a(x)</td>
<td>9.42 ± 6.30 a(x)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tsunami + SDS+ ultrasound</td>
<td>9.81 ± 3.90 a(x)</td>
<td>9.78 ± 6.81 a(x)</td>
<td>10.80 ± 8.20 a(x)</td>
<td></td>
</tr>
<tr>
<td>Iceberg</td>
<td>Water</td>
<td>NA</td>
<td>10.78 ± 5.50 a(x)</td>
<td>11.90 ± 5.33 a(x)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chlorine + ultrasound</td>
<td>12.35 ± 6.20 a(x)</td>
<td>6.76 ± 3.68 a(y)</td>
<td>8.50 ± 4.36 a(xy)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tsunami + ultrasound</td>
<td>7.81 ± 4.40 a(x)</td>
<td>9.64 ± 3.60 a(x)</td>
<td>8.93 ± 3.19 a(x)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tsunami + SDS+ ultrasound</td>
<td>11.33 ± 6.00 a(x)</td>
<td>11.31 ± 6.28 a(x)</td>
<td>11.60 ± 8.60 a(x)</td>
<td></td>
</tr>
</tbody>
</table>

_a-c_ Treatment means within time (columns) with different letters are different at α 0.05.

_x-y_ Treatment means within treatments (rows) with different letters are different at α 0.05.

* SE Standard Error.
4.3.6 Aerobic plate count and yeasts and molds

The total aerobic plate counts of the Iceberg and Romaine lettuce during storage are presented in Table 4.3 and Table 4.4. At day 0, the survival count of aerobic bacteria on Iceberg lettuce only washed with water was higher than samples treated with combinations of sonication and sanitizers. Treatment with Tsunami 100® + SDS + sonication achieved the highest reduction of aerobic microorganisms, significantly higher than the control only washed with water (P<0.05). The samples treated with chlorine or Tsunami 100®, either in combination with ultrasound, had the lowest mean survival counts of aerobic bacteria on Romaine lettuce; however, there are no significant differences among the treatments (P>0.05). At day 7, a sharp increase in total aerobic plate count for both Iceberg and Romaine is observed; and might be due to tissue damage, availability of O₂ inside the packages, or the presence of moisture and nutrients on produce surfaces that support microbial growth. Notably, the Romaine lettuce treated with chlorine + ultrasound had the lowest aerobic plate count at day 7, significantly different from other treatments (P<0.05). Additionally on day 7, the Romaine lettuce treated with chlorine received the highest overall quality rating, showing a good correlation between the low natural micro-flora count and produce quality. At the end of storage, the total aerobic plate count remained unchanged with no significant differences observed in both lettuce samples. This can be interpreted as stabilization in microbial growth during storage; the microorganisms reached a stationary phase of growth, with consumption of nutrients leading to decay in produce quality (Jacxsens et al., 2002). A similar trend was reported by Akbas & Olmez (2007) who treated lettuce samples with organic acid and stored them at 4°C for 12 days. Their counts of aerobic and psychrotrophic bacteria sharply increased from day 0, but remained constant after the mid-point of storage. Growth of yeasts and molds for the two lettuce types and among the three sanitization
treatments were all below 0.7 Log10 CFU/g, indicating the effectiveness of sanitization (Table 4). On the contrary, the Romaine lettuce only washed in water recorded 1.85 ± 0.99 and 1.57 ± 0.75 Log10 CFU/g growth of yeasts and molds at days 7 and 14, respectively. The results clearly demonstrate the importance of disinfection with sanitizers in order for fresh-cut lettuce to maintain microbial quality.

Table 4.3 Aerobic plate count (APC) of Romaine and Iceberg lettuce through 14 days of storage.

<table>
<thead>
<tr>
<th>Lettuce type</th>
<th>Treatments</th>
<th>APC day 0 log$_{10}$ CFU/g Mean ±SE</th>
<th>APC day 7 log$_{10}$ CFU/g Mean ±SE</th>
<th>APC day 14 log$_{10}$ CFU/g Mean ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Romaine</td>
<td>Water</td>
<td>4.55 ± 0.21 a(y)</td>
<td>6.97 ± 0.38 a(x)</td>
<td>7.14 ± 0.21 b(x)</td>
</tr>
<tr>
<td></td>
<td>Chlorine + ultrasound</td>
<td>2.65 ± 1.43 a(z)</td>
<td>6.15 ± 0.17 b(y)</td>
<td>6.88 ± 0.12 b(x)</td>
</tr>
<tr>
<td></td>
<td>Tsunami + ultrasound</td>
<td>2.69 ± 1.47 a(y)</td>
<td>6.84 ± 0.21 a(x)</td>
<td>7.24 ± 0.17 ab(x)</td>
</tr>
<tr>
<td></td>
<td>Tsunami + SDS+ ultrasound</td>
<td>4.01 ± 0.15 a(z)</td>
<td>7.06 ± 0.18 a(y)</td>
<td>7.52 ± 0.05 a(x)</td>
</tr>
<tr>
<td>Iceberg</td>
<td>Water</td>
<td>4.27 ± 0.27 a(y)</td>
<td>7.34 ± 0.25 a(x)</td>
<td>7.56 ± 0.23 a(x)</td>
</tr>
<tr>
<td></td>
<td>Chlorine + ultrasound</td>
<td>2.80 ± 1.38 ab(y)</td>
<td>7.14 ± 0.90 a(x)</td>
<td>7.37 ± 0.03 a(x)</td>
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<tr>
<td></td>
<td>Tsunami + ultrasound</td>
<td>2.46 ± 0.81 ab(y)</td>
<td>7.05 ± 0.35 a(x)</td>
<td>7.35 ± 0.23 a(x)</td>
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<tr>
<td></td>
<td>Tsunami + SDS+ ultrasound</td>
<td>2.00 ± 0.01 b(y)</td>
<td>7.12 ± 0.41 a(x)</td>
<td>7.20 ± 0.14 a(x)</td>
</tr>
</tbody>
</table>

a-c Treatment means within treatments (columns) with different letters are different at α 0.05.

x-y Treatment means within days (rows) with different letters are different at α 0.05.

*SE Standard Error.
Table 4.4 Yeasts and molds of Romaine and Iceberg lettuce during storage.

<table>
<thead>
<tr>
<th>Lettuce type</th>
<th>Treatments</th>
<th>Yeasts &amp; molds day 0 Log₁₀ CFU/g Mean ±SE</th>
<th>Yeasts &amp; molds day 7 Log₁₀ CFU/g Mean ±SE</th>
<th>Yeasts &amp; molds day 14 Log₁₀ CFU/g Mean ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iceberg</td>
<td>Water</td>
<td>&lt;0.70&lt;sup&gt;a&lt;/sup&gt; (x)</td>
<td>1.85 ± 0.99&lt;sup&gt;a&lt;/sup&gt; (x)</td>
<td>1.57 ± 0.75&lt;sup&gt;a&lt;/sup&gt; (x)</td>
</tr>
<tr>
<td></td>
<td>Chlorine + ultrasound</td>
<td>&lt;0.70&lt;sup&gt;a&lt;/sup&gt; (x)</td>
<td>&lt;0.70&lt;sup&gt;a&lt;/sup&gt; (x)</td>
<td>&lt;0.70&lt;sup&gt;a&lt;/sup&gt; (x)</td>
</tr>
<tr>
<td></td>
<td>Tsunami + ultrasound</td>
<td>&lt;0.70&lt;sup&gt;a&lt;/sup&gt; (x)</td>
<td>&lt;0.70&lt;sup&gt;a&lt;/sup&gt; (x)</td>
<td>&lt;0.70&lt;sup&gt;a&lt;/sup&gt; (x)</td>
</tr>
<tr>
<td></td>
<td>Tsunami + SDS+ ultrasound</td>
<td>&lt;0.70&lt;sup&gt;a&lt;/sup&gt; (x)</td>
<td>&lt;0.70&lt;sup&gt;a&lt;/sup&gt; (x)</td>
<td>&lt;0.70&lt;sup&gt;a&lt;/sup&gt; (x)</td>
</tr>
<tr>
<td>Iceberg</td>
<td>Water</td>
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<td></td>
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<td>&lt;0.70&lt;sup&gt;a&lt;/sup&gt; (x)</td>
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<td></td>
<td>Tsunami + ultrasound</td>
<td>&lt;0.70&lt;sup&gt;a&lt;/sup&gt; (x)</td>
<td>&lt;0.70&lt;sup&gt;a&lt;/sup&gt; (x)</td>
<td>&lt;0.70&lt;sup&gt;a&lt;/sup&gt; (x)</td>
</tr>
<tr>
<td></td>
<td>Tsunami + SDS+ ultrasound</td>
<td>&lt;0.70&lt;sup&gt;a&lt;/sup&gt; (x)</td>
<td>&lt;0.70&lt;sup&gt;a&lt;/sup&gt; (x)</td>
<td>&lt;0.70&lt;sup&gt;a&lt;/sup&gt; (x)</td>
</tr>
</tbody>
</table>

<sup>1</sup> Detection limit 0.70 log₁₀ CFU/g.
<sup>a-c</sup> Treatment means within treatments (columns) with different letters are different at α 0.05.
<sup>x-y</sup> Treatment means within days (rows) with different letters are different at α 0.05.

*SE Standard Error.

4.4 CONCLUSIONS

In this study, we compared the effect of washing Iceberg and Romaine lettuce in chlorine, Tsunami 100®, and in Tsunami 100® + 0.1% SDS, with and without ultrasound, on the quality of lettuce samples. For Romaine lettuce after 14 days of storage, the overall quality when washed in chlorine was better than the other treatments as shown by OQ scores whereas no significant differences among treatments were found for Iceberg lettuce samples. None of the washing treatments had a detrimental effect on the color of packaged lettuce, and no significant differences were observed in color changes for Iceberg and Romaine lettuces compared to the values on day 0. No significant differences among treatments were observed in plant tissue damage, as measured by either ECR or the firmness of fresh-cut lettuce. Treatments with sanitizers effectively reduced the initial count of natural flora compared to the water wash.
During storage, regrowth of bacteria as shown by total aerobic plate counts was observed for all treatments. The use of SDS at low concentration did not cause additional quality changes.

4.5 REFERENCES


CHAPTER 5

FUTURE WORK

In this project we evaluated the effect of washing carrots either uncut or cut into slices, sticks and shreds to see the effect of contact area in the decay of free chlorine and peroxyacetic acid as well as the reduction of *E. coli* O157:H7 counts. As an oxidizing agent chlorine reacts with organic matter in the solution, resulting in rapid depletion in its concentration and lower efficacy in pathogen inactivation (Luo et al., 2012). It was observed that chlorine and peroxyacetic acid availability was depleted as carrots were left uncut, cut into sticks or shredded, the decrease in free chlorine and peroxyacetic acid was nearly 60 and 20 % respectively as the surface area of contact increased (Figure 3.4). In the same it was observed greater reduction on *E. coli* O157:H7 counts on carrot that was washed with chlorine (60 mg l⁻¹) and left uncut compared to sticks and shredded treatments (P<0.05), evidencing that depletion of free chlorine affects inactivation of microorganisms (Figure 3.5). Thus, it is proposed to evaluate the efficacy ultrasound in combination with sanitizers such as peroxyacetic acid and acidic electrolyzed water in reducing *E. coli* O157:H7 from carrots as affected by different surface areas.

We also evaluated the effect of sonication, two sanitizers (chlorine and Tsunami 100®) and a surfactant (sodium dodecyl sulfate (SDS)) on the quality of fresh-cut Iceberg and Romaine lettuce stored for 14 days. It was observed that electrolyte leakage rate values among all the treatments of Iceberg and Romaine lettuce are high on day 1 of storage (Figure 4.3), an indication of loss of turgor and damage in cell membrane. After 7 days of storage, electrolyte leakage rate decreased in all samples, which is the result of self-induced healing by the plant (Fan, 2012), after 14 days tissue damage became permanent which resulted in an increase in electrolyte leakage rate (Wang, 2004; Kim et al., 2005). Since electrolyte leakage rate was only
measured during 3 point during storage time (days 0, 7 and 14) it is difficult to assess when is healing of tissue taking place, thus, it is proposed to evaluate electrolyte leakage rate during 14 days with measurements at each day of storage in order to assess when tissue healing takes place and when the tissue damage becomes permanent resulting in high electrolyte leakage rate. It is also proposed to use scanning electron microscopy to visually evaluate the tissue damage as the result of processing conditions such as cutting and washing in an ultrasonic washer.
APPENDIX A

EFFECT OF ULTRASOUND AND CHLORINE AT DIFFERENT WASHING TIMES ON THE POPULATION REDUCTION OF *E. coli* K-12 INOCULATED ON ICEBERG AND ROMAINE LETTUCE

![Graph showing the efficacy of chlorine and ultrasound in reducing *E. coli* k-12 on Romaine and Iceberg lettuce washed for different times.](image)

**Figure A.1** Efficacy of chlorine and ultrasound in reducing *E. coli* k-12 on Romaine and Iceberg lettuce washed for different times.

**a-c** Treatment means within treatment time with different letters are different at α 0.05
Figure A.2 Effect of different Ultrasound Power Levels in the reduction of *E. coli* k-12 from Iceberg and Romaine lettuce.

a-c Treatment means within treatment time with different letters are different at α 0.05
APPENDIX B

ESTIMATION OF SURFACE AREA OF DIFFERENT CUTS OF CARROTS

1. \textit{surface Area of cylinder (Whole carrot)} = 2\pi r^2 + h(2\pi r)  
   = 2\pi 0.38^2 + 5(2\pi 0.38) = 12.83 \text{ cm}^2 \times 4 \text{ pieces} = \textbf{51.34 cm}^2

   \begin{figure}[h]
   \centering
   \includegraphics[width=0.3\textwidth]{cylinder}
   \caption{Estimation of surface area whole carrot.}
   \end{figure}

2. \textit{surface Area of cylinder (carrot slices)} = [2\pi r^2 + h(2\pi r)] + 4(14\pi r^2)  
   = [2\pi 0.38^2 + 5(2\pi 0.38)] + 14\pi 0.38^2 = 51.34 \text{ cm}^2 + 25.40 \text{ cm}^2 = \textbf{76.74 cm}^2

   \begin{figure}[h]
   \centering
   \includegraphics[width=0.3\textwidth]{cylinder_slices}
   \caption{Estimation of surface area carrot slices.}
   \end{figure}

3. \textit{surface Area of rectangle(carrot sticks)} = 2ab + 2bc + 2ac  
   = 2[2(0.10 \times 0.38) + 2(0.10 \times 5) + 2(0.38 \times 5)] \times 32 \text{ pieces} = \textbf{312.06 cm}^2

   \begin{figure}[h]
   \centering
   \includegraphics[width=0.3\textwidth]{rectangle_sticks}
   \caption{Estimation of surface area carrot sticks.}
   \end{figure}
4. \textit{surface Area of rectangle (shredded carrot)} = 2ab + 2bc + 2ac
   = 2[2(0.6 \times 5) + 2(5 \times 0.1) + 2(0.6 \times 0.1)] \times 160 \text{ pieces}=\textbf{2278.4cm}^2

\textbf{Figure B. 4} Estimation of surface area shredded carrot.