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# USE OF ELECTROLYZED WATER AS A TOPICAL ANTIMICROBIAL AND MINIMAL PROCESSING TECHNIQUE FOR FRESH, WHOLE PEACHES

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Sciences
Food, Nutrition, and Culinary Sciences

By
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May, 2015

Accepted by:
Dr. Julie Northcutt, Committee Chair
Dr. Paul Dawson
Dr. Doug Smith

# ABSTRACT

Three experiments were performed to enumerate the natural microflora on unwashed peaches, known as “field” peaches, and to determine the efficacy of using acidified electrolyzed water as a topical antimicrobial to remove or reduce the number of the natural microflora or inoculated *Listeria innocua* from to peach surfaces. During the first experiment, field peaches were divided into four treatment groups: no wash (NW), tap water wash (TW), acidified electrolyzed water wash (AEW), and chlorinated water wash (CL). Peaches were dipped into each of the treatment solutions at ambient temperature and immediately removed (approximately 5 seconds). Peaches were then rinsed in 100 mL of 0.1% peptone and rinsates were plated on aerobic plate count agar for enumeration. For the second experiment, exposure time to the treatment solutions and the temperature of the same treatment solutions were studied. Field peaches were again divided into NW, TW, AEW, and CL but treatments were applied using two exposure times of 5 seconds and 40 minutes at a temperature of 2°C (samples were given either a “0” or “40” in their labels to denote exposure time in minutes where 5 second exposures = 0 minutes e.g. TW-0, TW-40, AEW-0, etc.). Rinsing and plating was conducted as mentioned above.

Experiment three investigated the efficacy of NW, TW, AEW, and Cl, in reducing numbers of *Listeria innocua* on peaches that were previously inoculated and held at 4°C for 24 hours. Inoculated peaches were dipped in treatment solutions for 5 second and 40 minute times at 2°C. Results showed that exposure time had a significant effect on bacterial reduction for both AEW and Cl treatments. Average aerobic counts from all NW peaches was 4.2 $\log_{10}$ CFU/g peach for natural microflora and 4.3 $\log_{10}$ CFU/g peach for samples inoculated with

*Listeria*. The following results show the number of bacteria recovered ($\log_{10}$ CFU/g peach) from natural microflora samples and *Listeria* inoculated samples, respectively: NW = 4.2 and 4.9, TW-0 = 3.8 and 4.3, TW-40 = 3.2 and 4.7, AEW-0 = 3.6 and 3.7, AEW-40 = 2.6 and 1.6, CL=0 = 3.7 and 3.7, and CL-40 = 2.3 and 1.9. Greatest reductions were found with AEW-40 and CL-40 at refrigerated temperatures against both aerobic microorganisms and *Listeria innocua*. They reduced natural microflora counts by approximately 1.6 and 1.9 $\log_{10}$ CFU/g peach, respectively and they also reduced *Listeria innocua* counts by 3.3 and 3.0 $\log_{10}$ CFU/g peach, respectively. *Listeria innocua*, like *monocytogenes*, thrives in cold environments and the analysis of this study's results suggest that *Listeria* in TW-40 may have reattached to peaches during exposure.

Color studies were also performed on the peaches from the preliminary experiment and Experiment 2 to determine the effects of exposing the peaches to low pH environment such as that of the AEW used in this study. Peaches were analyzed for L*a*b* color data prior to their exposure to treatment solutions then they were analyzed again after their treatment concluded and they had air dried until no visible moisture remained. There was no significant color difference shown in any of the peaches when the pre- and post-treatment data was compared.

Results from these studies demonstrate that total aerobic microorganisms and *Listeria* spp. may be reduced, but not eliminated, during washing (by dipping) with AEW or CL with similar reductions for both antimicrobial treatments.

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# CHAPTER 1

# LITERATURE REVIEW

## *Introduction*

Many people in the United States, and worldwide, are affected by foodborne illnesses due to insufficient processing or preservation methodologies coupled with additional mishandling by consumers. Despite the efforts of the USDA, FDA, EPA, and other agencies working to promote the manufacture and sale of safe food, food safety continues to be a growing concern. It has recently been reported that the estimated cost of foodborne illnesses in the United States alone is over $51 billion and that approximately 85% to 90% of that sum results from the five following microorganisms: *Salmonella enterica*, *Campylobacter spp*., *Listeria monocytogenes*, *Toxoplasma gondii*, and norovirus (Batz, 2013; Scallan et al., 2011a; Scallan et al., 2011b; Scharff, 2010). Many of these microorganisms are ubiquitous and can commonly be found in the natural environment, which presents a challenge for food producers and processors. Most foods are grown or raised in environments that contain large quantities of microorganisms that cause illness when ingested by humans. These environments are natural mediums and include things like the soil that crops are grown in, the areas where food production animals are raised, air, the water that harbors fish and shellfish, etc. (Abadias et al., 2008; Beuchat, 1996; Kim et at., 2012; Lee et al., 2014, Li et al., 2014; Natvig et al., 2003; Su and Lui, 2007). Because of this, it is clear that there are potential hazards involved in the production of any food item. These hazards must be accounted for in the process of getting foods from the farm to the table to ensure the health and safety of the public.

### *Global Implications of Produce Shelf-life Extension*

Fruits and vegetables are a vital source of nutrients in the human diet and compose a significant portion of the average diet for many people across the globe. According to the World Health Organization (2013), fruits and vegetables have many benefits including contributing to the prevention of certain cardiovascular diseases and cancer; and 1.7 million deaths (2.8%), worldwide, can be attributed to insufficient intake of fruits and vegetables. This means that many people are not consuming adequate amounts of fruits and vegetables (400g/day, excluding starch vegetables, according to the WHO) to help prevent issues like heart disease, cancer, diabetes, obesity, and other micronutrient deficiencies (Diet 2003). Fruits and vegetables are very perishable which makes them more difficult to harvest, process, transport, and sell before natural biochemical processes and/or naturally present microorganisms render the products unacceptable or unsafe for consumption. The perishability of fresh fruits and vegetables is one reason that many people groups, especially in countries without efficient transportation infrastructure, do not have access to much fresh produce (Gustavsson et al., 2011). Though processed produce is shelf stable and readily shippable, it is still difficult to get sufficient amounts of it to people due to transportation hurdles in many underdeveloped countries. The perishable nature of fresh produce is not only a hurdle for addressing global nutritional issues but also presents a problem for domestic production. Fruits and vegetables are often damaged or spoiled before safely making it into the market in either fresh or processed form. During three steps of food production (postharvest handling/storage, processing/packaging, and distribution) produce losses range from approximately 5%, 2%, and

10%, respectively, in industrialized countries to 10%, 20%, and 15% in lesser developed countries (Gustavsson et al., 2011).

### *Food Production in the United States*

In the United States, it is estimated that 30% of the food produced is wasted and most of this loss occurs at the farm and in retail sales environments (Jones, 2004; Lundqvist et al., 2008; Nellemann et al., 2009). This equals approximately 20 pounds per person per month (Jones, 2004; Lundqvist et al., 2008; Nellemann et al., 2009). Specifically regarding fruits and vegetables produced in the United States, the losses can separated by processing/handling steps; and losses are given as percentages based on the amount of food entering that step. They are as follows:

- agricultural production = 20%
- postharvest handling and storage = 4%
- processing and packaging = 2%
- distribution: supermarket retail = 12%, and consumption = 28% (Gustavsson et al., 2011).

To decrease these losses while maintaining or increasing the amount of available fresh produce in the market, continued research into novel, responsible preservation methodologies and technologies is needed. As these new methods are developed, produce can be made more readily available at lower costs to consumers, which would help break down barriers to purchasing and consuming fruits and vegetables needed to maintain good health. When consumers buy and consume produce before it degrades, waste is reduced and personal health could be improved. Thus, it can be seen that new food preservation strategies need to be developed in order to help eliminate the current high levels of food waste and decrease malnutrition.

Fruit and vegetable consumption in the United States has been a concern for many years. The “5 A Day for Better Health” program was in place in the United States for 15 years before recently being replaced by “Fruits & Veggies – More Matters,” which was established through a partnership between the Centers for Disease Control & Prevention (CDC) and the “Produce for Better Health Foundation” (PBH) (Rekhey and McConchie, 2014). This program was created to educate the American public on the importance of eating fruits and vegetables and to help them find ways to get recommended amounts into their diet. The United States Department of Agriculture (USDA) has stated its recommendation in the 2010 *Dietary Guidelines for Americans* as filling half of the plate with colorful fruits and vegetables (USDA, 2010). The “My Plate” nutrition guide is a reference guide for the general public that reflects many of the recommendations of the USDA and the “Fruits & Veggies – More Matters” program and is easily available online (USDA, 2010).

According to the current *Dietary Guidelines for Americans*, most Americans are consuming only around half of the amounts of fruits and vegetables that are recommended each day (USDA, 2010). In the past, the majority of fruit and vegetable intake has come in the form of processed items. However, as improvements in infrastructure, transportation, and food safety/preservation have been made, the high ratio of processed compared to fresh fruit and vegetable consumption has been changing. In 2002, the USDA published its “*Agricultural Fact*

*Book*,*"* which tracked the consumption of fresh versus processed fruits and vegetables and showed a substantial increase in annual fresh fruit and vegetable consumption from 250 pounds per capita to 328 pounds per capita between 1970 and 2000. While the ratio of the amount of fresh versus processed vegetables has not changed significantly (intake of processed vegetable consumption also rose), the ratio between the amount of fresh versus processed fruit has fallen by approximately 50% (USDA, 2003). This suggested a strong trend in the American population's shifting preference from processed to fresh fruit. Later, in 2012, the United States Census Bureau published its findings on fruit and vegetable per capita consumption in the United States between 2000 and 2009, which noted that the consumption of fresh vegetables, processed vegetables, and processed fruits steadily decreased over the 10 year time period (U.S. Census Bureau, 2012). Interestingly, the amount of fresh fruit consumed remained constant which further reduced the ratio of processed to fresh fruit consumption. Now there is only a negligible difference (2 pounds) in the amount of fresh compared to processed fruit consumed annually per person in the United States (U.S. Census Bureau, 2012).

Historically, many food safety issues have occurred with consumption of produce. Fruits and vegetables are grown in soils and are exposed to rodents, insects, and pests that inevitably contain or deposit large amounts of microorganisms on the produce (Abadias et al., 2008; Beuchat 1996; Kim et al., 2012; Lee et al., 2014; Natvig et al., 2003). The ubiquity of microorganisms has been known for years and serves as the primary reason that producers wash their crops and often treat them with topical antimicrobials and preservatives. In recent years, many consumers have become adverse to the use of "chemical" additives including those used during the washing of food. Because some of the public is concerned with food additives, it is becoming increasingly important for producers to find new ways to minimize food waste due to chemical and bacterial breakdown. Bearth et al. (2014) state that even though additives do not arrive in the food supply without rigorous scientific testing to ensure safety, the public does not always trust scientific consensus. One trend that many consumers support is the use of “natural” or “organic” ingredients, but even these terms are becoming targets of consumer skepticism. In a report published by Browne (2011) on Mintel, it was reported that only 34% of consumers trust the term “organic” and that only 24% trust the term “natural” when they appear on food and beverage labels. The same study, though, also noted that among the consumers who purchase natural and organic foods and beverages (NOFB), 84% did so for health reasons and 44% believed NOFB to be more nutritious than regular foods (Browne, 2011). Because there is confusion regarding some of the terms used on food labels (what they actually indicate) and because consumers and scientist do not evaluate food safety with the same perspective, the general public may be concerned about the food additives being utilized (Bearth et al., 2014; Hansen et al., 2003; Krystallis et al., 2007; Sparks and Shepherd, 1994)). Ultimately, the goal of food additives is to create safe, nutritious, wholesome, and appealing food with long shelf-life for consumers. In order to improve a sense of trust and security in the food supply and to prevent harm, the Food Safety Modernization Act was signed into law in 2011.

### *Food Safety Modernization Act (FSMA)*

“About 48 million people (1 in 6 Americans) get sick, 128,000 are hospitalized, and 3,000 die each year from foodborne diseases, according to recent data from the Centers for Disease Control and Prevention. This is a significant public health burden that is largely preventable”

(FDA, 2014a). This statement is a summation of the reasoning behind the passing of the FSMA. The purpose of the FSMA is “to amend the Food, Drug, and Cosmetic Act with respect to the safety of the food supply” (FDA, 2011). Previously, the overall focus of food safety regulations was to create a system to effectively respond (reactive) to food safety issues that were discovered. While this goal is important and should be maintained, it is being increasingly noted that it is incomplete. To go alongside an efficient response system, an efficient prevention system (proactive) for food safety is equally important. That is the aim of the FSMA according to the United States Food and Drug Administration. It was created to establish food safety protocol not only for response to food safety issues but for preventing them from ever occurring.

To do this, regulatory agencies have been given more authority to require compliance with food safety standards such as written “preventative controls” for hazards (such as HACCP plans), mandatory inspections at intervals deemed necessary by scientific evidence for specific products, import regulations, and collaboration among inspections agencies. More stringent testing of water supplies is being implemented, as are higher levels of training for inspectors, including scientific training, laboratory and sampling techniques, specialization of knowledge and skill for particular types of food, and increased knowledge of best practices. Lab accreditation requirements were also reviewed and accreditation maintenance is required, at minimum, every five years to ensure compliance (FDA, 2011). Furthermore, authority to require documentation on all imports is upheld, training of third-party auditors through accredited institutions is being pursued to provide more accountability, and the voluntary qualified importer program are in place (FDA, 2011). These steps help redirect the attention of food regulations towards preventing foodborne illness incidences with the hope that the number of cases can be reduced on a yearly basis.

One area determined to be of significance within the FSMA is in regards to fresh produce. From 1996 to 2010, there were 131 reported cases of produce-related foodborne illness outbreaks which resulted in 14,350 cases, 1,382 hospitalizations, and 34 deaths (FDA, 2014b). Because of these numbers the FSMA mandates that the FDA establish minimum standards, based on science, for the safe growing, harvesting, packing, and holding of produce on farms to prevent contamination with pathogens (FDA, 2011; FDA, 2014b). In South Carolina, approximately 25% of the landmass is dedicated to farming fresh fruits and vegetables. This makes meeting the FSMA requirements critical to the state's economic growth.

### *Peaches*

#### 1.) Production and Processing

Peaches are an important portion of the produce grown in the United States. In 2013, over 100,000 acres of land were dedicated to growing peaches in the United States and yielded over 900,000 tons of fruit. Of this, approximately 15,000 tons of peaches were not utilized due to many factors, including spoilage, mechanical damaging, deformity, and defects. During the same year, the average price per ton of peaches was $614 which generated nearly $550 million of revenue (USDA-NASS, 2013). However, economic loss from the 15,000 tons of unutilized peaches should also be considered and may be estimated to be $10 million.

Since 2004, the amount of acreage dedicated to peach farming has consistently lessened, decreasing from 140,000 acres in 2004 to just over 100,000 acres in 2013. Along with that, the amount of utilized peach production has dropped from around 1.25 million tons to about 0.9 million tons (USDA-NASS, 2013). With peach production acreage dropping, it is important for peach farmers to make the best use of the crops they plant. Currently, roughly half of all unutilized peaches were not harvested while the other half were harvested but were not sold (USDA-NASS, 2013). Though unharvested peaches would not be benefited by improvements in processing or preservation, the peaches that are harvested but not sold could be potentially saved and utilized as advances are made. In the United States in 2013, peach farmers lost over 5 million dollars simply because peaches that they harvested could not be sold.

According to the National Agricultural Statistics Survey in 2013, South Carolina alone produced an estimated 70,000 tons of peaches making it the second largest peach producing state in the United States, behind California. This crop produced a potential revenue of nearly 75 million dollars; however, approximately 8% of those peaches went unutilized. This equates to a loss of over 6 million dollars in South Carolina in one year. Of the peaches that were not utilized, 45% were unharvested and the remaining 55% were harvested but were not sold (USDA-NASS, 2013). There are various reasons why peaches may not have been sold; but, undoubtedly, spoilage, damage, and disease are large contributors and need to continue to be addressed.

During peach production and processing, there is one step that stands out as having high potential as a critical control point (CCP) for microorganisms. This step is the wash step referred to as hydrocooling and it is used heavily by peach farmers. The primary purpose of hydrocooling is the rapid reduction in temperature to slow fruit degradation (respiration) and to slow the growth of microorganisms. Temperature reduction of peaches needs to be done quickly after harvest to maximize production yields and minimize the amount of time bacterial growth is favored. If done properly, peaches should reach a final temperature of 40°F (4.4°C), preventing decay and softening of texture prior to reaching the consumer (Bennett et al., 1965). In the hydrocooling step, there are two primary methods in use: 1.) flood-type hydrocooling and 2.) bulk hydrocooling (Bennett et al., 1965). Bennett et al., (1965) note that flood-type hydrocooling has been in use longer than bulk hydrocooling; and it entails having peaches placed in their shipping baskets, set on a conveyor, and run through a large "shower" of cold water (32°F). Bulk hydrocooling involves submersing loose peaches into a large tank of chilled, circulating water which they pass through until they are lifted out by a conveyor which then carries them under a shower, similar to that of the flood-type hydrocoolers. Bulk hydrocooling may provide more opportunity for the bruising and damaging of peaches due to excess handling. Previous research has shown that a peach with a three inch diameter would need approximately 30 minutes exposure time to water at 35°F (1.7°C) to reduce their internal temperature from 90°F (32.2°C) to 40°F (4.4°C) (Bennett et al., 1965). This long exposure time provides a good opportunity for bacterial control if the control technology is applied using the rinse water as a medium.

### 2.) Quality

According to the United States Department of Agriculture (2004), peaches may be graded using four quality categories. The categories are listed below, along with their specifications (USDA, 2004).

**U.S. Fancy.**

"U.S. Fancy" consists of peaches of one variety which are mature but not soft or overripe, well formed and which are free from decay, bacterial spot, cuts which are not healed, growth cracks, hail injury, scab, scale, split pits, worms, worm holes, leaf or limb rub injury; and free from damage caused by bruises, dirt or other foreign material, other disease, insects or mechanical or other means. In addition to the above requirements, each peach shall have not less than one-third of its surface showing blushed, pink or red color.

**U.S. Extra No. 1.**

Any lot of peaches may be designated "U.S. Extra No. 1" when the peaches meet the requirements of the U.S. No. 1 grade: Provided, That in addition to these requirements, 50 percent, by count, of the peaches in any lot shall have not less than one-fourth of the surface showing blushed, pink or red color.

**U.S. No. 1.**

"U.S. No. 1" consists of peaches of one variety which are mature but not soft or overripe, well formed, and which are free from decay, growth cracks, cuts which are not healed, worms, worm holes, and free from damage caused by bruises, dirt, or other foreign material, bacterial spot, scab, scale, hail injury, leaf or limb rubs, split pits, other disease, insects or mechanical or other means.

**U.S. No. 2.**

> "U.S. No. 2" consists of peaches of one variety which are mature but not soft or overripe, not
>
> badly misshapen, and which are free from decay, cuts which are not healed, worms, worm holes and free from serious damage caused by bruises, dirt or other foreign material, bacterial spot, scab, scale, growth cracks, hail injury, leaf or limb rubs, split pits, other disease, insects, or mechanical or other means.

The highest quality peaches are often reserved for applications where they will be sold whole and fresh while lesser quality fruits may be used for purees, slices, juices, canning, or other applications where they can be trimmed or where visual quality is not of concern. Official USDA quality standards are assigned shortly after harvesting; but peaches will continue to ripen during holding and, if not consumed soon enough, they will begin to decay. Peaches may begin to degrade in quality and safety during postharvest processing, storage, and transportation due to various reasons including metabolic changes within the peach, microbial activity, mechanical damage, softening of the peach's flesh, and physiological decay. These effects can be caused by factors such as varietal differences, the extent of ripening at harvest, handling, and storage conditions (Crisosto and Mitchell 2002; Crisosto et al., 1999; Girardi et al., 2005; Lelièvre et al., 1997; Lill et al., 1989; Rombaldi et al., 2002). It should be noted that much of a peach's degradation is intrinsic and cannot be prevented. Once harvested, peaches continue to respire and increasingly lose weight (and thus per pound value) as the nutritional constituents of the peach (sugar, amino acids, vitamins and minerals, and organic acids) are used for metabolic processes. Perez-Lopez et al., (2014) found that peaches lost up to 9% of their harvest weight after five days of storage at 20°C. The same study tested peaches with the following percentages of their skin still yellow (yellow color denotes unripe peaches in this case): 25%, 50%, and 100%. The weight loss after five days varied by only 7% to 9%. The respiration rate, however, was differentiated in terms of $CO_2$ consumed. At ambient holding temperatures, peaches with 50% yellow consumed almost twice as much $CO_2$ as the peaches that were 100% yellow, while the 25% yellow peaches fell approximately midway between the 50% and 100% yellow peaches. This shows that, as expected, climacteric fruit respiration and subsequent changes in skin color and texture, are self-propagating. As the fruit's flesh increasingly softens from starches being converted into various mono- and disaccharides, from the increased methylation of pectin, and from polygalacturonase activity, it becomes less resilient; and there is a higher likelihood of lacerations that breach the skin of the fruits and provide excellent growth sites for microorganisms due to the nutrient availability from the mesocarp.

### 3.) Color

Peaches vary in color from white to yellow to red flesh with skins that transition from green (unripe) to yellow, orange, and red to during ripening and light exposure (Frett, 2012; Layne and Bassi, 2008; Zhang, 2014). There are several pigments in peaches that contribute to their color but carotenoids and anthocyanins are most notable when considering color for specific applications (Layne and Bassi, 2008). Carotenoids and anthocyanins impart yellow/orange and red colors to peaches, respectively, and their chemical structures make them both favorable for different applications. Peaches intended for sale as unprocessed and whole are often selected for higher anthocyanin content because blush (red skin pigmentation) makes the fruits visually appealing and favorable for marketing (Frett, 2012).

Anthocyanin production in the exocarp and mesocarp of peaches is independent, and that high blush does not inherently denote high anthocyanin content in the mesocarp (Layne and Bassi, 2008). Anthocyanin production in the exocarp is dependent on light exposure, but production of the pigment in the mesocarp is not, and the concentration of anthocyanins in the mesocarp varies largely among cultivars and from tree to tree (Layne and Bassi, 2008). Though anthocyanins are aesthetically pleasing when in the natural environment maintained in the peach's cell, they are not good for processing. This is largely because they are water soluble and are not heat or pH stable (Bakowska-Barczak, 2005; Giusti and Wrolstad, 2003; Layne and Bassi, 2008). When peaches are processed in syrups or water, anthocyanins will leach into the liquid and will also discolor due to heat which yields an unappealing brown color in the fruit and in the syrup (Layne and Bassi, 2008). Therefore, peaches intended for heat processing (e.g. canning) are usually grown with strong selection against anthocyanins and in favor of carotenoids (Layne and Bassi, 2008). Peaches for heat processing applications are typically peeled by spraying or immersing the whole fruits into hot caustic solutions of 1 - 2.5% lye (NaOH) for 30 - 60 seconds (Burrows, 2011; Dauthy, 1995; Layne and Bassi, 2008). This eliminates the aesthetic need for blush development in the skins and requires pigments with heat and pH stability in the mesocarp to maintain color. Carotenoids are heat stable and lipid soluble, which prevents both discoloration and leaching into hydrophilic processing liquids; this is why processors uses peaches for canning prefer peaches with high carotenoid to anthocyanin ratios (Layne and Bassi, 2008).

*Microbiology*

1.) General

Microorganisms, while extrinsic to peaches, are ubiquitous and are found on the surface of all fresh produce. Bacterial contamination of fresh produce, such as peaches, is inevitable during the growing process; and the surfaces of fruits contains high numbers of natural microflora, some of which are pathogenic, after harvest and before processing (Kalia and Gupta, 2006). These microorganisms are naturally found in the soil and water that peach trees are grown in, and their transfer from the growing medium to the food is unavoidable (Abadias et al., 2008; Beuchat 1996; Kim et al., 2012; Lee et al., Li et al., 2014; Natvig et al., 2003; Su and Lui 2007). Although fresh peaches have a natural pH between 3.30 and 4.05, which should be too low for supporting the growth of known pathogens, the skin of the peaches can easily harbor bacteria. This makes the washing of peaches critical control for biological hazards (pathogenic bacteria), since most fresh produce in the United States is not subjected to a kill step during processing or handling (FDA, 2007 and Omac et al., 2015). Alegre et al., (2010) tested fresh peach flesh for its ability to support the growth of *Escherichia coli* O157:H7, *Salmonella,* and *Listeria innocua* and found that several varieties of peach with pH < 4 still resulted in up to 2.5 $\log_{10}$ CFU/g increase in bacterial population after one day of storage at 5°C and 25°C. It is unclear as to why low pH, alone, may not always be sufficient to inhibit microbiological growth but this result is of concern when considering how different environments may affect microbial responses to different treatment mechanisms. Because of the molecular complexity that comprises fruits, the effectiveness of antimicrobial treatments for one specific type or variety of fruit may not be as effective for another. This being the case, additional research needs to be conducted to determine the factors that work synergistically or antagonistically with pH to inhibit bacterial growth on fresh fruit. The decontamination of produce, however, also eliminates the naturally present, non-pathogenic, competitive microflora which could then create an environment that more readily supports the growth of pathogens if they are present (Alegre et al., 2012; Carlin et al., 1996; Li et al., 2002).

### 2.) Listeria

Listeria is a gram (+), facultative anaerobic, psychrotrophic pathogen that is halotolerent at NaCl concentration of up to 25% (Bell and Kyriakides 2005). The International Commission on Microbiological Specifications for Foods (1996) notes *Listeria* spp. minimum and maximum pH for growth at 4.39 and 9.4, respectively, though some research reports its ability to survive between pH 3.0 and 12.0. It has a minimum water activity for survival of 0.92 (Bell and Kyriakides 2005; Liu 2008; Liu et al., 2005). As a human pathogen, *Listeria* is less common than others such as *E. coli* or *Salmonella* but is a high priority because listeriosis has a high mortality rate (Bell and Kyriakides 2005; Liu 2008). In the United States, the Centers for Disease Control and Prevention (CDC) reports that 123 cases of foodborne illnesses are caused by *Listeria monocytogenes* each year. Of those, nearly 112 illnesses resulted in hospitalizations; and over 24 illnesses resulted in death (Crim et al., 2014). Listeriosis outbreaks are commonly traced back to processed meat products like deli meats and hot dogs, fresh cheeses, and produce. Though listeriosis is less common than other foodborne pathogens, it has the third highest fatality rate of over 20% (Bennion et al., 2008; CDC 2011; Omac et al., 2015; Sant'Ana et al., 2012; Todd and Notermans 2011). Due to the nature of listeriosis, peach growers, processors, and distributors should begin to invest in more preventative technologies to stop future contamination and potential foodborne disease outbreaks (CDC 2011). This is the type of proactive thinking encouraged by the FSMA. In 2013, Wawona Packing Co. in California issued a voluntary recall of whole peaches and several other stone fruits due to possible *Listeria* contamination. The amount of fruit lost due to the recall is unpublished at this time; but the recall extended from peaches harvested on June 1st, 2014 to those harvested on July 17th, 2014. The large scale loss in product and revenue should be cause for concern about *Listeria* contamination among members of the National Peach Council. It is for this reason the many peach processors are investigating the use of "*Listeria* management protocols" for the packing sheds.

### 3.) Fungi

There are several fungi that are, historically, problematic in regard to peaches. The most prevalent ones are spoilage molds that will grow under refrigeration, albeit more slowly than at ambient temperatures. These molds cause various diseases in peaches such as blue mold rot from *Penicillium expansum*, *Rhizopus* rot from *Rhizopus stolonifer*, gray mold rot from *Botrytis cinerea*, and brown rot from *Monilinia fructicola* (Cao et al., 2011; Kalia and Gupta, 2006; Karabulut et al., 2002; Wang et al., 2013; Yu et al., 2012; Zhang et al., 2007a; Zhang et al., 2007b; Zhang et al., 2008; Zhou et al., 2008). It has been noted by Karabulut et al., (2002) that synthetic chemicals have been the most common fungicides; but, as the general public continues to ask for less synthetic treatments to be used in food, new and "natural" treatment options against fungi need continued research (Yang et al., 2011; Yu et al., 2012; Zhang et al., 2007a). Yu et al., (2012) found that applying some yeast saccharides to peaches would stimulate chitinase, β-1,3-glucanase, phenylalanine ammonia-lyase, and peroxidase activity and promote phenolic synthesis within the peaches, which may result in fungal inhibition. Guentzel et al., (2010) showed that using neutral electrolyzed water, with total residual chlorine from 25 to 100 ppm, as a dip (with 10 minute exposure time) for peaches was effective in reducing *Monilinia fructicola* and *Botrytis cinerea* to undetectable levels for up to three days. There are various other technologies being researched for antifungal properties, and that research will become increasingly important from an economic standpoint as time progresses. While molds do cause degradation in produce, they do not typically pose health threats to humans and are thus, primarily, a quality concern, not a safety concern; however, previous reports have suggested that mold growth on fruit can elevate the pH to levels that may allow for the growth of pathogenic bacteria (Ryser and Marth, 2007).

### *Antimicrobials*

#### 1.) General

The preservation of fresh produce is a great concern economically. Because of the highly perishable nature of fresh fruits and vegetables, they must be harvested, processed, stored, shipped, and sold under controlled conditions which most often entails low temperature storage to slow respiration and inhibit microbial propagation. Currently the most common method for washing produce after harvest is chlorinated water at concentrations between 50 and 200 ppm (Beuchat, 1998; Graca et al., 2011). Alegre et al., (2012) state that a 1 to 2 log reduction in bacterial populations on minimally processed produce can be expected when using chlorine as a treatment. Growing disapproval, though, has occurred relative to using chlorine as an antimicrobial food treatment. Additionally, chlorine reacts with organic materials, such as soil, causing reduced efficacy against microorganisms. Because chlorine is currently facing some obstacles as antimicrobial treatment for produce, alternative technologies that are practically feasible for manufacturers (including biopreservatives or “green” preservatives) are continuing to be researched to determine their efficacy against pathogenic microorganisms like *Listeria, E. coli* O157:H7, *Salmonella,* and *Shigella*.

### 2.) Essential Oils

Research has already been done regarding essential oils (EOs) and their applicability in food preservation, and it has been shown that they have significant antimicrobial potential. There are various ways by which EOs can work to destroy microorganisms; some of these ways are mitochondrial membrane degradation, inhibition of metabolic and respiratory activity, and cytoplasmic mutagenicity (Bakkali et al., 2008). Research has shown EOs to be viable antimicrobials in a diverse number of food systems such as lettuce and leafy greens (Yossa et al., 2013; Gunduz et al., 2012; Moore-Neibel et al., 2012); fresh water fish (Desai et al., 2012); salt water fish (Kykkidou et al., 2009); chicken (Giatrakou et al., 2010); pork (Chen et al., 2013; Gill et al., 2002); and citrus fruits (Chafer et al., 2012), other fruits, vegetables, and grains such as cabbage, barley, tomato, and papaya (Catherine et al., 2012; Yun et al., 2013); and bovine milk (Shah et al., 2013b), to name a few. Because of the wide range of essential oils and the unique proportion of constituents within each, there are many potential options for use with food applications. This is important because of the characteristic organoleptic properties associated with each particular EO. An EO cannot be selected for use with a food based solely on its general antimicrobial capabilities; further screening must be done to ensure that the EO and target food are compatible from a sensory perspective (Seow et al., 2014). Desai et al. (2012) conducted a study testing orange EO and carvacrol (shown by Burt (2004) to be a major constituent in some EOs (such as thyme and oregano) for use with catfish. Though the carvacrol had the highest antimicrobial strength, sensory panelists noted that it had a piney off flavor. Because of the off flavors they impart to food, EOs containing high levels of carvacrol should be avoided in applications like catfish. Klein et al. (2013) also noted that the concentration of thymol and carvacrol required to achieve bacteriostatic/bacteriocidal results produced negative organoleptic effects; however, Giatrakou et al., (2010) showed positive sensory effects of thyme oil when used in a chicken application, thus demonstrating the need to match EOs to the flavor profiles of the foods they will be used with. Furthermore, EOs should also be selected for use in specific foods known to typically contain certain types of microorganisms that the chosen EO is known to be effective against, especially concerning whether gram (+) or gram (-) bacteria are commonly found on the food. While it is generally noted that gram (+) bacterial cells are more susceptible to EOs than gram (-) cells (Akrayi 2012; Burt 2004; Seow et al., 2014), there are studies identifying EOs that are effective against both categories (Akrayi 2012; Catherine et al., 2012; Jiang et al., 2011; Singh et al., 2007; Tajkarimi et al., 2010; Wang et al., 2012). The effectiveness of EOs against gram (+) and gram (-) bacteria has much to do with the mechanisms of inhibition utilized by the oils for the destruction of bacterial cells.

Research has been conducted to explore different delivery methods to apply EOs onto food products. The stability of EOs in aqueous solutions is being studied and improved through the use of emulsions, microemulsions, nanoemulsions, and encapsulation techniques; and the antimicrobial/antifungal capacity of the oils within these systems is also being studied (Bhavini et al., 2012; Buranasuksombat et al., 2011; Chafer et al., 2012; Cramp et al., 2004; Engels et al., 1995; Francesco et al., 2011; Ghosh and Coupland 2008; Lim et al., 2011; Magnusson et al., 2011; McClements and Rao 2011; Rao and McClements 2011; Rao and McClements 2013; Rodriguez-Rojo et al., 2011; Shah et al., 2013a; Shah et al., 2013b). Yun et al. (2013) tested the possibility of applying EOs in the vapor phase to cherry tomatoes. They tested EOs including cinnamon, oregano, and mustard, and compared them to using only isolated primary components which are cinnamaldehyde, carvacrol, and allyl isothiocyanate, respectively. These tests showed positive results, especially with mustard EO and isolated allyl isothiocyanate, as both yielded no salmonella growth when used in either 16.7 or 33.3 µL/L, respectively, *in vitro* and reduced Salmonella to undetectable levels (> 5-log reduction) *in vivo*.

Essential oils applied both holistically and separated, isolated compounds derived from them have shown positive results for antimicrobial work. Though EOs do work more effectively against gram (+) bacteria, research is beginning to identify more ways to use EOs that are also effective against gram (-) bacteria which is extremely important when considering pathogen control. Delivery of the EOs onto food products is also an important area needing evaluation. The use of emulsions and encapsulation techniques is being researched and is showing good results but is not feasible for most large scale operations at this time. Specifically regarding the anti-listerial effects of EOs, variable results have been reported and suggest that the amounts of anti-listerial compound occurring naturally in foods is often too low to provide satisfactory log reductions of *Listeria* and should not be relied on as a sole prevention strategy (Bell and Kyriakides 2005; Friedman et al., 2002; Hammer et al., 1999; International Commission 1998). Because the anti-listeria compounds naturally present in foods is too low to be considered effective in reduction *Listeria* populations, using EO concentrations and isolated compounds from the EOs is the suggested topic of study moving forward. With continued work, EOs and novel delivery systems for their application could be another method by which to meet a rising need in the market for “natural” food preservatives.

3.) Peracetic Acid

Peracetic acid is a highly effective antimicrobial agent against bacteria, spores, viruses, and fungi (Kitis, 2004). It is more expensive to produce than chlorine and electrolyzed water but has shown strong antimicrobial results against coliforms even at low concentrations (Baldry, 1983; Baldry and French, 1989; Fraser et al., 1984, Kitis, 2004). When Greenspan and MacKeller (1951) tested its efficacy against various microorganisms including *E. coli, Bacillus subtilis, Pseudomonas aeruginosa, Aspergillus niger*, and others; they found it to be bactericidal at 0.001%, fungicidal at 0.003%, and sporicidal at 0.3% (Kitis, 2004). This demonstrates that peracetic acid is most effective against bacteria, though still effective against other microorganisms such as molds, yeasts, and fungi (Kitis, 2004; Liberti and Notarnicola, 1999; Rudd and Hopkinson; 1989).

Peracetic acid acts as a strong oxidant to alter cytoplasmic membrane lipoproteins, to disrupt cell walls, and to denature proteins and enzymes. This makes it effective against both gram (-) and gram (+) bacteria (Baldry and Fraser, 1988; Block, 1991; Kitis, 2004; Leaper, 1984; Liberti and Notarnicola, 1999). It has also been suggested as an inactivation compound against catalase, which makes it especially detrimental to aerobic bacteria (Block, 1991; Kitis, 2004). Another benefit of peracetic acid is that it does not form toxic or mutagenic byproducts after it reacts with organic materials and its decomposition products are acetic acid, hydrogen peroxide, oxygen, and water (Baldry and Fraser, 1988; Colgan and Gehr, 2001; Gehr et al., 2002; Kitis, 2004; Lefevre et al., 1992; Monarca et al., 2001; Monarca et al., 2002; Sancher-Ruiz et al., 1995; Wagner et al., 2002).

With the release of acetic acid, though, is the availability of extra organic acids that any surviving bacteria may be able to utilize to grow if pH is favorable (Kitis, 2004; Sanchez-Ruiz et al., 1995). Another disadvantage of peracetic acid is its combustibility and instability at concentrations over 15%, but most industry users apply it at 12% with sufficient results (Block, 1991; Kitis, 2004). This concentration of 12% has been estimated, by Kitis (2004), to cost four to five times more than sodium hypochlorite (chlorine) in the United States and is a current hurdle to more widespread use in industry.

### 4.) Electrolyzed Water

Electrolyzed water (EW) is an antimicrobial treatment that has begun to be studied for its potential use in food safety. EW is generated by using a machine called an electrolyzer to pass an electrical current through water containing a salt, typically either sodium chloride (NaCl) or potassium chloride (KCl) (Al-Haq et al., 2005). The solution is exposed to a chamber in the electrolyzer containing an anode and a cathode; and the salt water is fractioned into two water streams: a basic stream (predominantly containing NaOH) and an acidic stream (predominantly

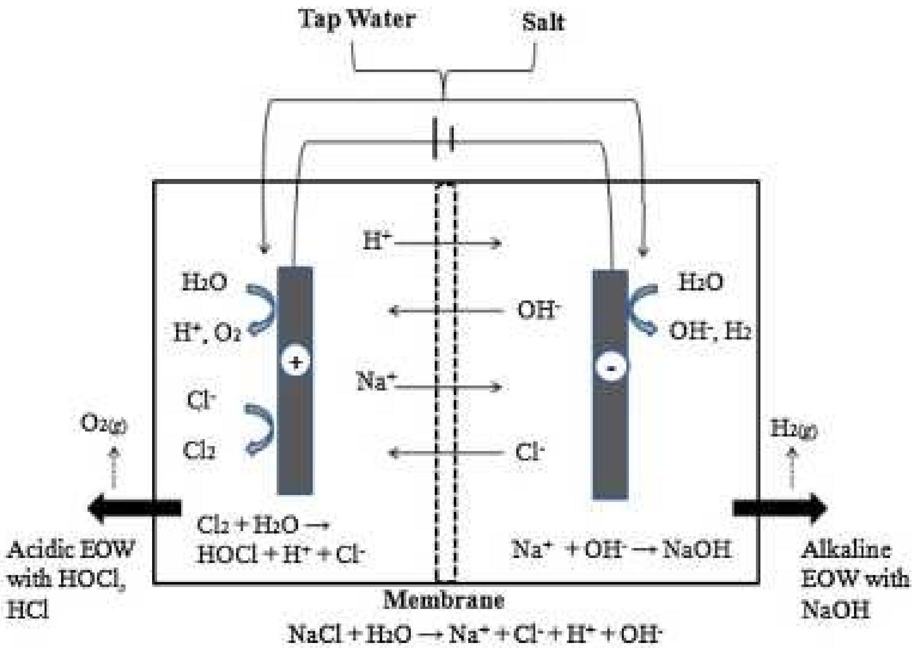
Tap Water
Salt
H+
H2O
H+, O2
+
OH-
H2O
OH-, H2
-
Na+
Cl-
Cl2
Cl-
O2(g)
H2(g)
Cl2 + H2O →
HOCl + H+ + Cl-
Na+ + OH- → NaOH
Acidic EOW
with HOCl,
HCl
Alkaline
EOW with
NaOH
Membrane
NaCl + H2O → Na+ + Cl- + H+ + OH-

**Figure 1**: Schematic of EW generation (Dev et al., 2014; Huang et al., 2008)

containing HOCl). Thus, electrolyzed water can either be classified as acidic electrolyzed water (AEW), basic electrolyzed water (BEW), or neutral electrolyzed water (NEW) (Al-Haq et al., 2005). This process is depicted in Figure 1.

The amperage of the electrolyzer and initial salt concentration can be altered to adjust the final pH of the streams and either the free chlorine content (AEW) or the NaOH content (BEW). The only components required to make EW are water and salt. Because these ingredients are presumably familiar to all consumers, this may improve the acceptability of this treatment. Some consumers may be concerned with the increased Na and Cl levels in the food treated with EW; however, due to the low concentration used and the topical application, sodium (Na) levels will be negligible and chloride (Cl) levels will not exceed what has been deemed safe to use for fruit and vegetable wash, which current scientific research has established at 200 ppm $NaOCl^-$ (FDA, 2013). Studies have shown promising results when using original salt solutions of only 0.1% NaCl and available chlorine levels ranging from 4-200 ppm (Rahman et al., 2011; Shimizu and Hurusawa 1992; Torlak 2014; Wang et al., 2014; Xu et al., 2014). Using EW as a preservative may also be a more inexpensive method for producers/processors to use than current additives. After the initial purchase of a water electrolyzing system, the EW may be generated on-site using only tap water and salt, both of which are very inexpensive ingredients.

Though EW is a relatively new technology for use as a topical antimicrobial, it has undergone some research primarily investigating three antimicrobial mechanisms it employs: pH, free chlorine, and oxidation-reduction potential (ORP). Each of these components, individually, are known to have antimicrobial affects; thus, it can be expected that the combination of the three would at least have additive affects, if not synergistic (Al-haq et al., 2000; Kim et al., 2000; Lee et al., 2014; Li et al., 2014; Park et al., 2004).

The mechanism by which pH affects bacterial cells is the altering of the permeability of the cell membrane, causing the cell's regulation of selective diffusion to become ineffective (McArthur, 2006; Srivastava and Srivastava, 2003). The tertiary and quaternary structure of proteins embedded within the cell membrane begin to degrade and lose polarity as they are exposed to increasing concentrations of hydrogen ions, and this degradation weakens, the integrity of the cell membrane. When the cell membrane integrity weakens excess hydrogen ions can pass into the cell; or the leakage of intercellular constituents into the environment may occur (McArthur 2006; Srivastava and Srivastava 2003). Intercellular proteins may then begin to be denatured by the invading hydrogen atoms and/or the cell could lose vital constituents to the environment rendering it incapable of reproducing (Brock, 1967; Jay et al., 2005; White 2010).

Another important mechanism that is involved in EW's antibacterial property is chlorine concentration. Chlorine has three primarily proposed mechanisms by which it inhibits bacterial propagation. These include the alteration of nucleic acids, the denaturing of cellular enzymes, and the disruption of the cell membrane structure (Fair et al., 1947; Green and Stumpf 1946; Knox et al., 1948; Sconce 1962; Marks and Strandskov 1950; White 2010; Wyss 1962). Chlorine's efficacy, however, is highly effected by pH. When mixed with water, it is most effective between pH = 4 and 6 because the predominant form it takes in that range is hypochlorous acid (HOCl) which has the highest efficacy against bacteria (White, 2010). Because HOCl has no charge and a low molecular weight (52.46 g/mol), it is easy for it to pass into a cell because the cell simply perceives it as water (Sconce 1962; White 2010). Green and Stumpf (1946) and Sconce (1962) note that, upon entering the cell, the weak acid can begin affecting vital enzymes and organelles and denaturing them such that they can no longer replicate. To an extent, low pH increases HOCl content; however, as is passes below 4 and lower, chlorine gas is also generated at the low pH levels; and this has the potential for becoming a health hazard (White, 2010). On the opposite side, pH increases will also cause HOCl decreases and hypochlorite ions ($ClO^-$) become the predominant species in the solution before later converting into sodium hypochlorite (NaClO) (Marriott and Gravani 2006; White 2010; White 2010). The negative effect of increasing pH on the activity (not simply the concentration) of hypochlorite has also been established (Block 2001; Johns 1934; Rideal and Evans 1921). One experiment noting the negative effect of alkalinity on the bactericidal activity of chlorite ions ($ClO^-$) was conducted by

Charlton and Levine in 1934. When they tested *Bacillus metiens* in calcium hypochlorite solutions, the researchers observed that 100 ppm of free chlorine at pH 8.2 would inactivate an approximately equal amount of bacterial cells as 1000 ppm of free chlorine at pH 11.3. Later, in 1941, Rudolf and Levine tested a solution of 25 ppm free chlorine against *B. metiens* spores and noted how much exposure time was required at various pH levels in order to achieve a 2-log reduction in spores. They found the following pH and exposure combinations that eliminated spores: pH 6 = 2.5 minutes, pH 7 = 3.6 minutes, pH 8 = 5 minutes, pH 9 = 19.5 minutes, pH 9.35 = 35.5 minutes, pH 10 = 131 minutes, and pH 12.86 = 465 minutes (Block 2001; Rudolf and Levine, 1941). Again, undissociated hypochlorous acid concentration was thought to be the limiting factor causing reduced sporicidal activity (Block 2001; Rudolf and Levine 1934). Because of the tendency of HClO to dissociate above pH = 6 and the increased production $Cl_2$ gas below pH 4, it is best to keep the pH of hypochlorite solutions between pH 4 and 6 when using them as antimicrobial agents (Marriott and Gravani 2006; White 2010). This will result in the most efficient balance of bactericidal activity and chlorine gas production.

The last antimicrobial mechanism seen in EW is oxidation reduction potential (ORP). ORP refers to the level of oxidizing or reducing strength within a given system (Jay 1996; McPherson, 1993; Robbs et al., 1995; Venkitanarayanan et al., 1999). Negative ORP values denote a reducing environment, and positive values denote an oxidizing environment. In the realm of EW, chlorine is another oxidizing agent that increases ORP values. Most pathogens and viruses will be killed on contact or within a few seconds by solutions with an ORP of over +800 mV due to oxidizing the cell walls, which alters their chemical makeup and physical structure. High ORP can also cause damage to nucleic acids, enzymes, and other proteins if reactive compounds enter the cell as the cell wall becomes more permeable (Jay 1996; McPherson, 1993; Robbs et al., 1995; Venkitanarayanan et al., 1999). This damage prevents bacterial growth. According to Venkitanarayanan (1996) and Jay (1999), aerobic microorganisms have an optimal growth rate in an ORP range of +200 mV to +800 mV, and favor growth in an ORP range between -200 mV and -400 mV.

Though all of the mentioned aspects of EW may contribute to its effectiveness as an antimicrobial, they are not all thought to contribute to bactericidal activity to the same degree. Li et al., (2014) tested the efficacy of AEW ice against *Listeria monocytogenes* and *Vibrio parahaemolyticus* and monitored the contributions of available chlorine concentration, pH, and ORP to the overall reductions seen in the presence of the pathogens. They reported that the most significant contribution to bactericidal activity in AEW ice was due to available chlorine content, followed by pH, and lastly ORP. They also showed that pH remained stable over time but available chlorine content and ORP decreased over time (Li et al., 2014). Because EW contains all three of these factors, the strengths and limitations of each can be used together to provide an antimicrobial treatment that is very versatile as it inherently implements hurdle concepts for microbiological control.

EW has been tested and shown effective in many different mediums including herbs, vegetables, fruits, seeds, seafood, pork, poultry, and in non-food media both alone and in conjunction with other control measures like temperature and added acids (Abadias et al., 2008; Goodburn and Wallace 2013; Graca et al., 2011; Hao et al., 2015; Kim et al., 2000; Kim et al., 2013; Lee et al., 2014; Mansur et al., 2015; Northcutt et al., 2008; Park et al., 2004; Rahman et al., 2011; Torlak 2004; Wang et al., 2014; Xu et al., 2014). On apples, both neutral electrolyzed water (NEW) and acidic electrolyzed water (AEW) have been shown by Graca et al., (2011) to have antimicrobial effects against *L. innocua* with AEW at 100 ppm, free chlorine concentration showing the greatest reduction in counts. The results from the AEW 100 ppm free chlorine samples were not statistically different from samples treated with HOCl diluted to 100 ppm free chlorine. The application of EW to most products can be easily implemented by substituting commonly used tap water, either partially or in total, with AEW. As is noted by Goodburn and Wallace (2013), tests need to be conducted for different products that receive topical AEW treatments to ensure that the acidity and ORP of the AEW would not alter the properties of the products in a negative way. Because of its seemingly widespread efficacy and manufacturing feasibility, the use of electrolyzed water as a topical antimicrobial for fresh produce appears to be very promising.

### 5.) Other Antimicrobial Treatments

DiPersio et al., (2004) researched the use of sodium metabisulfite ($Na_2S_2O_5$) and ascorbic and citric acid solutions for treating peaches prior to dehydration and found that it was effective in reducing *Listeria* populations by 1.5 to 2.0 log CFU/g. Sodium metabisulfite was developed initially as a replacement to trisodium phosphate ($PO_4$), which had negative environmental effects. Sodium metabisulfite decomposes in water, resulting in free $SO_2$ at a theoretical yield of 67.4%. The high levels of free $SO_2$ are more active against bacteria at pH levels below 5, and the sodium metabisulfite solutions used in this study were approximately 4.2, resulting in high antimicrobial activity. Rose and Pilkington (1989) and Barnett (1985) suggested that the boost in antimicrobial activity of sulfites at low pH could be due to un-ionized $SO_2$ which can easily pass through cell membranes and interfere with intercellular metabolic functions within bacterial cells (DiPersio et al., 2004). However, Davidson and Branen (1993) have expressed concern over negative health effects from sulfites when ingested by some individuals who have sulfur sensitivities; and, thus, sulfites should be used cautiously and with proper labeling on food products. Citric acid and ascorbic acid also showed reduction in *Listeria* by approximately 5 logs when assessed on dried peaches; but the drying process alone yielded *Listeria* reductions of approximately 3 $\log_{10}$ CFU/g. This suggests that the acids account for no more than a 2 $\log_{10}$ reduction when using this process of drying then treating with acid (DiPersio et al., 2004). The drying process may have also weakened bacterial cells leaving them more susceptible to acid treatment and facilitating the additional 2 $\log_{10}$ CFU/g reductions produced by post-drying exposure the acids.

Another possible treatment is ozone, either as a gas or by bubbling it in water for liquid application. Ozone in gaseous form was tested on bell peppers by Alwi and Ali (2014). After dipping bell pepper plugs into *Listeria* solution ($10^4$ CFU/ml) for one minute and drying for one hour, the plugs were exposed to 9 ppm of gaseous ozone for six hours, and a 3.06 $\log_{10}$ reduction in *Listeria* was observed (Alwi and Ali 2014). While these numbers are good, it is likely not applicable in large scale operations simply due to the amount of product that would need to be treated. For effective treatment, complete coverage of the ozone over the entire surface of each product must be ensured. This would be a difficulty to large producers.

All of the aforementioned antimicrobial treatments are non-thermal and can be applied at 1 atm of pressure. This is an important regulation concerning the treatment of fresh peaches. Due to the soft nature of the flesh and skin of peaches, treatments must be applied that will not degrade the texture or appearance of the peach (such as thermal or barometric processes). Peaches contain enzymes such as polyphenol oxidase and catechol oxidase that facilitate transformation of phenols aggregated quinones that yield a brown color. Because of this, the cell structure of peaches must not be damaged during treatment or the enzymes will become de-compartmentalized and begin creating a brown color on the fruit's flesh.

### *Conclusion*

Food safety is of extremely high importance to ensure the health and wellness of the people who trust food manufacturers to provide them with wholesome and nutritious foods. In regards to fresh produce, the traditionally used chlorine wash is coming under more and more disapproval amongst consumers who are requesting new, "natural" treatments to remove microorganisms from food. This has produced a need amongst food scientists to face the challenge of creating new food treatment technologies that can achieve equal results as chlorine not only in microbial reduction, but also in cost effectiveness and technical feasibility for manufacturers. Multiple alternative methods are available, including irradiation, ozone treatment, bacterial competition, essential oils, electrolyzed water, and many others. At this point, each of these techniques shows potential, but most of them are not feasible outside of laboratory settings or for use in high-volume processing environments. Currently, electrolyzed water seems to be an outstanding choice for alternative treatment methods for produce. Though it would require an initial investment for a high-volume electrolyzer, the subsequent treatment process requires nothing more than tap water and salt (NaCl or KCl) and can be applied, in the same step, as a substitution for traditional chlorine wash. Though more validation for antimicrobial efficacy should be obtained, current research suggests results may be comparable to traditional chlorine but additional research is needed. Because of the high potential EW shows for the treatment of fresh produce for both quality and safety, the following research was conducted as to provide further documented validation supporting electrolyzed water's use within the food industry. The purpose of this study is to test the efficacy of using acidified electrolyzed water (AEW) as a topical antibacterial agent for use on fresh, whole peaches. This research project involved the following objectives:

1.) Determining the amount of natural microflora found on peaches
2.) Testing the efficacy of AEW, in comparison to chlorine, as a topical antimicrobial for fresh, whole, unwashed peaches.
3.) Testing the efficacy of AEW, in comparison to chlorine, as a topical anti-listeria agent on fresh, whole, unwashed peaches.

The above objectives were evaluated in three experiments.

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# CHAPTER 2

# EFFECT OF ANTIMICROBIAL TREATMENTS ON THE RECOVERY OF MICROORGANISMS FROM THE SURFACES OF FRESH, WHOLE, UNWASHED PEACHES

## Abstract

New technologies are being investigated to maintain the safety and quality of fresh peaches during processing while reducing cost and meeting the demands of some consumers who are adverse to buying foods treated with chlorine. The goal of this study was to determine the amount of natural microflora found on fresh, whole, unwashed peaches and to compare acidified electrolyzed water (AEW) to traditional chlorine as a topical treatment for the reduction or elimination of total aerobic microorganisms on those peaches. This experiment compared peaches receiving no wash (NW), a tap water wash (TW), an acidified electrolyzed water wash (AEW), and a chlorine wash (CL) for effectiveness at removing microorganisms naturally present on peaches right after harvest. Each treatment was applied for two different time exposures (5 seconds or 40 minutes) for comparison. NW peaches received no treatment while tap water washed (TW) peaches were washed with water chilled to 2 ± 2°C. AEW was produced immediately before each application with the following characteristics: 21 ± 0.2 ppm free chlorine, pH ≈ 2.5, ORP ≈ 1100 mV, and a temperature of 2 ± 2°C. CL solution was also prepared immediately before each application to contain 21 ± 0.3 ppm free chlorine with a resulting pH of approximately 8.

Analysis of results showed that the length of time of treatment exposure had a significant effect on the number of aerobic microorganisms recovered from the surfaces of peaches ($P < 0.05$). AEW reduced the counts of microbes recovered from peaches by 0.9 and 1.7 $\log_{10}$ CFU/gram peach after 5 second or 40 minute exposure times, respectively. Peaches

treated with CL had similar microbiological reductions as those treated with AEW as CL was found to reduce counts of aerobic microorganisms recovered from peaches by 2.0 and 0.6 $\log_{10}$ CFU/gram peach after 5 second or 40 minute exposure times, respectively. AEW and CL reduce the numbers or microorganisms naturally found on the surfaces or harvested peaches. Based on these findings, the incorporation of AEW into the hydrocooling step used by peach processors seems feasible from a microbiological safety standpoint.

## Introduction

The sales of peaches accounts for roughly $550 million in revenue generation in the United States each year (USDA-NASS, 2013). Peaches are a seasonal crop, typically marketed from April until early October, but this varies based on location within the United States (USDA-NASS, 2013). The fruit is largely used for commercial canning or selling fresh but, as with all produce, peaches must be minimally-processed for microbiological control prior to selling. Many bacteria are naturally occurring in the environment and are transferred to all types of produce during production, harvest, and transportation to the pack-house (Abadias et al., 2008; Bell and Kyriakides, 2005; Beuchat 1996; Kim et al., 2012; Lee et al., 2014; Natvig et al., 2003). However, numbers of these microorganisms can be reduced during processing and lowered levels can be maintained with proper storage and distribution conditions.

According to the National Agriculture Statistics Survey (2013), nearly $10 million in lost revenue occurs each year from peaches that are defective and unutilized. This inability to use harvested peaches can be attributed to many factors but, when considering usable peaches, spoilage (typically from mold) is the factor that will eventually deem a peach unusable. It is therefore critical to sell or process peaches into products before they begin to spoil and have to be condemned. Because of this, technologies that reduce total aerobic microorganisms found on peaches, thus inhibiting their spoilage, are of great importance to the industry.

The treatment of peaches with chlorinated water has been a commonly used method to prolong the shelf life of the fruits and to ensure safety to consumers (Beuchat 1998; Graca et al., 2011). Typically peaches are treated with low levels of chlorine (between 50 and 200 ppm) which have been shown to reduce bacterial population by 1 to 2 $\log_{10}$ CFU/g (Alegre et al., 2010;

Beuchat 1998; FDA, 2001; Graca et al., 2011). The mechanisms by which chlorine inhibits bacterial cell propagation are still being investigated; however, three strong conjectures have been drawn thus far. The alteration of nucleic acids within the cells, the inactivation of cellular enzymes, and the disruption of the cell membrane are currently the proposed mechanisms by which chlorine is thought to inhibit bacteria (Fair et al., 1947; Green and Stumpf 1946; Knox et al., 1948; Marks and Strandskov 1950; Sconce 1962; White 2010; Wyss 1962). Chlorine is most active in the form of hypochlorous acid (HClO) which is chlorine's predominant form when dissolved in water at pH 4 to 6 (Marriott and Gravani 2006; White 2010). HClO is an oxidizing agent that will disrupt cell membranes and, thus, prevent cell regeneration from occurring as it damages structural proteins and causes the leakage of vital organelles and other cellular components (Marriott and Gravani 2006). Due to its low molecular weight (52.46 g/mol) and the fact that it carries no charge, HClO can also readily migrate into bacterial cells where will begin to disrupt enzymatic processes (White 2010; Sconce 1962). AEW shares the antibacterial benefits of low pH, chlorine content, and high ORP and is thought to be similar to the traditional chlorine that is used (sodium hypochlorite) in its bactericidal efficacy. AEW is produced from salt water (NaCl or KCl) which would makes it a more "natural" preservative for concerned consumers and it could be implemented with comparatively minimal disruptions to large scale processors (Bearth et al., 2014; Browne, 2011; Hansen et al., 2003; Krystallis et al., 2007; Sparks and Shepherd, 1994).

The red skin pigmentation (blush) of peaches is caused from the light regulated development of anthocyanin pigments (Bakowska-Barczak, 2005; Frett, 2012; Layne and Bassi, 2008; Schijlen, 2004). Anthocyanins are glucosides of anthocyanidins (Figure 2) whose resonance structure accounts for the wide array of colors they can reflect (Castañeda-Ovando et al., 2003; De-Xing et al., 2004; Pauling, 1939; Wrolstad et al., 2005).

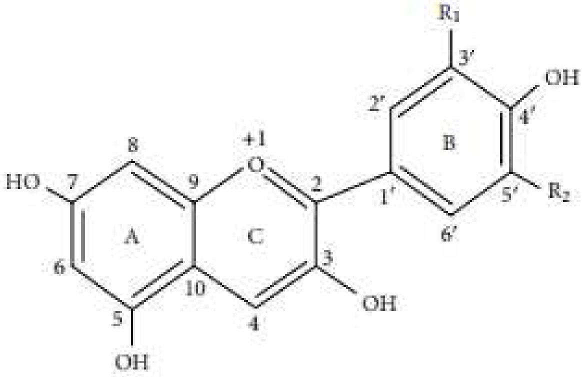

Figure 2: Anthocyanidin structure (De-Xing et al., 2004)

The production of anthocyanins in peach skin is inversely proportional to chlorophyll production which can be seen during peach ripening as the fruits transition from green and begins developing red colors (Layne and Bassi, 2008). This blush development is beneficial for the marketing of fresh, whole peaches because it improves the fruit's visual appeal to consumers (Frett, 2012). A potential problem for the use of AEW on peaches is the instability of anthocyanins to pH. When the pH of the pigment's environment changes, the aromatic rings undergo chemical changes (at various pH levels) that alter the light absorption, of the molecules and thus produce a color change (Bakowska-Barczak, 2005; Gould, et al., 2009; Iqbal and Mido, 2005). This is predominantly due to substitutions to the anthocyanidin base structure and degradation reactions that take place as higher pH increases the degree of hydroxyl substitution on the B-ring (Bakowska-Barczak, 2005; Castaneda-Ovando et al., 2009; Giusti and Wrolstad, 2003). Anthocyanidin structure can take different forms based on pH levels (Figure 3)

(Casteneda-Ovando, 2003; de Costa et al., 1998; Fleschhut et al., 2006; Heredia et al., 1998; Kennedy and Waterhouse, 2000). The purple/red flavylium cation (shown in Figure 3A) is predominant at pH 1, while the blue quinoidal species (Figures 2B-D) is predominant at pH 2 – 4. The colorless carbinol pseudobases (Figure 3E) and chalcones (Figure 3F) are the predominant anthocyanin structures at pH 5 – 6. At pH 7 and higher the pigment with begin to degrade and produce dark arrays that eventually blacken as pH continues to increase (Figure 3 Degradation reaction, Castaneda-Ovando et al., 2009).

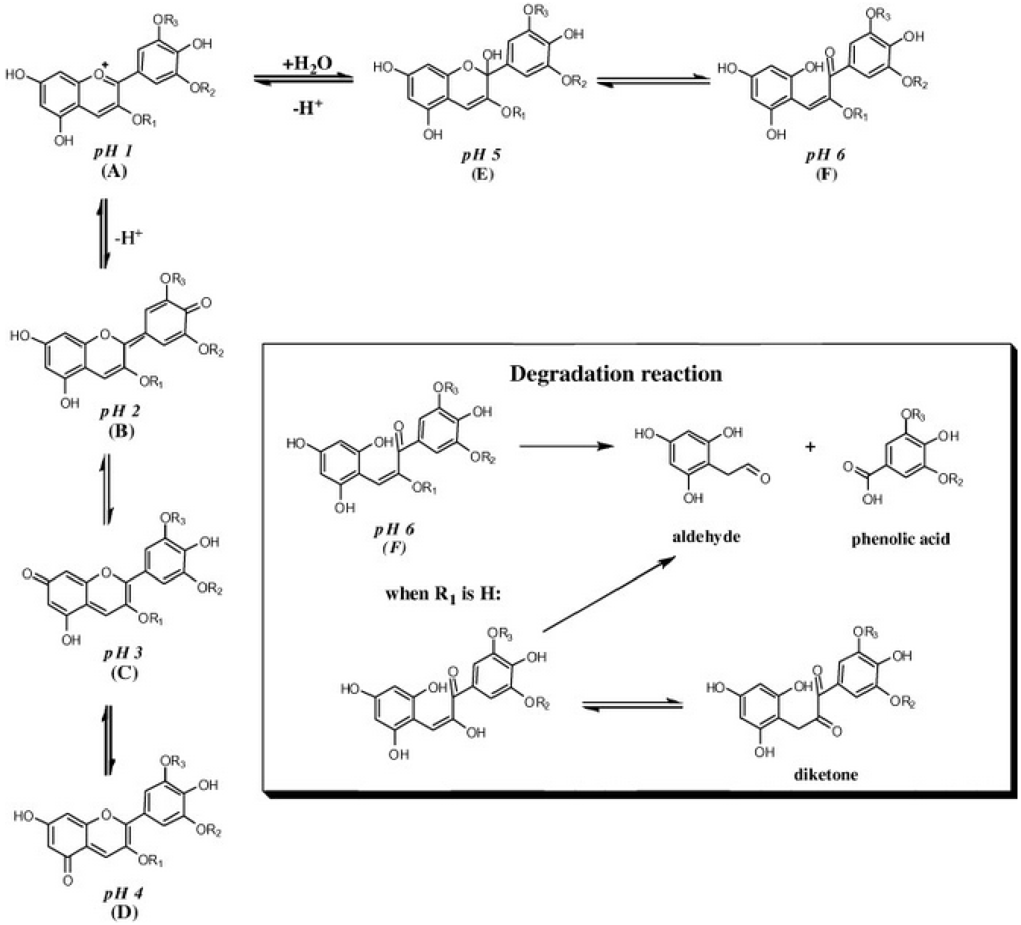
pH 1
(A)
+H2O
-H+
pH 5
(E)
pH 6
(F)
-H+
pH 2
(B)
pH 3
(C)
pH 4
(D)
Degradation reaction
pH 6
(F)
aldehyde
phenolic acid
when R1 is H:
diketone

Figure 3: “Anthocyanins chemical forms depending on pH and degradation reaction for anthocyanins. Where $R_1$ = H or saccharide, $R_2$ and$R_3$ = H or Methyl” (Castañeda-Ovando et al., 2009).

Anthocyanins are also hydrophilic pigments and have a tendency to leach into water if exposed for long periods of time; however, if the cell walls containing them are not damaged then loss of the pigment will not occur (Iqbal and Mido, 2005; Bakowska-Barczak, 2005). Because of the water soluble and pH instable nature of anthocyanins, it is important to consider the effects of AEW on the pigments in peach skin which has not been previously reported.

The goals of this study were two-fold: 1.) to determine the efficacy of AEW to act as a topical antimicrobial for reducing populations of aerobic microorganisms on the surfaces of fresh, whole, unwashed peaches; and 2.) to assess the effects of AEW and chlorine on the surface skin color of fresh, whole, unwashed peaches.

## Materials and Methods

### *Peaches*

Sweet September peaches were obtained from a commercial grower located in South Carolina. All peaches were procured within 24 hours after harvest and before processing (washing). Peaches with preexisting signs of microbial degradation (mold growth) were not used in the experiment.

### *Treatments*

A preliminary experiment was conducted to compare the antimicrobial efficacy of four treatments when applied to peaches at 25°C for less than 5 seconds. The four treatments were:

no wash (NW), tap water wash (TW), acidified electrolyzed water wash (AEW), and chlorinated water wash (CL). NW (control) peaches did not undergo a wash step. TW peaches used were dipped into municipal tap water. AEW peaches were dipped into electrolyzed water that was generated using a Hoshizaki ROX-10WA-E Water Electrolyzer that was set at 10 A and was generated from a 100 g/L NaCl solution at ambient temperature. The chlorine concentration of the AEW was approximately 22 ± 0.2 ppm. CL treatment was made using commercial bleach (8.25% sodium hypochlorite) and was diluted in tap water (previously chilled to 2°C) to be similar to the AEW chlorine concentration (22 ± 0.3 ppm). Chlorine concentration of both the AEW and chlorine (CL) treatments was tested using a portable CHEMetrics CHLORINE 2 SAM test kit with Vacu-vials to analyze *N*,*N* diethyl-*p*-phenylenediamine (DPD) colorimetric reactions.

After conducting the preliminary experiment, another experiment (Experiment 2) was conducted to test the effects of time and temperature on the effectiveness of the treatment solutions. Experiment 2 included additional samples that underwent increased exposure time to the TW, AEW, and CL treatment solutions.

For all treatments in the preliminary experiment and in Experiment 2 (excluding NW), 500 mL of freshly prepared solution was placed into individual 1000 mL beakers and one peach was dipped into one beaker. The use of 500 mL of solution per peach was implemented replicated the maximum amount of water that can be estimated to contact each peach in the hydrocooler used by this study's peach supplier. All 500 mL allocations of the treatment solutions were used to treat only one peach and were then discarded. Four peaches per treatment were tested per replication plus four peaches that were not washed in each experiment. Therefore 16 peaches for Experiment 1 and 28 peaches for Experiment 2 were tested per replication.

After preparing the treatment solutions, peaches were weighed and the skin color of the peaches was measured as C. I. E. L*a*b* color values at four equidistant points around the midsection of each peach. A HunterLab UltraScan PRO spectrocolorimeter was used to measure lightness (*L), redness (+a*), and yellowness (+b*) using the settings for 2° and 10° observer in illuminate C light. Prior to each analysis, the spectrocolorimeter was standardized using a light trap and standard white tile (EVU 000746).

After weighing and measuring skin color, treatments were applied with exposure times as follows: preliminary experiment - 5 seconds; Experiment 2 - 5 seconds and 40 minutes. For the preliminary experiment, the treatment solutions were only tested at 25°C. For the second experiment, only refrigerated treatments (2 ± 2°C) were used. This was done to simulate commercial peach hydrocooling procedures (≈0°C). At the end of the exposure, peaches were removed from the treatments, allowed to drip for 1 minute, and then immediately placed into clean bags (1 gallon) along with 100 mL of sterile 0.1% peptone solution. Peaches and peptone were shaken for approximately 1 minute to recover surface microorganism. NW peaches were also shaken in peptone. The peaches were aseptically removed from the rinsate and placed into pre-numbered weigh boats. The rinsate was used to prepare serial dilutions and these were spread plated on aerobic plate count (APC) agar plates. Plates were inverted and incubated for 48 hours at 37°C. After incubation, visible colony forming units (CFUs) were counted, converted to $\log_{10}$ CFU/g peach, statistically analyzed, and reported as $\log_{10}$ CFU/g peach. Rinsate was also tested for residual chlorine to determine if neutralization was required. No residual chlorine was found using the DPD colorimetric test and therefore no neutralization was used. After the peaches had visibly dried, post-treatment color measurements were taken, using the same procedure as mentioned above.

### Preliminary Experiment

Immediately after harvest, unwashed peaches were transported in corrugated boxes to the laboratory. Peaches were aseptically weighed and surface skin color was measured. Skin color of peaches was determined before and after treatments to determine if the treatments had an effect on skin color. All peaches that were tested were at ambient temperature and all treatments were freshly prepared (as described above) and applied at ambient temperature for less than 5 seconds.

### Experiment #2

Experiment 2 included the same treatments used in the preliminary experiment; however, exposure time varied and treatments were applied at refrigerated temperatures to mimic industry processes. All peaches that were treated were at ambient temperature. All treatments were applied for either < 5 seconds or 40 minutes by dipping peaches into freshly prepared solutions as described above. Color analysis of all peaches was performed in the same manner as is discussed for the preliminary experiment.

*Statistics*

Both the preliminary experiment (N=48) and Experiment 2 (N=84) were performed in triplicate. Data were analyzed using the General Linear Model procedure of SAS to test the main effects of antimicrobial treatment, replication, and time (only in Experiment 2; SAS, 2001). The residual error served as the model error term in the model. Means were separated using least square means with Tukey's mean separation procedures of SAS at a $P < 0.05$ level. Color data across both experiments were pooled for pre- and post-treatment. Pooled values were reported after analyses showed no statistical difference for treatment exposure time ($P > 0.05$).

## Results and Discussion

In the preliminary experiment, the weight of peaches ranged from approximately 297 g to 346 g (Table 1). The pH of CL treatment was not adjusted to optimum (pH 4 – 6) to simulate commercial conditions as most processors would not control for pH of chlorine washing treatments. No significant difference existed between any of the treatments. Unwashed peaches were found to have approximately 4 $\log_{10}$ CFU/g peach of total aerobic microorganisms and this did not change substantially after dipping in TW, AEW, or Cl (Table 1). New time and temperature variables were then incorporated into a second experiment for evaluation.

In Experiment 2, increasing exposure time from 5 seconds to 40 minutes produced significant differences in microbial reductions for the TW, AEW, and Cl treatments when compared to NW peaches as all three treatments removed > 1 $\log_{10}$ CFU/g of aerobic microorganisms from peaches ($P < 0.05$) (Table 3). Previous research has suggested that chlorinated water may primarily function as an antimicrobial by eliminating bacteria in residual wash water and preventing microorganisms from re-attaching to surfaces (Northcutt et al., 2008). This principal has been demonstrated by showing little or no difference in the reduction of *Escherichia coli* O157:H7 counts in lettuce leaves using a chlorinated wash from 20 ppm to 200 ppm versus non-chlorinated treatments or deionized water (Adams et al., 1989; Beuchat, 1999; FDA, 2001, Li et al., 2001). Even so, it has been reported that at least one minute of exposure to chlorine is necessary for fresh produce to achieve a 1 to 2 $\log_{10}$ CFU/g reduction in bacteria when being used with produce (Beuchat, 1998; FDA, 2001; Graca et al., 2011). These data demonstrated that either additional time or agitation would be required to produce significant reduction in the microorganisms on peach surfaces. Because industry practice often incorporates a 40 minute hydrocooling step utilizing approximately 0°C water, Experiment 2 was conducted to reflect industry conditions.

Studies have been conducted utilizing various exposure times between 2 and 5 minutes and achieving approximately 2 $\log_{10}$ CFU/g reductions or aerobic bacteria; however little research has been conducted using a 40 minute exposure time even though this is a washing time period common for manufacturers (Hao et al., 2015). The results of Experiment 2 agree with those reported by Graca et al. (2011) and Beuchat (1998) who state the necessity of at least 1-2 minutes exposure time for chlorine to be effective against microorganisms (Table 3). Rahman et al. (2010) noted an approximate 2 $\log_{10}$ CFU/g reduction in total bacteria and Hao et al. (2015) noted an approximate 3 $\log_{10}$ CFU/g reduction of *Escherichi coli* when using AEW on spinach and cilantro, respectively. Chlorine concentration in those studies was higher than that used in this study (Rahman et al. (2010) used 50 ppm free chlorine and Hao et al. (2015) used 68 ppm free chlorine). When considered together, Experiment 2 along with the work by Rahman et al. (2010) and Hao et al. (2015) validate the efficacy of AEW at multiple chlorine levels and exposure times. The aforementioned studies report similar bacterial reductions from 22 ppm chlorine up to 68 ppm chlorine and with exposure times from 5 seconds to five minutes (Rahman e al., 2010 and Hao et al., 2015). Rahman et al. (2010) also noted that increasing exposure time from 30 seconds to 7 minutes further reduced bacterial counts by 1 $\log_{10}$ CFU/g using AEW. Their data are in agreement with the 0.8-$\log_{10}$ CFU/g reductions found in this study as exposure time increase from 5 seconds to 40 minutes.

AEW-0 and AEW-40 did not show significant differences in $\log_{10}$ CFU/g peach, but CL-0 and CL-40 were significantly different. Chlorine is one of the mechanisms in AEW that reduces microbial populations and its effectiveness was shown to be time dependent suggesting that is it not the primary mechanism for antimicrobial activity in AEW which was not shown to be time dependent. Therefore, pH or ORP are thought to be the primary antimicrobial mechanism in AEW in the ≈ 22 ppm chlorine concentration used for this experiment. This could either be a direct effect of oxidation and excess hydrogen ion concentration on bacterial cells or it could pertain to the low pH of AEW maintaining higher levels of hypochlorous acid than that seen in the CL samples. This contrasts Rahman et al. (2010) who reported significant differences in total bacteria concentrations between samples subjected to AEW exposures of less than 1 minutes and samples subjected to AEW exposures over 3 minutes. The differences in AEW's effectiveness over time may be due to minor chlorinated species that are generated during AEW generation. These species may include hydrochloric acid (HCL), chlorine ($Cl_2$), hypochlorite ions ($ClO^-$), and trichloride ions ($Cl_3^-$) and are all measured in DPD colorimetric analysis. The presence of these minor species (though most likely very low) may contribute to overall effectiveness of

AEW and, since their concentrations are unknown, could have contributed to the differences in the present work and the work conducted by Rahman et al. (2010) (White, 2010). However, when compared to unwashed peaches, AEW-40 and CL-40 showed reductions in aerobic microorganisms of 1.7 $\log_{10}$ CFU/g peach and 2.0 $\log_{10}$ CFU/g peach, respectively, which indicates that they are both sufficient antimicrobials for use on food products. It should also be noted that all CL samples were approximately at pH 8 which is not within chlorine's maximum bactericidal range of 4 to 6 (Marriott and Gravani 2006; White 2010). Nevertheless, the CL solutions (60% hypochlorite ions at pH 8 according to White (2010)) produced a 2.0 $\log_{10}$ CFU/g peach reduction. Additionally, the control sample (TW-40) also reduced total aerobic bacterial counts by 1.1 $\log_{10}$ CFU/g peach, implying that the process of washing peaches alone can achieve large reductions in surface bacteria. This denotes the importance of AEW's aqueous nature on its bactericidal efficiency.

Temperature of treatment solutions was compared from peaches in the preliminary experiment (ambient with 5 second exposure time) and Experiment 2 (refrigerated with 5 second exposure time) and no statistical difference was found on this basis ($P < 0.05$). However, there is a numerical trend that suggests temperature has some of an effect since bacterial reductions from the preliminary experiment (Table 1) are consistently lower than those the 5 second samples in Experiment 2 (Table 2).

Three things can be derived from the results of this work. First, when taking into account the noted dependency of chlorine on exposure time and AEW's efficacy being independent of exposure time, it is suggested that either pH or ORP are the primary reasons for AEW's efficiency in solutions containing < 25 ppm of free chlorine. This could be due to direct inhibition or it could be indirect as pH maintains an environment that allows chlorine to perform at its maximum antimicrobial capacity in the form of HOC. Secondly, because AEW-40 and CL-40 showed no difference in bactericidal activity, it is thought that AEW (pH ≈ 2.5 and ORP ≈ +1100 mV) and traditional chlorine treatments (with equal free chlorine concentrations < 25 ppm) result in equal amounts of reduction in aerobic microorganisms on fresh, whole peaches. Lastly, the confirmation that AEW-40 and CL-40 both reduce aerobic microorganisms by 1 to 2 logs demonstrates that the amount of reduction in viable aerobic microorganisms achieved by AEW-40 and/or CL-40 is sufficient for use on fresh, whole peaches. In short, though the mechanisms used by AEW (pH ≈ 2.5, ORP ≈ +1100 mV, and [Cl] ≈ 22 ppm) for bacterial inactivation may be different than those used by traditional sodium hypochlorite, AEW and NaHClO are equal in antimicrobial propensity, and they are both sufficient for use on fresh, whole peaches.

Table 3 shows the colorimetric measurements (L*, a*, b*) for peaches before and after the washing treatments. Statistical analyses revealed no differences due to exposure time to treatment and these data were pooled and reported together. Measurements were performed to determine the effect of using an acidic treatment (AEW) on the color of the anthocyanin pigments in the peach skins. Iqbal and Mido (2005) state the even though anthocyanins are hydrophilic and prone to leaching into aqueous solutions, as long as the cell containing the pigments remain intact no leaching should occur. In this case, excluding small lacerations or mechanical damage to peach skins that would expose cellular constituents to the environment, it appears from the data that anthocyanins in the peach skins had minimal contact with the wash treatments and thus were not be chemically effected by the pH levels of the treatments nor would the overall concentration of the pigment be altered. After analyzing peaches both before and after treatment, no statistical difference ($P < 0.05$) between the pre- and post-treatment peaches in any of the test groups (NW, TW, AEW, and CL). On average, peaches were found to have lightness of approximately 55, redness of approximately 20, and yellowness of approximately 35. Although not significant, the greatest variation among peach color was observed with redness. Results from the colorimetric data can be seen in Table 3.

Results from the present study demonstrate that washing peaches may remove nearly 1 $\log_{10}$ CFU/g of the total aerobic microorganisms on the surfaces of peaches and reductions may be increased by incorporating antimicrobial factors into the wash water such as the of chlorine and/or acid. Results also demonstrate that AEW may be an acceptable alternative to chlorine with comparable biocidal effects without compromising the color of the peaches.

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Table 1: Chemical characteristics[1] of treatment solutions and their effects on numbers of total aerobic microorganisms[1] recovered from peaches after 5 second treatment exposure time.

| **Treatment** | **Peach Weight (g)** | **pH** | **Oxidation Reduction Potential (ORP)** | **[Cl] (ppm)** | **Log$_{10}$ CFU/g** | **Reduction (log$_{10}$ CFU/g)** |
|---|---|---|---|---|---|---|
| No Wash (NW) | 346.45 ± 13.50 | N/A | N/A | N/A | 3.95 ± 0.10 | N/A |
| Tap Water Wash (TW) | 297.29 ± 15.03 | 6.64 ± 0.00 | 390.00 ± 0.00 | 0.00 ± 0.00 | 3.94 ± 0.35 | 0.01 |
| Acidified Electrolyzed Water Wash (AEW) | 325.62 ± 15.21 | 2.77 ± 0.00 | 1118.00 ± 0.00 | 22.2 ± 0.0 | 3.79 ± 0.12 | 0.16 |
| Chlorine Wash (CL) | 340.6 ± 13.0 | 8.2 ± 0.0 | 753.3 ± 0.0 | 21.1 ± 0.0 | 3.7 ± 0.2 | 0.2 |

[1] Means ± standard error of means

[2] N=12

Table 2: Chemical characteristics of treatment solutions and their effects on numbers of total aerobic microorganisms recovered from peaches after 5 second and 40 minutes treatment exposure times at 2°C[1].

| **Treatment** | **Exposure Time (min.)** | **Peach Weight (g)** | **pH** | **Oxidation Reduction Potential (ORP)** | **[Cl] (ppm)** | **Log$_{10}$ CFU/g** | **Reduction (log$_{10}$ CFU/g)** |
|---|---|---|---|---|---|---|---|
| NW | N/A | 335.14 ± 14.37 | N/A | N/A | N/A | $4.30^{a}$ ± 0.23 | N/A |
| TW | 0 | 336.32 ± 18.78 | 6.96 ± 0.05 | 526.33 ± 34.87 | 0.03 ± 0.01 | $3.47^{abc}$ ± 0.17 | 0.83 |
| AEW | 0 | 343.43 ± 12.76 | 2.87 ± 0.01 | 1126.33 ± 8.04 | 21.87 ± 0.24 | $3.41^{abc}$ ± 0.19 | 0.89 |
| CL | 0 | 333.89 ± 13.46 | 8.39 ± 0.02 | 744.33 ± 12.38 | 21.47 ± 0.31 | $3.67^{ab}$ ± 0.31 | 0.63 |
| TW | 40 | 304.12 ± 11.97 | 6.96 ± 0.05 | 526.33 ± 34.87 | 0.01 ± 0.1 | $3.20^{bcd}$ ± 0.20 | 1.10 |
| AEW | 40 | 320.54 ± 18.08 | 2.89 ± 0.02 | 1126.33 ± 8.04 | 21.87 ± 0.24 | $2.57^{cd}$ ± 0.32 | 1.73 |
| CL | 40 | 335.63 ± 19.52 | 8.39 ± 0.02 | 744.33 ± 12.38 | 21.47 ± 0.31 | $2.34^{d}$ ± 0.36 | 1.96 |

[a-d] Means ± standard error of means with differing superscripts are significantly different ($P < 0.05$).

[1] N=12

Table 3: Pre- and post-treatment colorimetric data. All numbers are averages including measurements from every peach tested within each treatment. No statistical difference was seen in color for any individual peach in any of the treatment groups.[1, 2, 3]

| | **NW** | | | **TW** | | | **AEW** | | | **Cl** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **L*** | **a*** | **b*** | **L*** | **a*** | **b*** | **L*** | **a*** | **b*** | **L*** | **a*** | **b*** |
| **Initial** | 55.9 ± 1.1 | 18.9 ± 0.6 | 35.2 ± 1.3 | 55.8 ± 1.3 | 20.0 ± 0.9 | 35.7 ± 1.4 | 55.4 ± 1.2 | 20.9 ± 0.8 | 35.2 ± 1.4 | 56.4 ± 1.2 | 19.6 ± 0.8 | 35.7 ± 1.3 |
| **Post** | 55.5 ± 1.0 | 20.1 ± 0.6 | 35.5 ± 1.2 | 55.7 ± 1.3 | 21.8 ± 1.0 | 37.8 ± 1.6 | 55.1 ± 1.2 | 22.3 ± 0.8 | 35.1 ± 1.44 | 57.3 ± 1.3 | 19.3 ± 0.8 | 37.5 ± 1.5 |
| **Difference** | 0.5 ± 1.4 | -1.23 ± 0.8 | -0.4 ± 1.6 | 0.1 ± 1.9 | -1.8 ± 1.2 | -2.1 ± 2 | 0.4 ± 1.3 | -1.4 ± 0.9 | 0.1 ± 1.5 | -0.9 ± 1.4 | 0.3 ± 0.8 | -1.8 ± 1.6 |

[1] Means ± standard error of means

[2] N=132

[3] L* indicates lightness with "0" being black and "100" diffuse white; a* indicates green/red where negative values denote green and positive values denote red; b* indicates blue/yellow where negative values denote blue and positive values denote yellow.

# CHAPTER 3

# ACIDIFIED ELECTROLYZED WATER AS A TOPICAL ANTIMICROBIAL AGAINST *LISTERIA INNOCUA* ON FRESH, WHOLE, UNWASHED PEACHES

## Abstract

Pathogenic contamination is of great concern to the produce industry, regulators, and consumers. Organisms like *Escherichia coli* O157:H7, *Salmonella spp., Listeria monocytogenes*, and many others are common sources of foodborne illness outbreaks associated with fresh produce. Because *Listeria* is a psychrotrophic bacteria, it is well suited for cold, dark storage areas common in many packing sheds or distributors. This makes it increasingly important that fruit sanitization steps are effective in inactivating pathogens.

The goal of this study was to determine the efficacy of acidified electrolyzed water (AEW) on fresh, unwashed peaches inoculated with *Listeria innocua* and held for 24 hours before treatment. Peaches received 0.1 mL of *Listeria* inoculum containing $10^9$ cells/mL and after inoculation peaches were held overnight at 34°C. The AEW used for this experiment was prepared to contain 21.8 ± 0.3 ppm free chlorine, at pH ≈ 2.8, and was applied at a temperature of 2 ± 2°C. Unwashed and inoculated peaches were divided into the following treatment groups: no wash (NW), a tap water wash (TW), an acidified electrolyzed water wash (AEW), and a chlorine wash (CL) containing 20.9 ± 0.1 ppm free chlorine. Each treatment was performed with two exposure times for comparison - 5 seconds or 40 minutes. In both AEW and CL samples, a significant difference was observed in numbers of *Listeria* recovered from peaches washed for 40 minutes versus peaches washed for 5 seconds. When exposure time for AEW was increased from 5 seconds to 40 minutes, numbers of *Listeria* removed from peaches decreased 2.2 $\log_{10}$ CFU/g of peach. Similarly, when the exposure time for CL was increased from 5 seconds to 40 minutes,

peaches achieved $1.6_{10}$ log CFU/g greater reduction in numbers of *Listeria* recovered. Both the 40 minute CL and the 40 minute AEW samples reduced *Listeria innocua* by over 3 $\log_{10}$ CFU/g when compared to the total *Listeria* counts recovered from the NW samples which contained 4.9 $\log_{10}$ CFU/g peach.

Results from the present study demonstrate that washing peaches will remove *Listeria* from peaches (0.4 $\log_{10}$ CFU/g) and that greater removal of *Listeria* occurs with longer washing times and the use of AEW of CL, which have similar results (3.5 and 3.02 $\log_{10}$ CFU/g reduction).

## Introduction

*Listeria* is a gram (+) pathogen that is psychrotrophic, halotolerant up to sodium chloride (NaCl) concentrations of 25%, and is a facultative anaerobe (Bell and Kyriakides 2005). These properties make *Listeria* a good competitor in storage environments commonly used for fresh produce. *Listeria*'s ability to grow under chilled conditions and in low oxygen environments means that is can migrate into small crevices in fruits and vegetables and propagate where it is very difficult for sanitizers to reach. It is also capable of surviving at a wide pH range (pH 3.0 to 12.0) (Liu et al., 2005). All of these things combined make *Listeria* an increasingly difficult pathogen to eliminate in a food processing environment.

Though it is less prevalent in produce outbreaks than other pathogens like *Escherichia coli* and *Salmonella*, *Listeria* is still known to occur on fresh produce and causes an estimated 123 cases of foodborne illness in the United States each year (Crim et al., 2014). Furthermore, it has the third highest human fatality rate among all pathogens which has been estimated at approximately 20% (Bell and Kyriakides 2005; Bennion et al., 2008; CDC 2011; Hoelzer et al., 2014; Omac et al., 2015; Sant'Ana et al., 2012; Scallan et al., 2011a; Scallan et al., 2011b; Todd and Notermans 2011). In 2014, Wawona Packing Company in Cutler, California issued a voluntary recall of several peach lots spanning from June 1st to July 17th due to the possibility of *Listeria* contamination. Though Listeria is not commonly associated with peaches, this recall shows the importance of focusing on preventative action when considering foodborne pathogens that affect peaches and other fresh produce. Peaches are not commonly associated with *Listeria* because of their naturally low pH but, given the recent recall, more emphasis is being placed on preventative action regarding the pathogen. *Listeria* has been shown to survive in high acid environments with pH levels as low as 3.0, especially when refrigerated which gives it a competitive edge over mesophilic bacteria (Gahan et al., 1996; Liu et al., 2005; Ryser and Marth, 2007). When refrigerated, *Listeria* will upregulate genes for cold-adaptive response and will also increase production of $\sigma^B$ protein which enhances the resistance of *Listeria* spp. against acid and several other factors (Becker et al., 1998; Ferreira et al., 2001; Liu et al., 2002; Ryser and Marth, 2007). .

Batz (2013) and Scharff (2010) suggest that, when considering direct and indirect monetary costs, more than $51 billion dollars are lost due to foodborne illnesses each year in the United States and that 85% to 90% of that is attributed to only five major pathogens; one of which is *Listeria*. Clearly *Listeria* is of high importance from a food safety standpoint and must be controlled for during processing. The goal of this study was to determine the efficacy of acidified electrolyzed water (AEW) against *Listeria innocua* on fresh, whole, unwashed peaches in comparison to the currently used chlorine wash.

## Materials and Methods

Sweet September peaches were obtained from a commercial grower located in South Carolina. All peaches were procured immediately after harvest but before processing (washing). Peaches with preexisting signs of microbial degradation, mold growth, or decay were removed from the experiment. Immediately after harvest, peaches were placed into corrugated boxes and were transported to the laboratory for testing. Upon arrival at the laboratory, peaches were weighed and inoculated with 0.1 mL of *Listeria innocua* inoculum containing $10^9$ cells per mL.

### *Inoculum Preparation*

Non-pathogenic *Listeria innocua* was chosen for this study because of its genetic similarity to *Listeria monocytogenes*. *Listeria innocua* cultures were grown on PALCAM agar with PALCAM *Listeria* selective supplement. After incubation for two days at 37°C, colonies were removed from plates using a sterile loop and were vortexed in 0.1% sterile peptone. Absorbance at 495 nm was used to estimate numbers of *Listeria* in the inoculum (Thermo Scientific Genesys 10S UV-Vis spectrophotometer). The inoculated peptone was then plated on PALCAM with supplement for CFU/mL confirmation. All inoculum used in this experiment contained initial concentrations of 9 ± 0.2 $\log_{10}$ CFU/mL of *Listeria innocua*. After inoculation and 24 hour storage, peaches were randomly divided into the following treatments: NW, TW, AEW, and CL. All treatments except NW were applied for 5 seconds or 40 minutes using solutions chilled to 2°C. For each of the treatments (excluding NW), 500 mL were placed into individual 1000 mL beakers into which the peaches were dipped. Only one peach was dipped into one beaker and treatment solutions were then discarded.

### *Inoculation of Peaches*

All peaches were inoculated with 0.1 mL of *Listeria* inoculum containing $10^9$ cells/mL . Peaches were inverted and inoculum was pipetted onto the bottom surface of each peach. The inoculum was spread across the bottom surface of the peach using a sterile loop before allowing the peach to rest until visibly dry. Peaches were then packed into a foam cooler and place under refrigeration (2°C) overnight for bacterial attachment as well as to mimic potential overnight storage prior to a wash step that could be required at farms during harvesting.

### *Treatment Application*

Peaches were exposed to four treatments in order to determine the comparative antimicrobial efficacy of each treatment. NW (control) peaches did not undergo a wash step. TW peaches used unaltered tap water as the wash for the treatment. AEW peaches used electrolyzed water that was generated using a Hoshizaki ROX-10WA-E Water Electrolyzer that was set at 10A and was drawing from a 100g/L NaCl solution at ambient temperature. The chlorine concentration of the AEW was approximately 20.9 ± 0.1 ppm and the chlorine concentration of the AEW was approximately 22 ± 0.3 ppm. CL treatment was made using commercial bleach (8.25% sodium hypochlorite) and was diluted in tap water to be similar to the AEW chlorine concentration (22 ± 0.3 ppm). Chlorine concentrations were tested on each batch of AEW or CL using a portable CHEMetrics CHLORINE 2 SAM test kit with Vacu-vials to analyze *N*,*N* diethyl-*p*-phenylenediamine (DPD) colorimetric reactions.

The treatments applied in this experiment were denoted as follows: NW, TW-0, TW-40, AEW-0, AEW-40, CL-0, and CL-40. The numbers following the abbreviations represent the exposure time (minutes) that peaches were in contact with the treatments where 0 minutes = denotes a 5 second exposure.

Aside from NW, each treatment was applied by dipping individual peaches into 500 mL of treatment solution in 1-liter beakers with the following exposure times: 5 seconds and 40 minutes. Each of the treatments used were refrigerated (2 ± 2°C). All 500 mL allocations of the treatment solutions were used to treat only one peach and were then discarded. Four peaches per treatment were tested per replication (N=28).

After dripping, peaches were removed from the treatment solutions; "0" time samples were immediately placed into clean 1 gallon bags along with 100 mL of sterile 0.1% peptone solution and shaken for 1 minute. The peaches were allowed to drip for 1 minute prior to rinsing in peptone. After rinsing, peaches were retained and residual peptone was diluted and plated on PALCAM agar plates with selective nutrient supplement (0.1mL residual peptone/plate). Plates were inverted and incubated for 48 hours at 37°F at which point visible colony forming units (CFUs) were counted. Numbers of bacteria were converted to $\log_{10}$ CFU/g peach and reported.

### *Statistics*

The experiment was replicated three times with four peaches per treatment group per replication (N=12/treatment). Data were analyzed using the General Linear Model procedure of SAS to test the main effects of antimicrobial treatment, replication, and time. The residual error served as the error term in the model. Means were separated using least square means with Tukey's mean separation procedures of SAS at a $P < 0.05$ level.

## **Results/Discussion**

The ability of *Listeria* to implement a cold shock response and continue propagating in cold conditions presents potential safety concerns for processors of fresh produce, especially if they store their products in chilled environments (Becker et al., 1998; Ryser and Marth, 2007). Because *Listeria* is also know to become more acid tolerant as it is exposed to low temperatures

(≈4°C), it was important to evaluate the efficacy of chilled AEW against this pathogen (Becker et al., 1998; Fereira et al., 2001; Ryser and Marth, 2007). Table 5 shows that AEW is effective against *Listeria* even at refrigerated temperatures. AEW and CL exhibit the same bactericidal effect against *Listeria* with a 1.2 and 1.6 $\log_{10}$ CFU/g reduction after 5 seconds as compared to number recovered from NW. No statistical difference was found in *Listeria* reduction between AEW-0 and Cl-0 treatments. There was also no statistical difference in reduction of *Listeria* in AEW-40 and Cl-40 treatments. After 40 minutes of exposure time, AEW and Cl treatments reduced *Listeria* by approximately 3.5 $\log_{10}$ CFU/g and 3.2 $\log_{10}$ CFU/g, respectively, when compared to NW samples. AEW-40 and CL-40 also produced significantly higher reductions in *Listeria* when compared to control samples (TW-40) which showed 0.2 $\log_{10}$ CFU/g reductions. These results are in agreement with Rahman et al. (2010) and Park et al. (2009) who showed AEW to reduced *Listeria* populations on spinach and green onion/tomatoes, respectively. Rahman et al. (2010) observed an approximate 2.7 $\log_{10}$ CFU/g reduction in *Listeria* (7 minute exposure time) while Park et al. (2009) observed an approximate 2 $\log_{10}$ CFU/g reduction (5 minute exposure time). Another study by Graca et al. (2011) showed over 1 $\log_{10}$ CFU/g reductions in *Listeria innocua* after treating apple slices with AEW (pH≈3, ORP≈1111, [Cl]≈53 ppm) for 5 minutes. In this experiment, exposure time was shown to be a significant variable in reductions in *Listeria* with both AEW-40 and CL-40 treatments, as compared to reductions of 1.2 $\log_{10}$ CFU/g and 1.6 $\log_{10}$ CFU/g in AEW-0 and CL-0, respectively. This supports the conclusion of Kim et al. (2000), who tested 30 second and 60 second exposures, that exposure time does affect the antimicrobial capacity of electrolyzed water.

From a commercial standpoint, results suggest that the implementation of AEW in place of traditional chlorine washes would pose no threat in terms of food safety and that the integration of AEW into the hydrocooling step, for peaches, or similar wash steps for other fresh produce seems feasible. The high pH (8) of the CL samples did not hinder the solution's effectiveness against *Listeria* even though Marriott and Gravani (2006) and White (2010) have reported chlorine's antibacterial capacity decreasing above 6. At pH 8, only 40% of free chlorine is in the form of hypochlorous acid, it's most bactericidal form. In this experiment, the presence of approximately 60% hypochlorite ions in the CL samples was still shown to be effective against *Listeria* with 40 minutes of exposure. Arevalos-Sanchez (2012) also tested electrolyzed water with 65 ppm chlorine at a neutral pH with exposure times of 5, 10, 20, and 45 minutes at 20°C and 37°C which yielded *Listeria* monocytogenes biofilm populations undetectable in most cases. Mansur et al. (2015), found that when used on fresh pork, AEW (pH≈2.3, ORP≈1159, [Cl]≈30 ppm) reduced *Listeria* by < 1 $\log_{10}$ CFU/g when applied at 25°C for 3 minutes and by approximately 1.5 $\log_{10}$ CFU/g when applied at 40°C. The low efficacy at 25°C is in contrast to what is suggested in this study; however, the difference in using a protein based media for testing or a higher surface moisture could be factors contributing to the discrepancy.

These findings were consistent with the results found by Hopkins (2015) who found low reductions (< 1 $\log_{10}$ CFU/g) in total aerobic microorganisms in chlorine solutions (pH 8) when samples were exposed to treatments for 5 seconds. This confirms the necessity of at least 1 – 2 minute exposure time to aqueous chlorine treatments for the surface decontamination of produce as is stated by Beuchat (1998) and Graca et al. (2011). Hopkins (2015) also reported no statistical difference ($P < 0.05$) in AEW and CL treatments in the reductions of aerobic microorganisms and is in agreement with the results found in the present study, pertaining to *Listeria* reduction, which showed no difference in AEW and CL treatments ($P < 0.05$).

Further research should be conducted to determine the threshold of bactericidal activity of AEW against *Listeria* in terms of exposure time. However, due to the fact that current bulk hydrocooling processes typically operate for 40 minutes and have been shown to take approximately 35 minutes to reduce a 3-inch peach from 32.2°C to 4.4°C (when using 1.7°C water), the enhanced efficacy of AEW with extended time can be utilized without adding more time in processing (Bennett, 1965). This study shows the viability of using acidified electrolyzed water (AEW) as an anti-*Listeria* treatment for the topical treatment of fresh, whole peaches. AEW proved to reduce equivalent amounts of *Listeria innocua* as compared to a chlorine wash (Cl) made from sodium hypochlorite solution. The elimination on 99.9% of *Listeria* by the treatments proves them both to be acceptable for use from a safety standpoint. These results show that AEW wash has strong potential as a “natural” anti-Listerial substitute for chlorine in the produce industry.

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Table 4: Effect of No Wash (NW), Tap Water Wash (TW), Chlorine Wash (CL), and Acidified Electrolyzed Water Wash (AEW) with five second and 40 minute exposure times on the *Listeria* populations on fresh, whole peaches inoculated with *Listeria innocua*.[1, 2]

| **Treatment** | **Exposure Time (min.)** | **Peach Weight (g)** | **pH** | **Oxidation Reduction Potential (ORP)** | **[Cl] (ppm)** | **Log CFU/g** | **Reduction (log CFU/g)** |
|---|---|---|---|---|---|---|---|
| No Wash (NW) | N/A | 246.75 ± 16.00 | N/A | N/A | N/A | $4.90^{a}$ ± 0.12 | 0.0 |
| Tap Water (TW) | 0 | 215.23 ± 9.35 | 6.39 ± 0.11 | 591.33 ± 30.77 | 0.00 ± 0.00 | $4.25^{ab}$ ± 0.12 | 0.65 |
| Acidified Electrolyzed Water (AEW) | 0 | 238.43 ± 19.07 | 2.83 ± 0.02 | 1159.67 ± 3.63 | 21.77 ± 0.27 | $3.68^{bc}$ ± 0.18 | 1.22 |
| Chlorine (CL) | 0 | 221.43 ± 15.08 | 8.18 ± 0.03 | 773.33 ± 8.18 | 20.87 ± 0.14 | $3.34^{c}$ ± 0.12 | 1.56 |
| Tap Water (TW) | 40 | 250.98 ± 12.56 | 6.39 ± 0.11 | 591.33 ± 30.77 | 0.00 ± 0.00 | $4.70^{a}$ ± 0.10 | 0.20 |
| Acidified Electrolyzed Water (AEW) | 40 | 222.75 ± 7.52 | 2.83 ± 0.02 | 1159.67 ± 3.63 | 21.77 ± 0.27 | $1.45^{d}$ ± 0.42 | 3.45 |
| Chlorine Wash (CL) | 40 | 216.90 ± 9.81 | 8.18 ± 0.03 | 773.33 ± 8.19 | 20.87 ± 0.14 | $1.73^{d}$ ± 0.28 | 3.17 |

$^{a-d}$ represent statistical differences within a column ($P < 0.05$)

[1] Means ± standard error of means

[2] N=12

# CONCLUSION

Acidified electrolyzed water (AEW) is an antibacterial agent effective against a wide spectrum of pathogenic and non-pathogenic bacteria. The results of these studies confirm that AEW can be used for the antibacterial treatment of fresh, minimally processed peaches replacing of chlorine solutions (sodium hypochlorite) that are commonly used at present. When tested for biocidal activity against aerobic microorganisms, AEW was found to be as effective as chlorine treatment and producing up to 1.7 $\log_{10}$CFU/g reductions. Exposure time of the AEW was considered and was shown to produce a significant difference in bacterial reductions. When implementing the use of AEW, it is suggested that exposure time be over one minute; however, a maximum exposure time has yet to be determined. Because the hydrocooling step is the proposed critical control point for the application of AEW, the exposure time tested was 40 minutes to mimic the hydrocooling step commonly used in industry by peach processors.

The results against *Listeria innocua* on inoculated peaches at 40 minutes of exposure was 3.5 $\log_{10}$ CFU/g peach. Compared to total aerobic microorganisms, AEW's effectiveness was no different than the effectiveness of chlorine samples tested in the same way at the same free chlorine concentration. The results of this work suggest that not only is AEW equivalent in antibacterial activity to sodium hypochlorite solutions (which are currently used) but it is also effective enough to ensure food safety by eliminating over 95% of native bacteria and over 99.9% of *Listeria innocua* on inoculated peaches.

Further research should be conducted to determine AEW exposure times yielding maximum bacterial reductions for use in applications that do not have an inherent 40 minute exposure time due to processing requirements. For applications using acidic electrolyzed water, uses for the basic electrolyzed water should also been investigated such as utilizing it for a peeling step for some fruits. If acidic and basic streams are recombined to reach a neutral pH, it is possible that the neutral electrolyzed water could be used in the water supply for livestock to prevent microbiological contamination.  Also, because *Listeria* is more susceptible to many antimicrobial treatments at ambient temperatures than it is at refrigerated temperatures and it is weakened by heat-cool cycling, a warm-cool hydrocooling study using AEW could be of benefit.