Investigating methods of conditioning fresh vegetables in retail establishments and exploring procedural modifications that improve product quality and safety

Thesis

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By

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Abstract

The safety and quality of fresh produce sold at grocery stores is a point of competition between many grocery store chains. Companies that can offer the most fresh, safest and least-expensive fresh produce are well-equipped to increase their customer base. Grocery stores may use antimicrobial agents to improve the quality of fresh produce and one of these agents is electrolyzed water. Solutions of antimicrobial agents may be used during washing or misting of fresh produce while it is on the produce rack shelves. Washing the produce with electrolyzed water is also referred to as conditioning and is typically done daily while the produce is held on the produce rack. Misting is performed periodically with diluted electrolyzed water sprayed onto the produce on the produce rack. This research first analyzed the effectiveness of using conditioning and misting during simulated produce rack storage. The effectiveness of using electrolyzed water as the conditioning agent versus tap water, along with the impact of trimming the stem of the produce during conditioning was then analyzed. Results were collected for both sensory scores for crispness and important microbiological counts.

Produce from a nationwide grocery store's regional distribution center was shipped to Ohio State University and subjected to simulated grocery store procedures. Asparagus, red leaf lettuce and romaine lettuce were the produce chosen for testing. The produce was stored for 72 hours simulating grocery store conditioning and misting procedures on an industrial produce rack. For the analysis of the effectiveness of electrolyzed water vs. tap water, simulation of consumer storage after purchasing the produce was added. The produce was stored for an additional seven days in a refrigerator following the 72 hour produce rack storage.

Results from the analysis of the effectiveness of conditioning and misting showed that conditioning had a positive impact on both the microbial counts and sensory scores for crispness of the produce used for testing. Analysis of the effectiveness of electrolyzed water as the conditioning solution indicated that it did not make produce significantly more crisp compared to produce treated with tap water as the conditioning solution. Electrolyzed water was also ineffective at reducing counts of aerobic psychrotrophic bacteria on the produce compared to tap water treatments. Electrolyzed water treated produce did have lower counts of aerobic mesophilic bacteria, Enterobacteriaceae and fungi, compared to tap water treated produce.

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Chapter 1 – An introduction to the fresh produce industry

1.1 - Fresh Produce

1.1a - Introduction

Fresh produce is an ubiquitous category of food enjoyed by billions of people around the world. Fresh fruits and vegetables are highly nutritious and generally considered as healthy foods to eat (Bruhn, 2002). They are in high demand by consumers causing companies globally to fiercely compete to offer the safest and freshest produce at their stores (Cook, 2002). This is a difficult task due to the perishable nature of fresh produce.

Fresh produce undergoes minimal processing only which makes it susceptible to microbial contamination (Harris, Zagory, & Gorny, 2002). It can be contaminated by a wide range of pathogenic and spoilage microorganisms. The contaminants include pathogenic species of *Escherichia, Salmonella, Listeria, Campylobacter and Clostridium* (Cantwell & Suslow, 2002). These dangerous microorganisms can infiltrate the fresh produce at different points between the farm and the grocery store shelf. Because of the minimal processing requirement of fresh produce, these dangerous microorganisms can be alive in dangerous quantities when the consumer purchases these products (Harris, et al., 2002). Outbreaks of foodborne illness linked to fresh produce are common and their occurrence is on the rise (Sivapalasingam, Friedman, Cohen, & Tauxe, 2004). A recent outbreak of *Escherichia coli* O104, a shiga toxin producing microorganism, was linked to fresh sprouts produced in Germany in 2011. According to the Center for Disease Control, 852 people contracted hemolytic uremic syndrome (HUS), with 32 deaths being caused (CDC, 2011). This review will cover the fresh produce industry, the challenges involved in fresh produce processing and the use of electrolyzed water as a part of fresh produce processing.

1.1b - Fresh Produce Popularity

In 2010, consumers purchased an estimated $122 billion U.S. Dollars' worth of fresh fruits and vegetables from supermarkets, retail outlets, farms, public markets and food service establishments in the United States (Cook, 2011b). Seventy five percent of the fresh vegetables purchased were grown in the United States, with 73% of those vegetables being grown in California, Florida, Arizona, Georgia and Washington. California alone accounts for 50% of the fresh vegetables grown in the United States each year (USDA/NASS, 2011). The $122 billion worth of fresh produce sold in 2010 can be compared to the $75.8 billion worth sold in 2000. Adjusted for inflation, this is a 27% increase in sales in 10 years (BLS, 2012; Cook, 2002). The United States has also seen an increase in the value of fresh produce imported and exported over the last two decades. Steady growth has been observed with imports increasing from $3 to $12.3 billion between 1994 and 2010, and exports increasing from $2.9 to $6.1 billion over the same time frame (USDA/FAS, 1994-2010).

The fresh produce industry has also been affected by the trend of increasing market share of the biggest grocery store chains. In 2009, 37% of total grocery sales were from the top four grocery store chains, compared to 28% in 2000 (Cook, 2011a). This shift in market share has caused the major grocery chains to become even more competitive amongst each other to secure a larger market share. A by-product of this trend has been increasing high standards of quality for fresh produce sold at retail stores (Cook, 2002). These increasing standards have motivated grocery store chains to find new ways to improve and maintain the quality of their fresh produce.

1.1c - Fresh Produce Diversity

Fresh produce includes fruits and vegetables that have been minimally processed before they are sold at the store. Because of the lack of processing, fresh produce items are susceptible to microbial contamination. Some fresh produce undergo a value-added step of cutting, chopping or shredding and are sold as fresh-cut produce. One subsection of fresh produce is leafy greens; these include vegetables such as spinach, iceberg lettuce, romaine lettuce and cabbage (Cantwell & Kasmire, 2002). Fruit and vegetable products such as canned applesauce, frozen vegetables, fruit and vegetable juices and shelf stable fruit cups are not considered fresh produce because they have been processed to extend their shelf life.

The variety of fresh produce available at the grocery store continues to increase. In 1999, it was estimated that on average 431 different products were available in a U.S. grocery store fresh produce department, compared to an average of 173 different products in 1987. Despite this diversity, in 1999, 41% of total sales were still from the six most popular items of fresh produce, bananas, lettuce, apples, tomatoes, potatoes and grapes (Cook, 2002). Fresh-cut produce also continues to increase in popularity; between 2010 and 2011, fresh-cut fruit sales increased by 10% and fresh-cut vegetable sales increased by 4% in US supermarkets (Cook, 2011a).

1.1d - Produce storage

Fresh produce is commonly displayed in grocery stores in refrigerated racks with an open face on one side. The unit is kept at refrigeration temperature by a condenser that is powerful enough to keep the unit chilled despite the cold air constantly escaping out the open face of the unit. The produce racks are sometimes equipped with misters designed to spray the produce with water in order to make the produce look more appealing to potential customers, and possibly extending the shelf life of the produce. The shelves the produce is stored on are usually perforated to allow excess moisture to drip down through the levels of the rack to a drain. This is done because excess moisture can quickly cause produce to loss quality characteristics such as crispness. All different types of fresh produce are stored together on the racks with grocery store chains treating the produce differently depending on the protocols they have in place.

Produce in grocery stores is usually sold within a few days of it arriving at the store and methods for keeping it fresh and crisp during its storage time vary greatly between different grocery stores. Some grocers mist their produce as described previously and others choose not to. Some grocers have added processing steps such as washing and trimming of th e produce every so often while it is on the rack (Kader, 2002). All of these steps are put in place to make the produce more appealing to potential customers. This works by either making it look nicer on the shelf, or potentially increasing its shelf life after the produce arrives in the consumer's home refrigerator.

1.1e - Produce storage problems

The storage of fresh produce in open faced refrigerated racks leads to several unique problems. Microbial cross-contamination (Doyle & Erickson, 2008) is one of these problems. Produce available for purchase in grocery stores is often examined by customers who pick it up and then return it to the rack after deciding not to purchase it. This can lead to microbial contamination of that piece of produce by yeasts, molds and bacteria from the customer's hands. These microorganisms can lead to increased spoilage rates of the produce or they can cause foodborne illness in the consumer that decides to purchase the previously handled piece of produce (Farrar & Guzewich, 2009). The problem is compounded by the fact that cross contamination can lead to the spread of microorganisms to other produce in physical proximity to the initially contaminated piece. Cross contamination can happen via air or water flow or direct physical contact between the pieces of produce. The problem can also worsen if the microorganism is a psychrotroph, which means it can grow at refrigeration temperatures of approximately 4° C. Most microorganisms can only survive at refrigeration temperatures and do not have the ability to replicate quickly at these temperatures. The issue of cross contamination makes misting produce occasionally counterproductive because the water that is supposed to be preserving the quality of the produce is now being used as a vehicle of transporting spoilage or pathogenic microorganisms that now inhabit the produce rack (Farrar & Guzewich, 2009).

Storing fresh produce for extended periods of time also leads to a decrease in the crispness of the produce. This can be caused by both dehydration and microbial growth. This problem is combated by grocery stores using different strategies. These strategies include the optimization of production timeline, installing misting equipment in produce rack and using conditioning and washing procedures on the produce during it's time at the grocery store (Gomez-Lopez, 2012).

1.2 - Challenges of fresh produce processing

1.2a – Maintaining freshness and susceptibility to microbial contamination

By nature, fresh produce items are susceptible to having high populations of microorganisms present when they reach the consumer. These microorganisms may include hazardous human pathogens and spoilage microorganisms (Cantwell & Suslow, 2002). The weakness in fresh produce processing revolves around keeping the produce fresh. It would be easy to reduce the microbial load of the produce by a variety of methods to make them safer; however these processes result in a major quality reduction of these typically fragile food products. Harsh processing also goes against what consumers are looking for in fresh produce. The word fresh is an important keyword for consumers and fresh produce companies strive to present to the public that their products are as fresh as possible. A consumer study in 2000 showed that the three most important factors that influenced consumer's fresh produce buying decisions were taste, ripeness and appearance. These three traits can be negatively affected by microorganism growing within the food product (Cook, 2002).

1.2b - Fresh Produce contamination and recent outbreaks

Microbial contaminants may survive on produce during its journey from the farm to the grocery store or these may be introduced by cross contamination or other means throughout the process. On the farm, sources of contamination include animals, insects, soil and irrigation water (Farrar & Guzewich, 2009; Johannessen et al., 2005). During processing and transportation to the grocery store, contamination can be caused by cross contamination, human handling and dirty equipment (Beuchat & Ryu, 1997). These contaminants can include dangerous species of *Escherichia, Salmonella, Campylobacter, Listeria, Bacillus* and *Clostridium*. Fresh produce can also be contaminated by species of spoilage microorganisms such as *Pseudomonas*¸ *Aspergillus* and *Penicillium* (Harris, et al., 2002). Reported outbreaks of foodborne illness associated with fresh produce have been on the rise in the last few decades (Sivapalasingam, et al., 2004).

The largest recent outbreak of fresh produce-related illness was in Europe in 2011. The outbreak was linked to sprouts produced in Germany, and cases surfaced throughout Europe and a few were reported in the United States. *Escherichia coli* O104:H4, a shiga toxin producing strain, was found to be the causative microorganism (CDC, 2011). As a result of this disease outbreak, 852 people contracted hemolytic uremic syndrome (HUS) and 32 individuals died. Contracting HUS can cause kidney failure and symptoms include fever, abdominal pain, fatigue, bruising and swelling (CDC, 2011). This outbreak illustrates the susceptibility of fresh produce to microbial contamination. These sprouts were most likely contaminated at some point while they were still on the farm, because illnesses were reported from multiple locations across Europe where these sprouts were consumed. This means that *Escherichia coli* O104 population survived during transportation, storage, and minimal processing it was subjected to before it infected at least 852 people that consumed the sprouts.

Another recent outbreak of foodborne illness linked to fresh produce, specifically cantaloupe, in the United States resulted in 261 illnesses and 3 deaths caused by *Salmonella* Typhimurium and *Salmonella* Newport. *Salmonella* infection symptoms include diarrhea, fever and abdominal cramps (CDC, 2010). The outbreak was traced back to cantaloupes from a farm in Indiana. Illnesses were reported in 24 different states, which indicates that the contamination of the cantaloupes with *Salmonella* serovars occurred at the farm before transportation. This outbreak also shows the susceptible nature of fresh produce to microbial contamination. The *Salmonella* population survived during transportation of cantaloupe from the farm and the minimal processing it received before consumption (CDC, 2012).

1.2c - Preharvest contamination of fresh produce

Fresh produce is susceptible to microbial contamination throughout their growth cycle at the farm. One source of contamination is irrigation water that has been contaminated by pathogenic bacteria before it is applied to the field (Johannessen, et al., 2005). This water can be sprayed directly onto the fresh produce or absorbed by the roots of the plants or trees.

Contaminated irrigation water can also lead to contaminated soil on fresh produce farms. Pathogens can survive extended periods of time in soil and were found in fresh produce harvested up to 6 months after initial soil contamination (M. Islam, Doyle, Phatak, Millner, & Jiang, 2004; Mahbub Islam, Doyle, Phatak, Millner, & Jiang, 2005). Other sources of preharvest contamination include insects, birds and other animals that can make physical or indirect contact with fresh produce while they are being grown (Farrar & Guzewich, 2009).

1.2d - Vacuum cooling and bacteria internalization

Vacuum cooling is a common step in fresh produce processing for many fresh produce items including leafy green vegetables. The product is rapidly cooled to refrigeration temperatures based on the rapid evaporation of water under vacuum conditions. Vacuum cooling is usually achieved in less than 30 minutes and 1% of the products weight is lost due to water evaporation for every 6° C reduction in product temperature (Thompson, Mitchell, & Kasmire, 2002).

Vacuum cooling has also been shown to cause bacterial internalization into lettuce (Li, Tajkarimi, & Osburn, 2008). Vacuum cooling caused the stomata on the lettuce surface to increase in surface area, allowing bacterial cells on the surface of the lettuce to internalize due to the suction caused by pressurization from the vacuum back to normal atmospheric pressure (Vurma, 2009). Internalization of bacteria is a serious safety concern. Internalization increases bacterial resistant to antimicrobial agents such as chlorine and ozone (Li, et al., 2008). Chlorine and ozone must make physical contact with their target microorganisms to inactivate them, and this is made more difficult when the bacteria are internalized and protected within the fresh produce product.

1.2e - Fresh-cut produce cross contamination concerns

Fresh-cut produce are at an increased risk for microbial contamination due to cross contamination concerns during production and processing. Fresh-cut processing steps can include trimming, cutting and shredding (Cantwell & Suslow, 2002). Each of these steps can cause cross contamination of an entire lot of produce due to residual microorganisms from a previously isolated contaminated piece of produce leftover on cutting or food contact surfaces. This problem can be compounded when water is present on these surfaces, due to the ability of microorganisms to create biofilms on most food contact surfaces when water is present (Kaneko et al., 1999). Biofilms are protective structures that are formed when microorganisms colonize a surface they have attached to. Biofilms protect the microorganisms from sanitizers such as chlorine, which are commonly used to clean processing equipment.

1.3 - Electrolyzed water

1.3a - Introduction

Electrolyzed water is an antimicrobial solution that is sometimes used on fresh produce in grocery stores. It is produced by the electrolysis of sodium chloride which produces water containing chlorine in a highly oxidative state (Al-Haw & Gomez-Lopez, 2012). Chlorine freely reacts with organic molecules and is a potent antimicrobial agent. Electrolyzed water can be produced at different pH's as well (Al-Haw & Gomez-Lopez, 2012; Hricova D, 2008). The pH of the water affects how chlorine molecules exist within the solution which can affect the reactions that occur between the chlorine and organic molecules (Hricova D, 2008).

1.3b - Electrolyzed water production

Electrolyzed water is produced by the electrolysis of an aqueous salt solution (Hricova D, 2008). Sodium chloride is dissolved in water and dissociates into two ions, negatively charged chlorine and a positively charged sodium. This free chlorine then reacts further to form reactive chlorine compounds including hydrochloric acid, hypochlorous acid and hypochlorite ions (Al-Haw & Gomez-Lopez, 2012). The production of these compounds also depends on the pH of the resulting electrolyzed water (Hricova D, 2008). Systems can be set up to produce electrolyzed water with a pH between 2 and 13.

Systems designed for fresh produce application are usually set up to produce an electrolyzed water with between 10 to 90 parts per million (ppm) chlorine that are readily available to oxidize organic matter (Hricova D, 2008). Electrolyzed water is usually generated on-site immediately before use to ensure the unstable reactive chlorine molecules are still present in the water when it is applied to the food product (Al-Haw & Gomez-Lopez, 2012). Multiple companies produce electrolyzed water units and they all produce electrolyzed water with different properties that are altered by changing the amperage level of the electrolyzing system, the water flow rate, the time allowed for electrolysis and the concentration of sodium chloride initially introduced to the system (Hricova D, 2008).

1.3c - Electrolyzed water applications

Electrolyzed water is used as a potent antimicrobial agent in a variety of fields. It is applied to food products to reduce their microbial load. Medical and dental practitioners use it to disinfect wounds and medical equipment (Hricova D, 2008). In the food industry, electrolyzed water is most commonly applied to fresh produce in order to reduce its microbial load (Abadias M, 2008). Grocery store workers also may apply it to their produce to help in maintaining more crisp product throughout storage time (Al-Haw & Gomez-Lopez, 2012). Electrolyzed water can be produced and applied at a pH range between 2 and 13, leading to three subgroups of electrolyzed water referred to as acidic, neutral and alkaline electrolyzed waters. Each type of electrolyzed water has its own advantages and disadvantages, and they are sometimes applied in conjunction with one another.

1.3d - Electrolyzed water antimicrobial efficacy

Studies have been conducted to determine the effectiveness of electrolyzed water at reducing microbial counts on fresh produce. None of these studies fully simulated the grocery store experience; however there results consistently showed that electrolyzed water had the ability to reduce microbial counts on the produce. Park et al. (2008) showed that electrolyzed water was effective at reducing the counts of pathogens including *Escherichia coli O157:H7*, *Salmonella* Typhimurium and *Listeria monocytogenes* on the surface of lettuce and spinach; the counts were reduced below the experimental detection limit (Park, 2008).

Koseki et al. analyzed the effect of electrolyzed water on cucumbers and strawberries and noted a 1-log reduction of aerobic mesophilic bacteria on the produce after a 10 minute electrolyzed water wash (Koseki et al. 2004). The same study also showed significant decreases in coliform and fungi counts on cucumbers after they were washed with a combined treatment of acidic and alkaline electrolyzed water. Other researchers analyzed the effect of storing lettuce in a container packed with frozen acidic electrolyzed water (Koseki et al. 2002). Aerobic bacteria on the lettuce were reduced by 1.5 log cfu/g after 24 hours of storage packed in the frozen electrolyzed water. This result was attributed to chlorine gas produced and contained in the closed environment (Koseki et al. 2002).

Use of electrolyzed water to degrade pesticides on fresh vegetables was investigated (Hao, 2011). The results indicated that pesticide residues were reduced by between 30-80% on spinach after 30 minutes of immersion in acidic electrolyzed water. Results for cabbage and leek were similar to spinach. Their results indicated that washing fresh produce with electrolyzed water was an effective method for degrading pesticide residues on fresh vegetables (Hao, 2011).

Bessi et al. (2014) analyzed the effect of dipping date fruits in electrolyzed water before extended storage. The results showed that electrolyzed water at pH 7, under ideal conditions could reduce the bacterial and fungi counts on the surface of the dates by 2.5 log cfu/g with only a four minute dip (Bessi, 2014). The resistance of shiga toxin producing *Escherichia coli* to electrolyzed water was tested by other researchers. Their results indicated that an electrolyzed water solution of the same concentration of free chlorine as a sodium hypochlorite solution was consistently more effective at reducing the counts of shiga toxin producing *Escherichia coli* than the sodium hypochlorite solution (Jadeja, 2013).

The efficacy of electrolyzed water against *Escherichia coli* O157:H7 on iceberg lettuce, cabbage and tomatoes simulating a food service kitchen conditions was tested by Pangloli et al (2009). Their results showed that washing iceberg lettuce with acidic electrolyzed water can reduce counts of *E. coli* O157:H7 by 3.0 log cfu/g (Pangloli et al. 2009). The same procedure was repeated with tap water which only yielded a 2.0 log cfu/g reduction. Results for the treatment of cabbage by electrolyzed water were similar to iceberg lettuce. *E. coli* O157:H7 was reduced by 4.7 log cfu/g on lemons treated with acidic electrolyzed water. The reductions seen on tomatoes were even greater; as high as 7.9 log cfu/g reductions were observed.

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Chapter 2 - Improving quality of fresh produce by modifying conditioning procedures used in retail establishments

2.1 – Abstract

Improving the safety and quality of fresh produce continues to be an important goal for grocery stores. Retailers use different methods to improve the quality of the produce in their stores. One of these methods is referred to as conditioning, which is the act of washing produce in different types of water daily while the product is held in refrigerated display cases. The second method is using misters placed in the produce rack to spray the produce with water periodically. Both of these methods were tested for their effectiveness at reducing counts of four groups of microorganisms. Contribution of these procedures to the crispness of the produce was measured by sensory analysis using a trained panel.

Asparagus, celery, romaine lettuce and red leaf lettuce were chosen as the fresh produce used for these experiments. These products were held under conditions that simulate retail store setting. A section of commercial fresh produce display case was assembled in a pilot plant to hold the fresh produce. The display case was equipped with refrigeration and misting equipment. Four treatments were tested; these are (i) conditioning with misting, (ii) conditioning with no misting, (iii) no conditioning with misting, and (iv) no conditioning with no misting. Conditioning was performed every 24 hours using electrolyzed water, and the misters were set to spray for seven seconds every two minutes. Results indicated that populations of aerobic psychrotrophic bacteria were decreased by conditioning treatments applied to both romaine and red leaf lettuce during the 72 hour simulated grocery store produce rack storage.

Fungi counts were significantly reduced when conditioning was applied to red leaf lettuce, romaine lettuce and celery. Counts of *Enterobacteriaceae* on three of the four types of produce were not affected by the treatments applied. Sensory results for crispness indicate d that on all four types of produce, treating them with conditioning with no misting produced more crisp produce than produce treated with no conditioning and no misting.

2.2 – Introduction

Fresh produce is consumed by billions of people around the world every day. It is perhaps the most ubiquitous food product around the world. Fresh fruits and vegetables are a nutritious part of many people's diet and every year billions of dollars are spent on fresh produce around the world (Bruhn, 2002). The most common way for people in developed countries to purchase their fresh produce is at a grocery store. Grocery stores compete fiercely to offer the freshest, safest produce to consumers (Cook, 2011a).

The sale of fresh produce in grocery stores brings its own unique challenges to the marketplace. Fresh produce is susceptible to microbial contamination due to the minimal processing it undergoes before it is sold (Harris, et al., 2002). Hazardous microbial contaminants include species of *Escherichia, Salmonella, Listeria, Campylobacter* and *Clostridium* (Cantwell & Suslow, 2002). An example of a major recent outbreak linked to fresh produce happened in Europe in 2011. Over 3000 people were infected by eating contaminated sprouts, 852 of those people contracted hemolytic uremic syndrome (CDC, 2011).

Electrolyzed water is a common sanitizer used by grocery stores to increase the shelf life of the fresh produce. Electrolyzed water is produced from the electrolysis of a salt solution and usually contains about 50 parts per million of chlorine in the form of sodium hypochlorite. Electrolyzed water can then be applied to the produce via misting or a process called conditioning.

Conditioning involves removing the produce from the shelves every 24 hours and soaking them in a tub of electrolyzed water for about 15 minutes. This is done with the goal of hydrating the produce in order to improve crispness and inactivating bacteria by chlorine (Gomez-Lopez, 2012; Philipus P, 2009). A literature search did not reveal any attempts to simulate grocery store produce storage conditions to evaluate the effectiveness of using these conditioning and misting treatments to extend the shelf life of fresh produce.

2.3 - Materials and methods

Experimental

An industrial produce rack equipped with a refrigeration unit and water spraying misters was installed in a pilot plant at Ohio State University. The misters were set to consistently spray the produce for ten seconds every two minutes with electrolyzed water containing 4 parts per million (ppm) active chlorine. The produce rack was set up to maintain 3° C throughout experimentation. The electrolyzed water used for conditioning and misting the produce was generated on-site via an electrolyzed water-producing unit which produced a solution containing 50 ppm chlorine that was used for conditioning and diluted to be used for misting.

Freshly-harvested asparagus, red leaf lettuce, romaine lettuce and celery were supplied by a national grocery chain and delivered via refrigerated truck and immediately transferred to a walk in refrigerators set at 4° C. The produce was then transferred to the produce rack area to be loaded onto the rack. Produce conditioning procedures, recommended by a grocery company, were followed. Bunches of asparagus was conditioned by examining the bottoms of their stalks (i.e., at the cut surface) for mold or damage and cutting them down as much as necessary to result in a healthy bottom of the stalk. Asparagus stalks were placed in a tub containing 50 ppm electrolyzed water solution to soak the bottom five centimeters of the stalks for 45 minutes before they were re-loaded onto the produce rack. Red leaf and romaine lettuce were treated in a similar manner for conditioning purposes. These two products were first examined for physical damage or molding of the outer leaves; any leaves that were deemed to be damaged enough to prevent someone from buying it at a grocery store were removed. The browned bottoms of the lettuce heads were cut down by approximately half a centimeter to reveal a fresh white bottom. The lettuce heads were then placed in 10 gallon tubs of 50 ppm electrolyzed water solution and soaked thoroughly for 15 minutes. The lettuce heads were then removed and allowed to dry for 15 minutes in a separate tub before being placed on the rack. The celery was first examined for damage to the stalks and any physical damage deemed sufficient to prevent someone from buying it at a grocery store were removed. The browned or dirty bottoms of the celery pieces were also cut down by less than a centimeter to reveal a whiter bottom. The tops of the celery stalks were also examined for mold and were cut down enough to remove any mold. The celery was then placed in 10 gallon tubs of 50 ppm electrolyzed water solution and soaked thoroughly for 15 minutes. The celery was then loaded onto the produce rack.

Asparagus was always loaded on the top shelf with red leaf lettuce on the shelf below, followed by romaine lettuce and then celery on the bottom shelf. The pieces of produce were bunched together tightly to simulate a grocery store atmosphere and were always orientated the same way throughout experimentation. Any time produce was removed from the rack during storage time for microbiological or sensory analysis, it was replaced with products that have been stored in a walk-in refrigerator. This was done to ensure consistent temperature and air flow throughout the product during the three days of storage. The replacement pieces were marked and any pieces of produce that came in direct physical contact with the replacement produce were not used for further testing to prevent cross contamination. Picture 2.1 illustrates the produce rack setup.

Picture 2.1: Example of the simulation of grocery store rack fresh produce storage

Sample preparation for microbiological and sensory analysis

Pieces of produce were sampled after 0, 24, 48 and 72 hours of storage, post receiving. The 0 hour samples were taken before any conditioning or treatment took place. The sampled produced was handled aseptically and each piece was cut in half with one half going for sensory analysis and the other half aseptically stored for microbiological analysis. In the case of asparagus, no cutting was required and the bunch of asparagus was simply separated in half.

Sensory analysis was performed within an hour after cutting occurred. Microbiological samples were stored at 4° C and testing was completed within three hours after the sample was taken.

Sensory analysis

Sensory analysis was performed by a trained panel of eight people with between four and seven people performing the analysis at each time point of 0, 24, 48 and 72 hours. Analysts examined only the crispness of the produce. They were allowed to touch and manipulate the produce and breaking of pieces of produce was allowed to help them assign a score as long as the analyst performed the action of breaking the piece of produce. Observations were scored on an anchored non-numeric line scale. The marks made were then measured and converted to a score out of 100 for crispness.

Microbiological analysis

Samples for microbiological analysis were homogenized and diluted in buffered peptone water in order to be plated for enumeration. The produce was analyzed for aerobic mesophilic, aerobic psychrotrophic, *Enterobacteriaceae* and fungi counts. For aerobic mesophilic and aerobic psychrotrophic counts, homogenized products were plated on tryptic soy agar (Becton, Dickinson and Company, Franklin Lakes, New Jersey) and incubated at 37° C for 48 hours and 4° C for ten days, respectively. Testing for *Enterobacteriaceae* was accomplished by plating on Violet Red Bile Agar (EMD Millipore, Billerica, Massachusetts) and incubation at 37° C for 48 hours. Testing for fungi was done by plating on yeast and mold petrifilms (3M, St. Paul, Minnesota) and incubation at 23° C for five days.

Statistical analysis

Statistical analysis was performed using the Statistical Analysis System program (SAS Institute Inc., Cary, North Carolina), version 9.3. A Generalized Linear Model was used that accounted for the random effect produced by the six separate weeks of testing that were performed. The model used was:

Sensory score or microbiological count = treatment applied + day of analysis + weekly batch

A Tukey's analysis was performed and the significant differences are represented in the figures below by differing letters associated with each treatment. A 95% confidence interval was used to calculate significant differences. All three days of testing were grouped together for analysis to increase statistical power and to better illustrate an average result for a piece of fresh produce on a grocery store produce rack shelf. The day 0 counts were not included in the results seen below.

2.4 – Results

Red leaf lettuce

Microbiological analysis of red leaf lettuce showed a reduction in the count of psychrotrophic bacteria on the lettuce during simulated grocery store storage when conditioning with no misting was applied to the red leaf lettuce rather than either treatment that did not contain conditioning (Fig. 2.1). Red leaf lettuces psychrotrophic population averaged 4.1 log cfu/g over the course of the 72 hour rack storage when the product was treated with conditioning and no misting, compared to 4.9 log cfu/g when treated with no conditioning and no misting. Fungi population on red leaf lettuce decreased significantly when the product was conditioned rather than not conditioned.

Figure 2.3 shows that the population of *Enterobacteriaceae* was not affected by the type of treatment applied to red leaf lettuce. Aerobic mesophilic bacteria were affected by the treatment applied to the red leaf lettuce, conditioning with no misting produced the lowest log cfu/g of aerobic mesophilic bacteria, 3.9. Conversely, no conditioning with no misting produced a 4.8 log cfu/g on red leaf lettuce during three days storage on the produce rack.

Figure 2.5 indicates a large difference in crispness detected by sensory testing of red leaf lettuce. Conditioning with misting and conditioning with no misting produce average scores of 66.4 and 73.8 respectively, while scores fell to 55.4 and 48.7 for no conditioning with misting and no conditioning with no misting, respectively. Statistical analysis indicated that conditioning with no misting produced the significantly highest crispness score for red leaf lettuce.

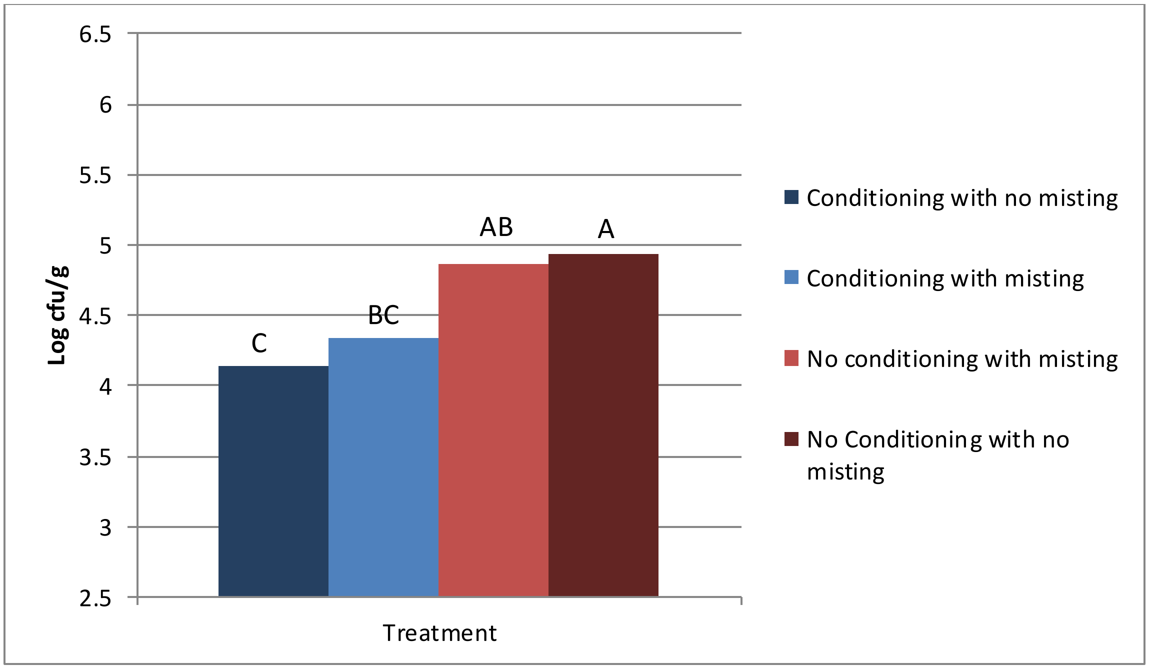

Figure 2.1: Average Log_{10} colony forming units per gram (cfu/g) of psychrotrophic bacteria found in red leaf lettuce during 72 hours of grocery store produce rack storage

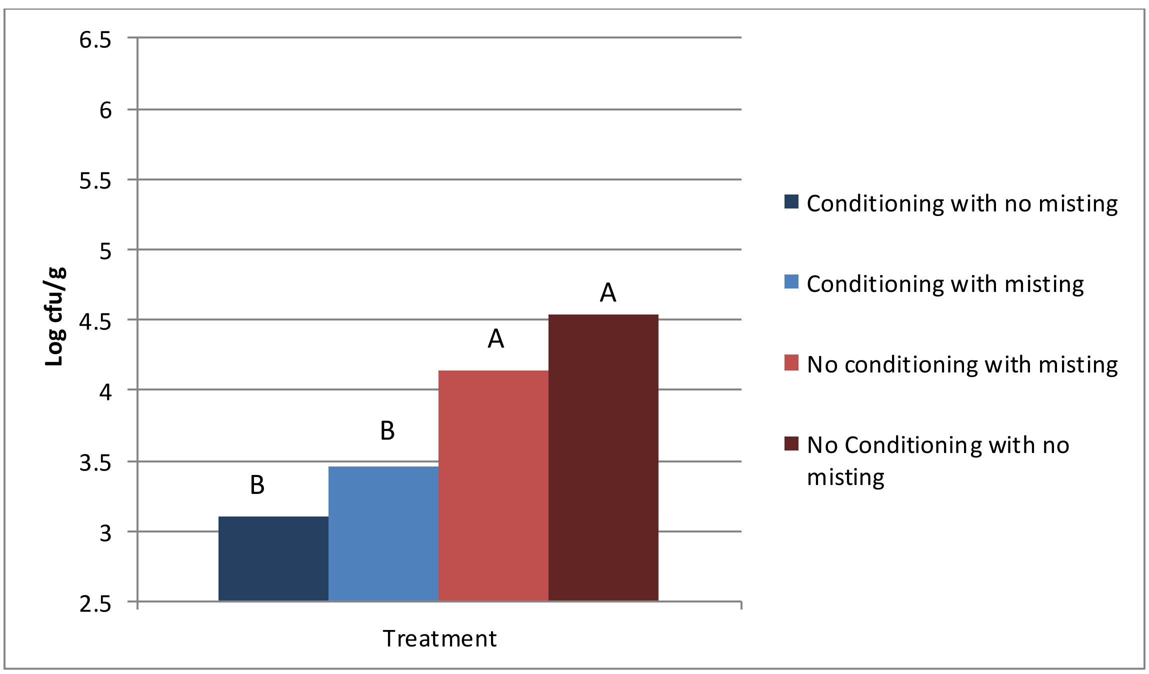

Figure 2.2: Average Log_{10} colony forming units per gram (cfu/g) of fungi found in red leaf lettuce during 72 hours of grocery store produce rack storage

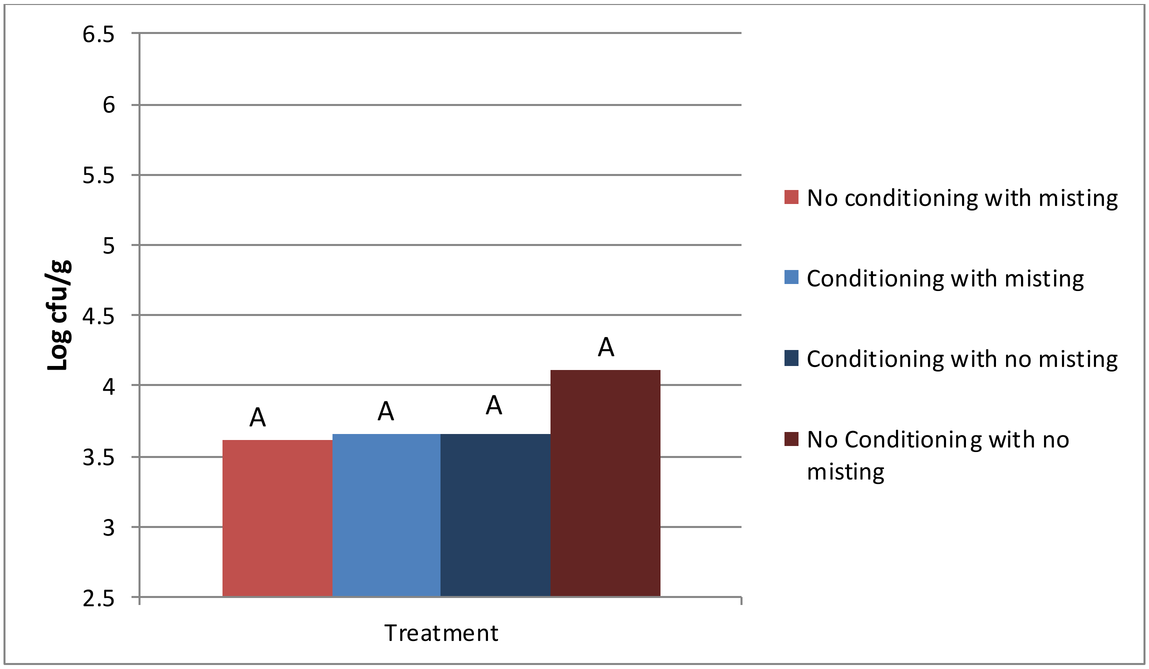

Figure 2.3: Average Log_{10} colony forming units per gram (cfu/g) of *Enterobacteriaceae* found in red leaf lettuce during 72 hours of grocery store produce rack storage

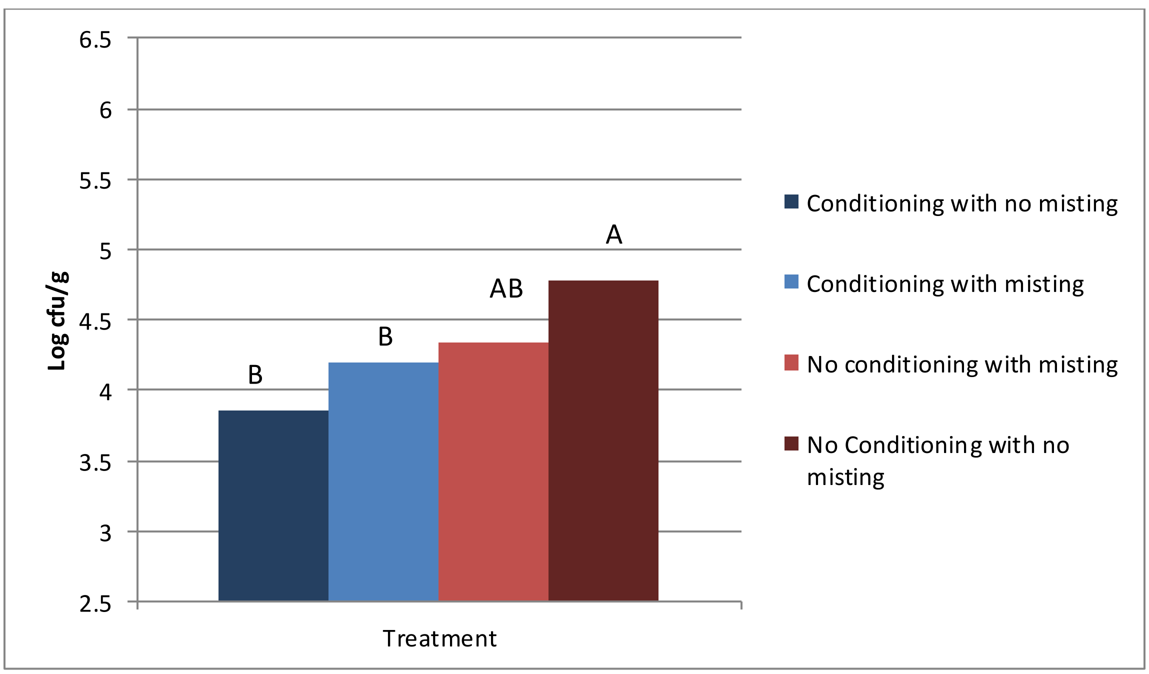

Figure 2.4: Average Log_{10} colony forming units per gram (cfu/g) of aerobic mesophilic bacteria found in red leaf lettuce during 72 hours of grocery store produce rack storage

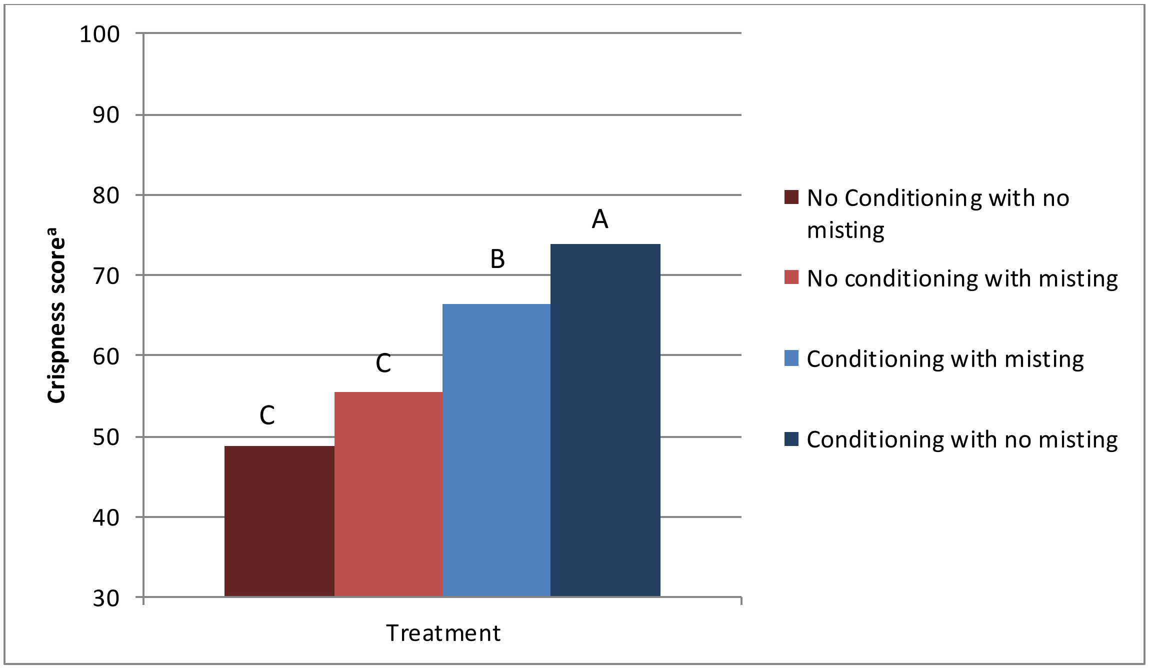

Figure 2.5: Average crispness sensory score of red leaf lettuce during 72 hours of grocery store produce rack storage

a = sensory score as measured by a non-numeric anchored line scale with the score converted to a score out of 100

Romaine lettuce

Figure 2.6 illustrates how conditioning with no misting produced an average log cfu/g for psychrotrophic bacteria on romaine lettuce of 3.7, while no conditioning with no misting produced an average log cfu/g of 4.7. The difference between those two treatments was statistically significant. Figure 2.7 shows a large difference between romaine lettuces treated with conditioning versus no conditioning, aerobic mesophilic bacteria counts were significantly reduced on romaine lettuce when subjected to conditioning rather than no conditioning. Conditioned romaine lettuce produced average log cfu/g of 2.7 and 3.4 for fungi during their three day storage on the produce rack. Unconditioned romaine lettuce produced average log cfu/g's of 4.0 and 4.3.

Figure 2.8 shows how the population of *Enterobacteriaceae* on romaine lettuce was affected when the product was treated with conditioning with no misting, compared to no conditioning with misting. Figure 2.9 indicates that aerobic mesophilic bacteria were significantly lower on romaine lettuce treated with conditioning with no misting compared to the other three treatments.

The sensory results shown in figure 2.10 indicate that conditioning romaine lettuce produced significantly higher average sensory scores compared to not conditioned romaine lettuce. The scores were 71.4 and 78.2 for conditioning with misting and conditioning with no misting respectively. No conditioning with misting and no conditioning with no misting produced scores of 58.9 and 62.4, respectively.

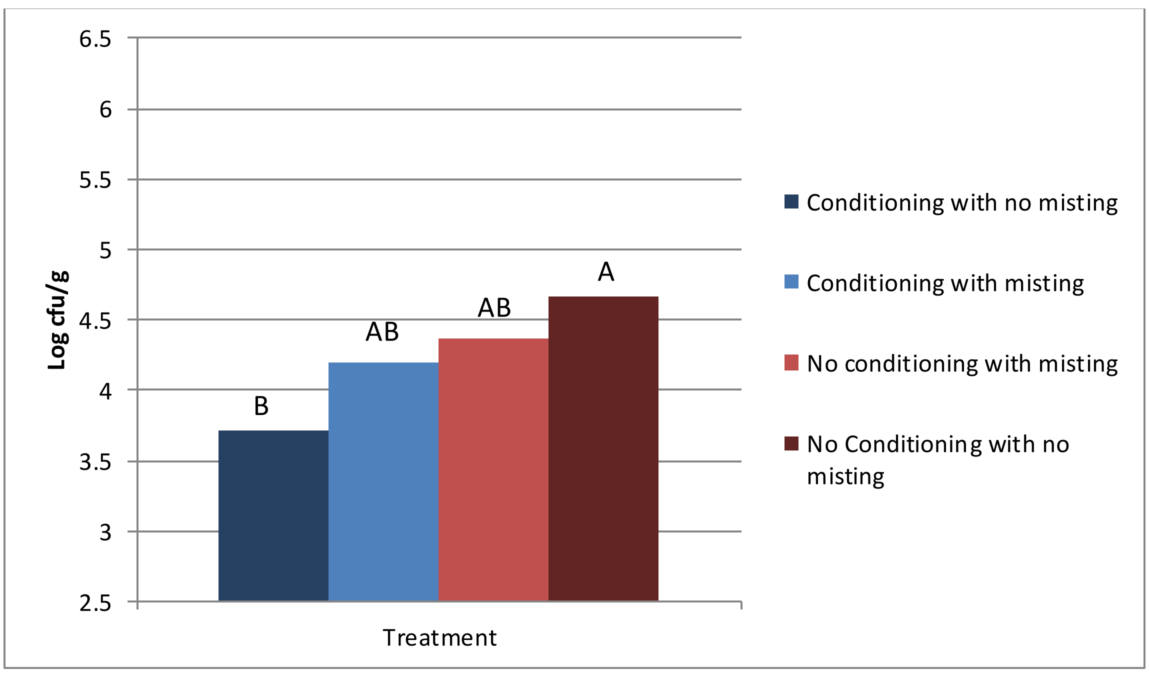

Figure 2.6: Average Log_{10} colony forming units per gram (cfu/g) of psychrotrophic bacteria found in romaine lettuce during 72 hours of grocery store produce rack storage

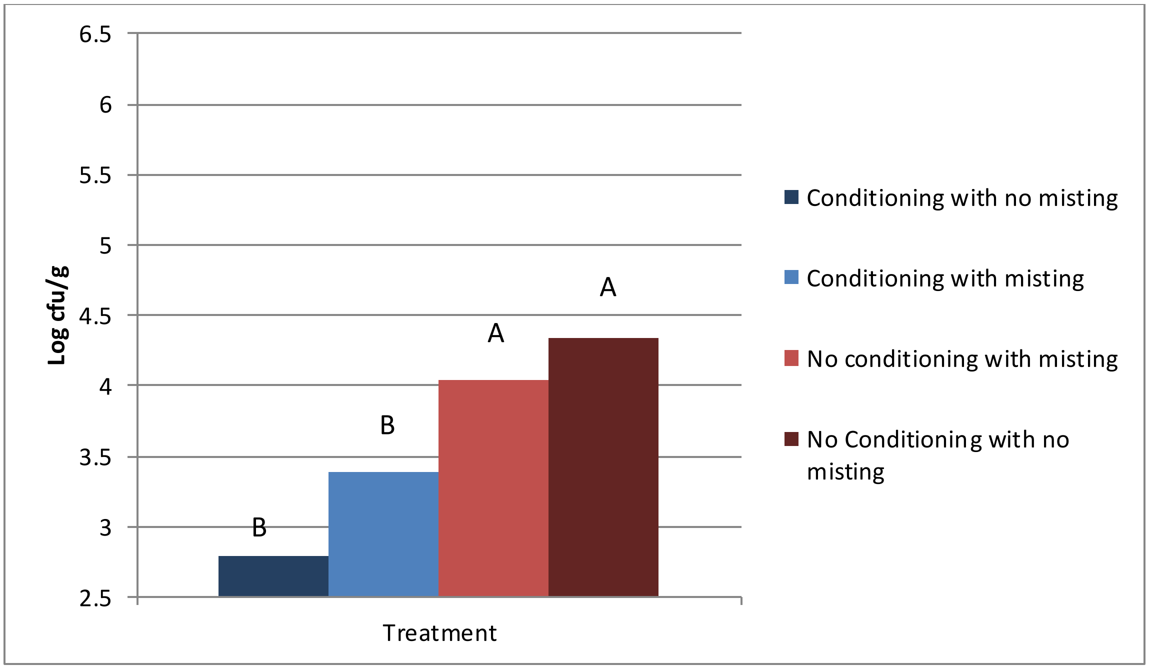

Figure 2.7: Average Log_{10} colony forming units per gram (cfu/g) of fungi found in romaine lettuce during 72 hours of grocery store produce rack storage

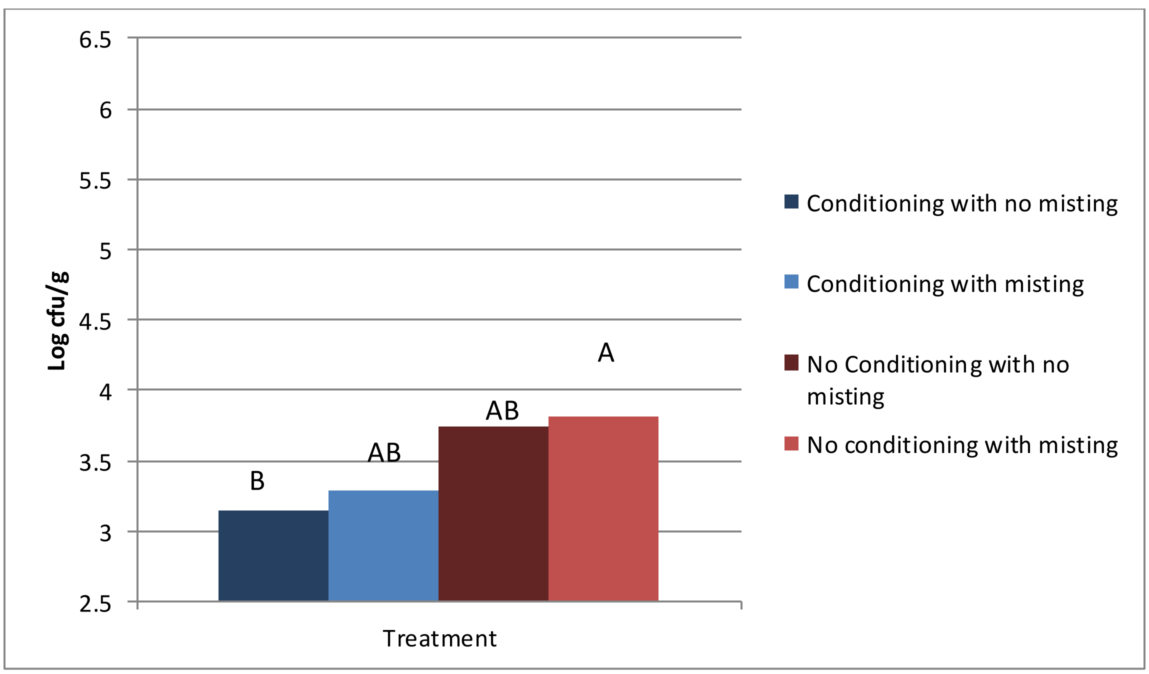

Figure 2.8: Average Log_{10} colony forming units per gram (cfu/g) of *Enterobacteriaceae* found in romaine lettuce during 72 hours of grocery store produce rack storage

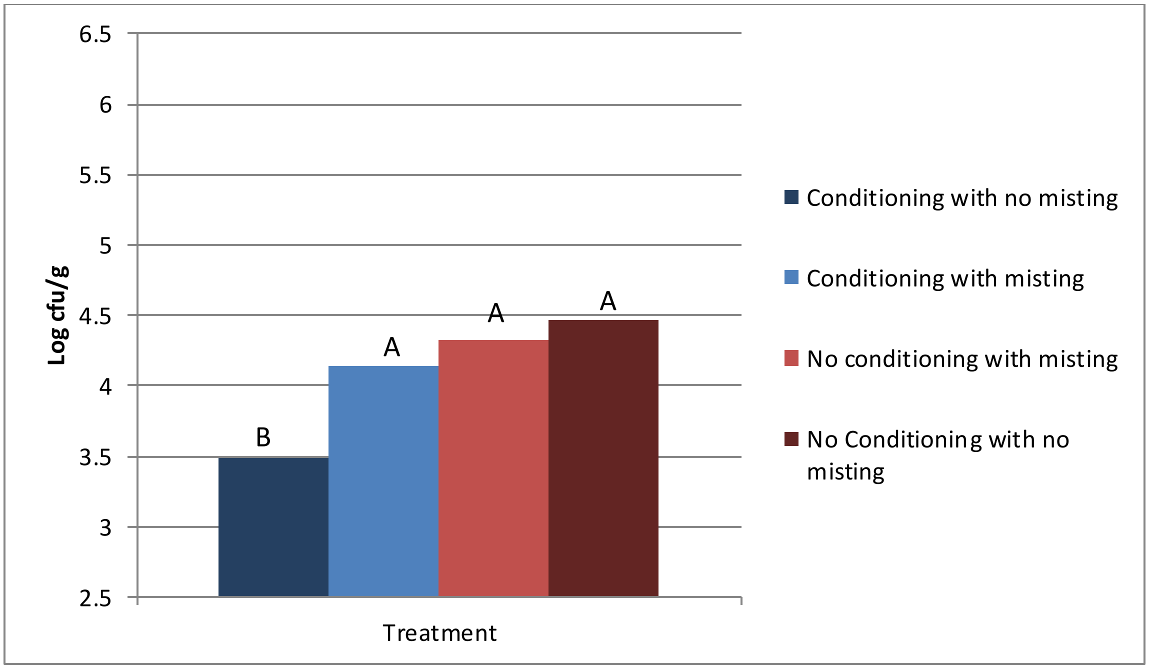

Figure 2.9: Average Log_{10} colony forming units per gram (cfu/g) of aerobic mesophilic bacteria found in romaine lettuce during 72 hours of grocery store produce rack storage

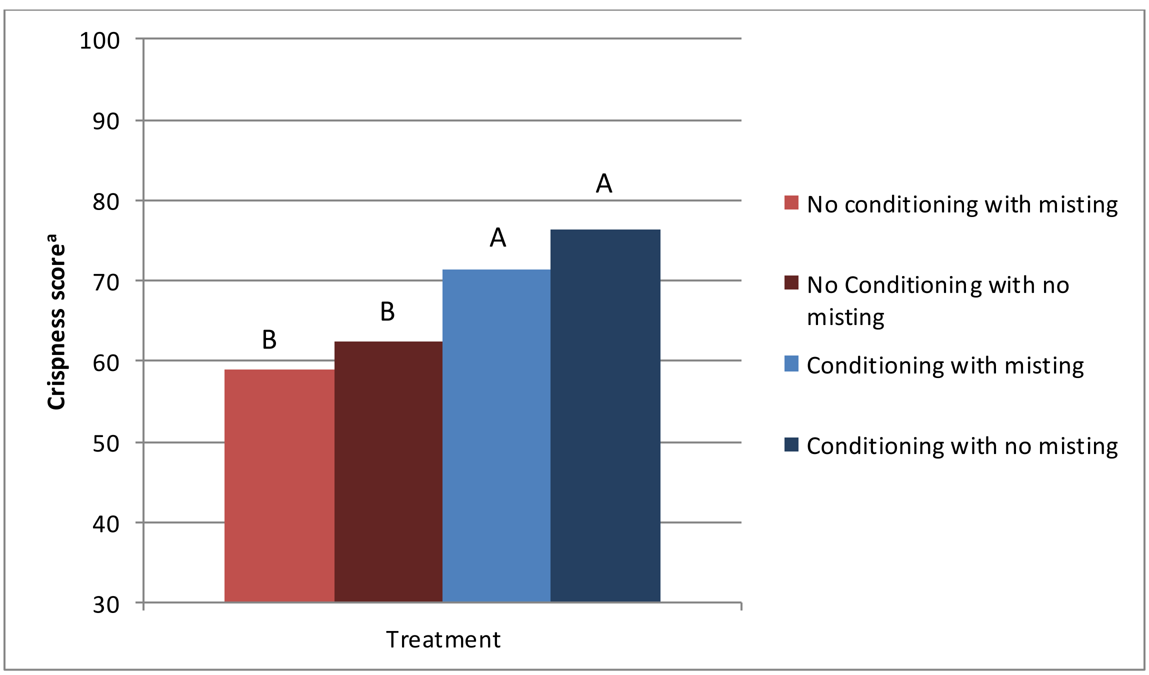

Figure 2.10: Average crispness sensory score of romaine lettuce during 72 hours of grocery store produce rack storage

a = sensory score as measured by a non-numeric anchored line scale with the score converted to a score out of 100

Asparagus

Psychrotrophic bacteria populations increased during storage of asparagus for 72 hours regardless of the treatment applied. Figure 2.11 illustrates the high counts of psychrotrophic bacteria that averaged 6.0 log cfu/g on the asparagus used for testing all four treatments. Figure 2.12 shows how fungi counts were affected by differing treatments applied to the asparagus. Significant differences were not detected; conditioning with misting produced the lowest counts of fungi at 4.7 log cfu/g, while no conditioning with no misting produced the highest fungi count of 5.1 log cfu/g.

Enterobacteriaceae populations were not affected by the type of treatment applied to asparagus. Figure 2.13 illustrates how regardless of treatment applied, *Enterobacteriaceae* counts grew to approximately 5.0 log cfu/g on asparagus. Conditioning with misting produced the lowest counts of aerobic mesophilic bacteria on asparagus, while no conditioning with no misting produced the highest counts.

Figure 2.15 illustrates the results of sensory testing for the crispness of asparagus during its 72 hour storage on the produce rack. Conditioning with no misting produced the highest average crispness score of 78.3, while no conditioning with no misting produced the lowest average score of 65.1. Scores for conditioning with misting and no conditioning with misting were similar at 70.5 and 72.6, respectively.

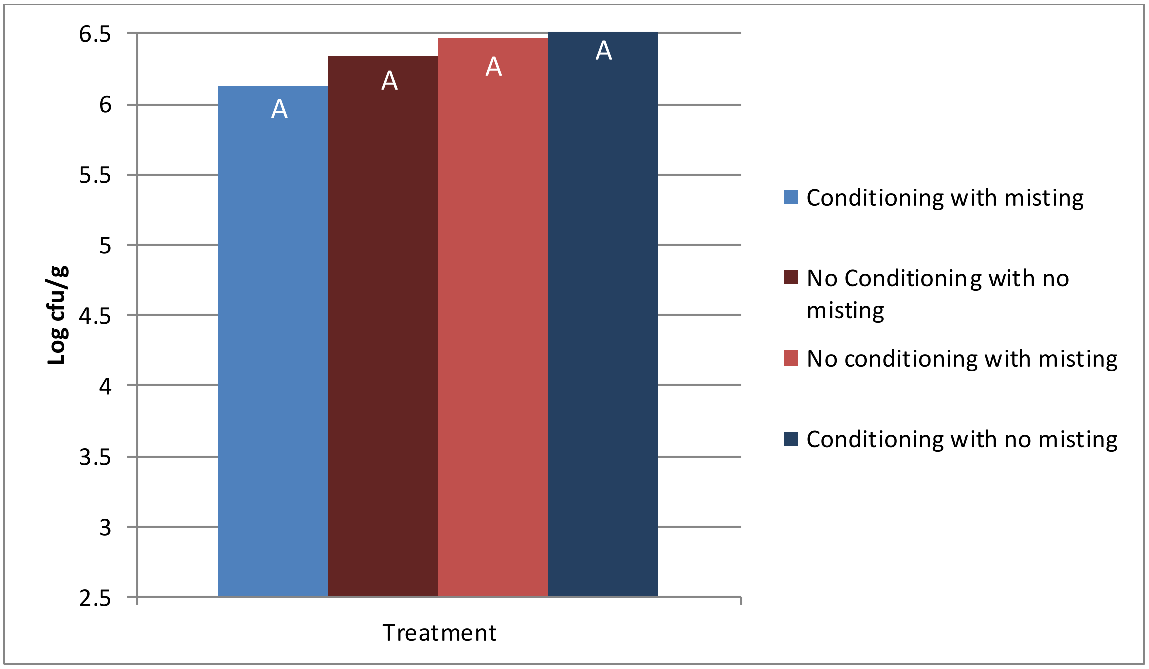

Figure 2.11: Average Log_{10} colony forming units per gram (cfu/g) of psychrotrophic bacteria found in asparagus during 72 hours of grocery store produce rack storage

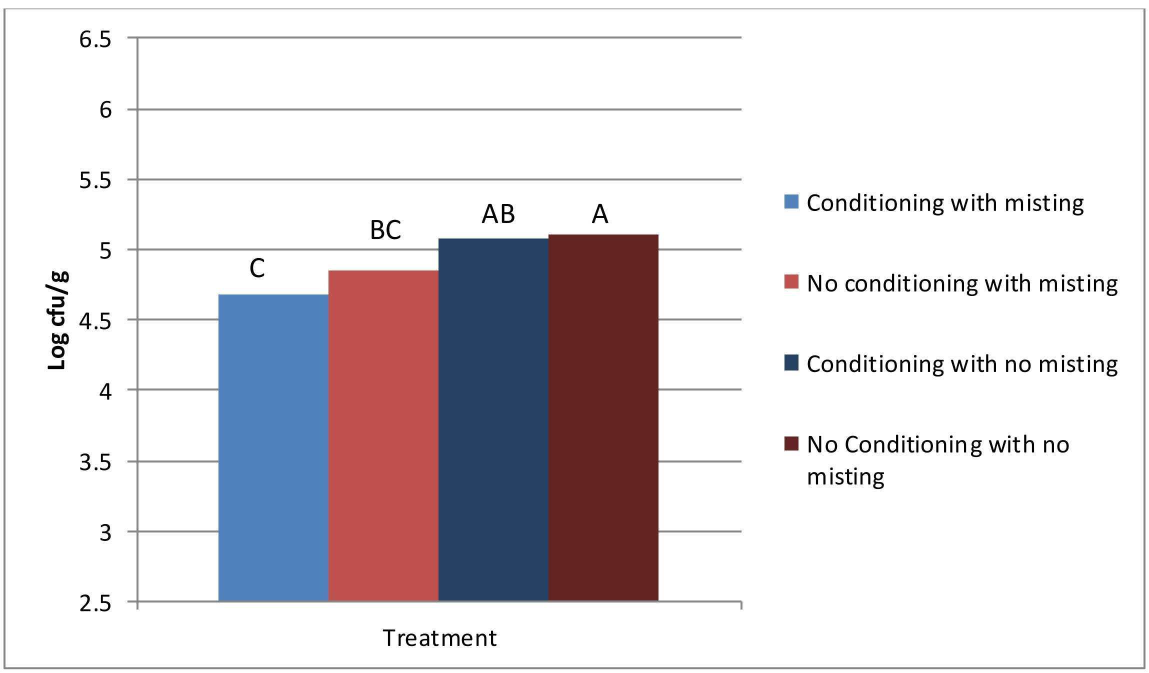

Figure 2.12: Average Log_{10} colony forming units per gram (cfu/g) of fungi found in asparagus during 72 hours of grocery store produce rack storage

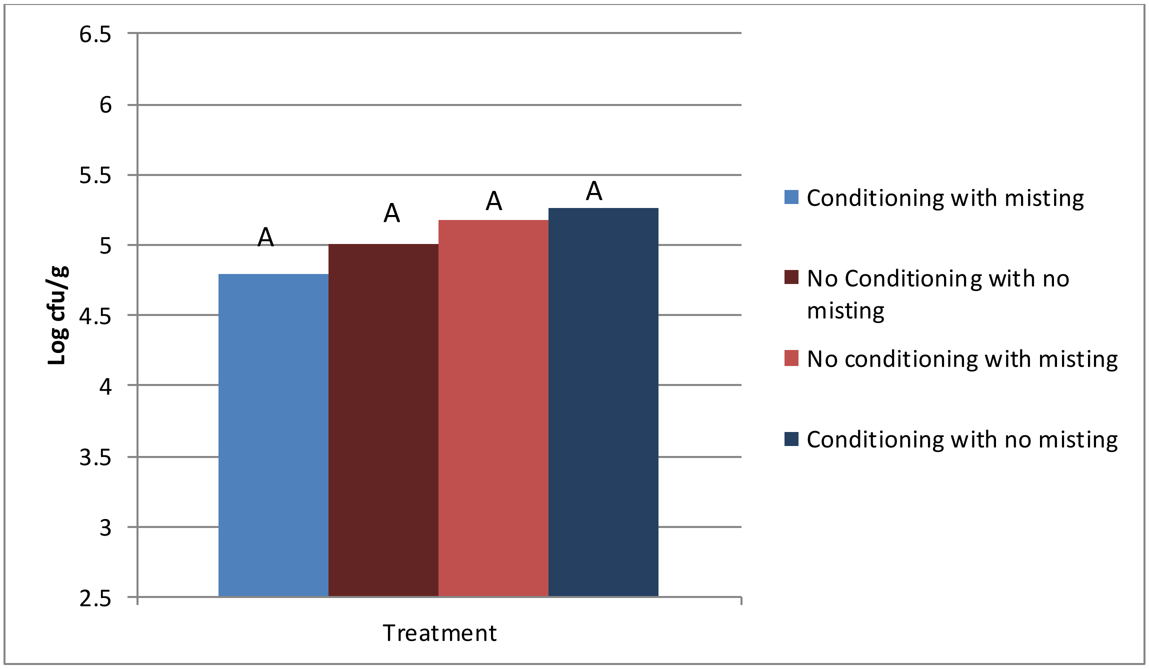

Figure 2.13: Average Log_{10} colony forming units per gram (cfu/g) of *Enterobacteriaceae* found in asparagus during 72 hours of grocery store produce rack storage

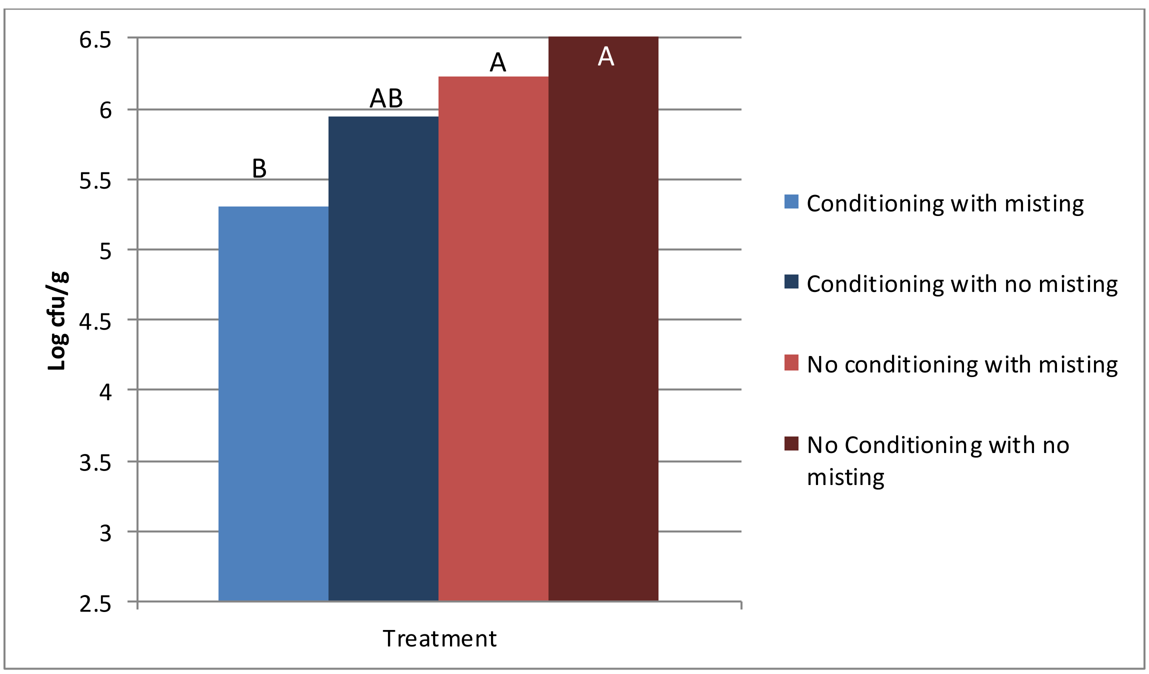

Figure 2.14: Average Log_{10} colony forming units per gram (cfu/g) of aerobic mesophilic bacteria found in asparagus during 72 hours of grocery store produce rack storage

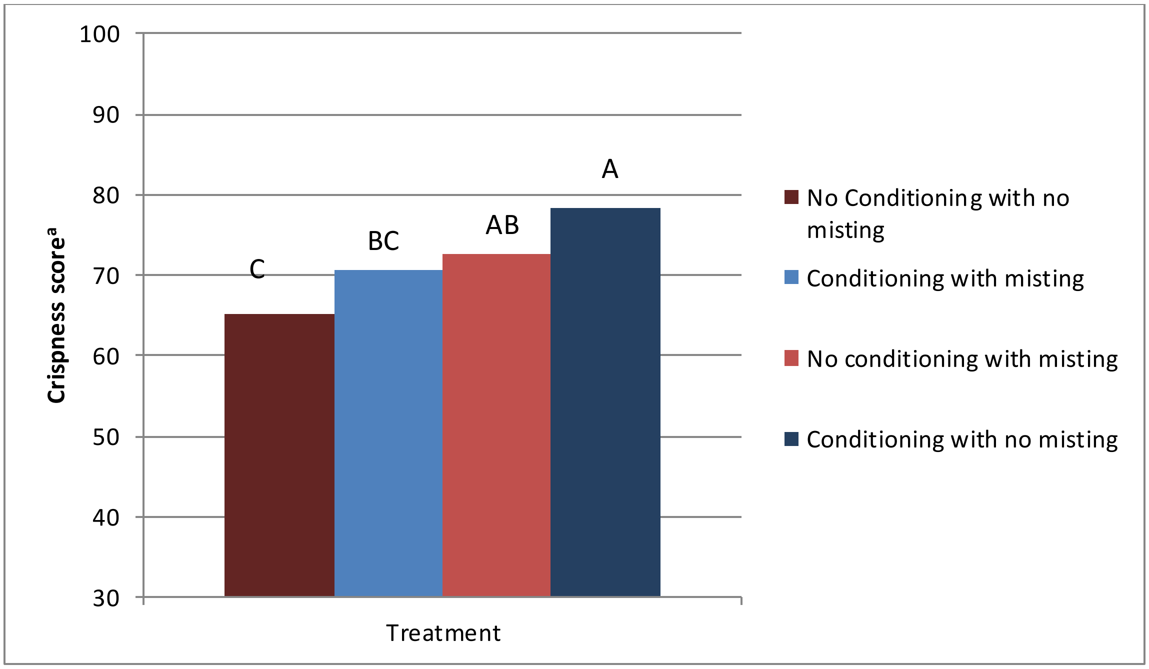

Figure 2.15: Average crispness sensory score of asparagus during 72 hours of grocery store produce rack storage

a = sensory score as measured by a non-numeric anchored line scale with the score converted to a score out of 100

Celery

Psychrotrophic bacteria counts on celery varied by about 0.6 log cfu/g depending on the treatment applied, however the treatments could not be statistically differentiated due to a high degree of variability in the data. Figure 2.17 illustrates how fungi counts on celery were decreased by treating the celery with conditioning. Treatments including conditioning produced fungi counts of 3.3 and 3.1 log cfu/g on celery, while treatments that did not include conditioning produced fungi counts of 3.9 and 4.1 log cfu/g on celery.

Figures 2.18 and 2.19 indicate that *Enterobacteriaceae* and aerobic mesophilic bacteria counts on celery were not significantly affected by the treatment applied to the celery. A high variability in results may have decreased statistical significance of differences between the treatments. Sensory results indicated that conditioning with no misting produced celery with the most crispness, which was statistically significantly more crisp than that treated with no conditioning and no misting.

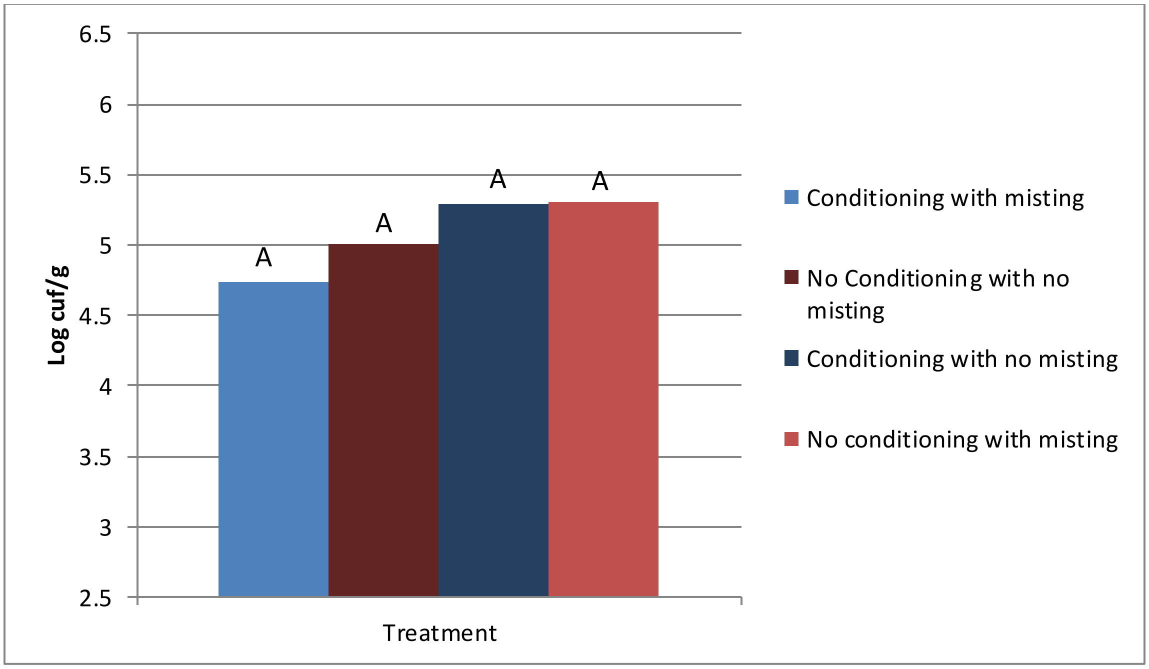

Figure 2.16: Average Log_{10} colony forming units per gram (cfu/g) of psychrotrophic bacteria found in celery during 72 hours of grocery store produce rack storage

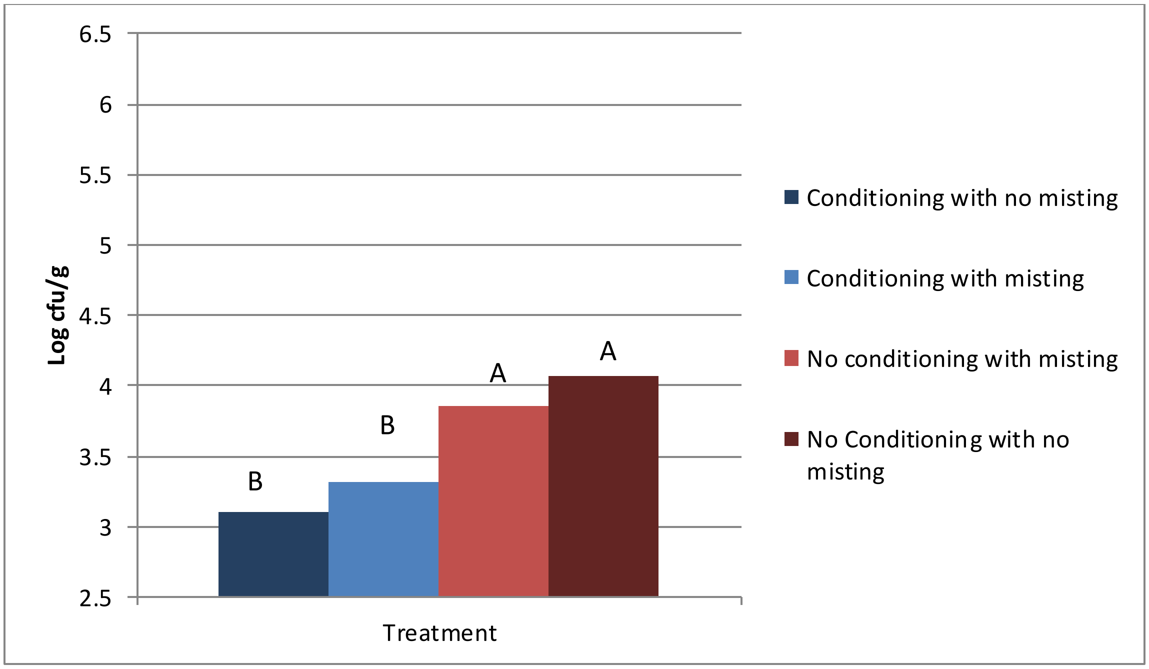

Figure 2.17: Average Log_{10} colony forming units per gram (cfu/g) of fungi found in celery during 72 hours of grocery store produce rack storage

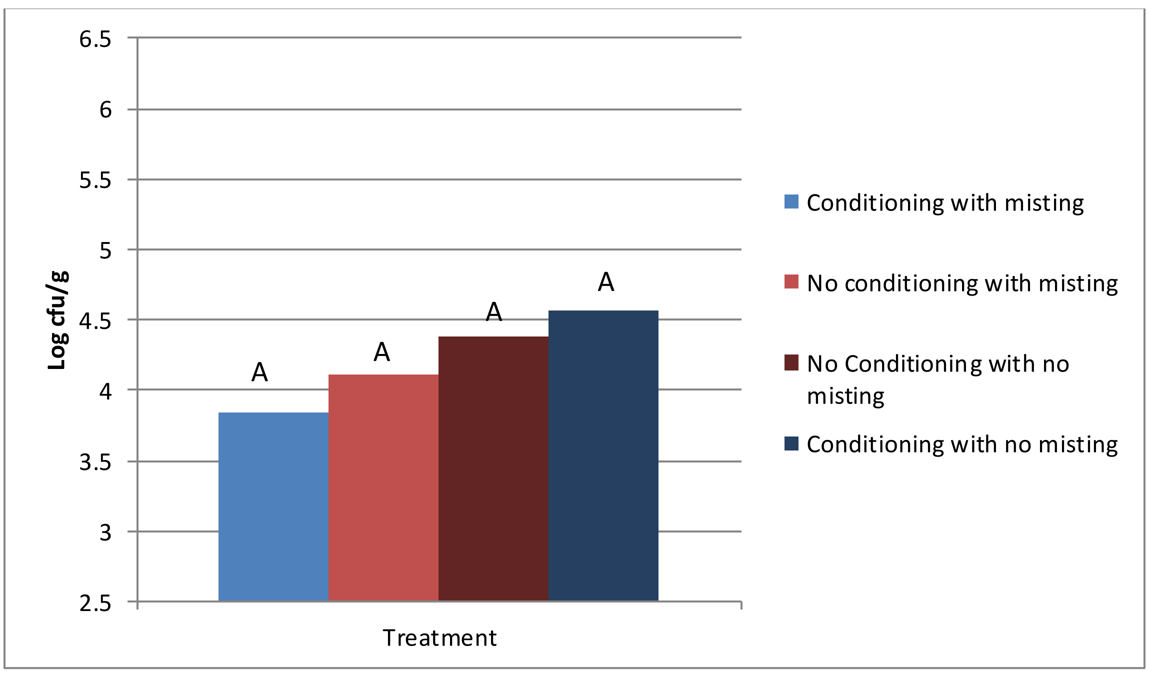

Figure 2.18: Average Log_{10} colony forming units per gram (cfu/g) of *Enterobacteriaceae* found in celery during 72 hours of grocery store produce rack storage

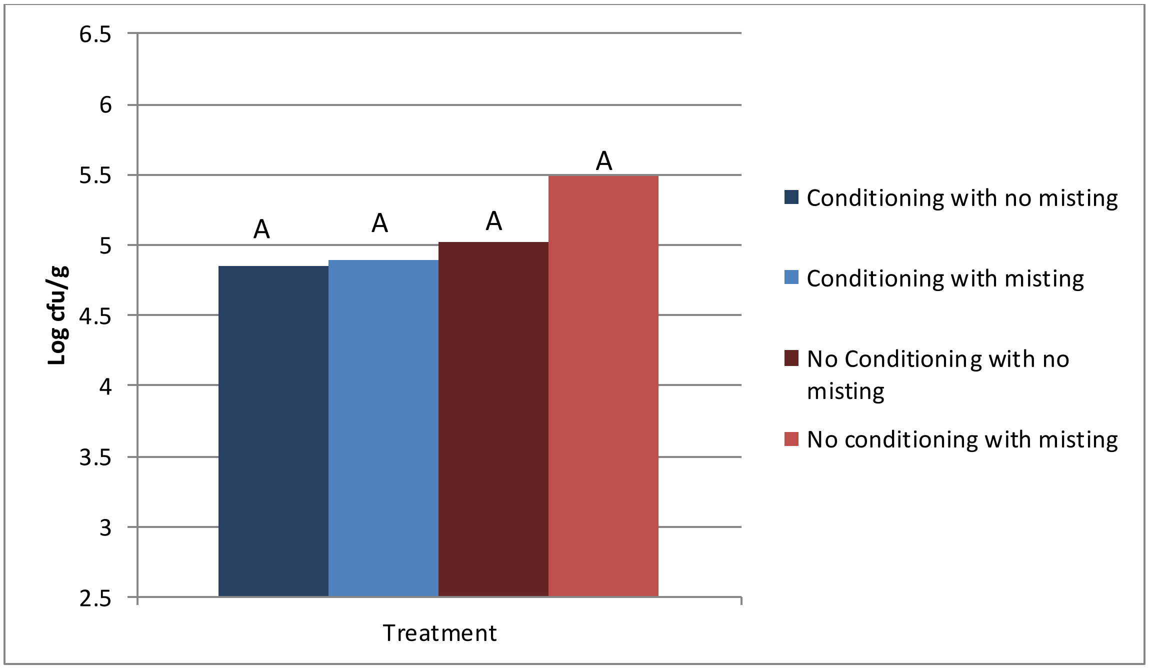

Figure 2.19: Average Log_{10} colony forming units per gram (cfu/g) of aerobic mesophilic bacteria found in celery during 72 hours of grocery store produce rack storage

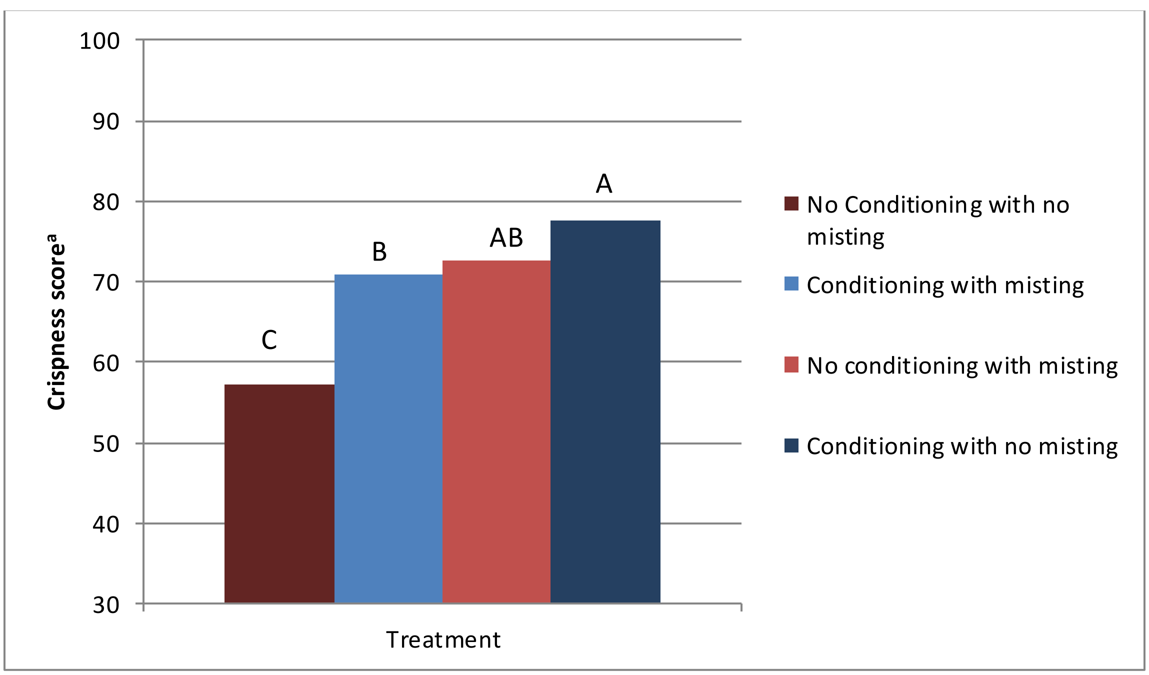

Figure 2.20: Average crispness sensory score of celery during 72 hours of grocery store produce rack storage

a = sensory score as measured by a non-numeric anchored line scale with the score converted to a score out of 100

2.5 – Discussion

Sensory analysis of crispness

Treating four types of produce with four different treatments during simulated grocery store produce rack storage results in significant differences in the crispness of this produce during the average day on the produce rack. Figure 2.5 indicates how the four different treatments affected the crispness of red leaf lettuce. The treatment that contained conditioning and no misting produced the most crisp red leaf lettuce. Both treatments that did not contain conditioning produced red leaf lettuce that was significantly less crisp than the red leaf lettuce that was conditioned without misting. Results from romaine lettuce were similar; romaine lettuce that was conditioned without misting was the most crisp during the average day on the produce rack. The results for both types of lettuce indicate that conditioning lettuce during grocery store produce storage can lead to an increase in crispness throughout a 72 hour shelf life. Misting for seven seconds every two minutes is potentially too frequent and is overhydrating the produce causing it to lose crispness rather than maintain it.

The sensory results for the crispness of asparagus indicated that conditioning with no misting produced the most crisp asparagus, while conditioning with misting and no conditioning with no misting produced the least crisp asparagus during the 72 shelf life. These results can be seen in Figure 2.15. The porous bottoms of asparagus allow them to soak up a large amount of water during conditioning which helps keep them hydrated and potentially more cri sp(Kader, 2002). This potentially explains why conditioning with no misting was the best treatment according to crispness scores, it does not explain why conditioning with misting produced lower scores. Results for the crispness of celery seen in figure 2.20 indicate that conditioning with no misting was a better treatment than both conditioning with misting and no conditioning with no misting. For all four types of produced, conditioning with no misting was the best overall treatment for maintaining the crispness of this produce, misting every two minutes for seven seconds was most likely overhydrating the produce, causing a loss of crispness when combined with a conditioning treatment.

Aerobic psychrotrophic bacteria

Aerobic psychrotrophic bacteria counts were analyzed because they are the organisms capable of growing at 4° C which is the storage temperature throughout fresh produce storage at the grocery store. They are the group of bacteria most associated with the spoilage of fresh produce (Cantwell & Suslow, 2002). Figure 2.1 indicates that treating red leaf lettuce with conditioning with no misting produced lower aerobic psychrotrophic bacteria counts over the three days storage time compared to no conditioning with no misting and no conditioning with misting treatments. Figure 2.6 shows that similarly, romaine lettuce treated with conditioning and no misting produced the lowest counts of aerobic psychrotrophic bacteria compared to romaine lettuce treated with no conditioning and no misting. This difference means that the growth of psychrotrophic bacteria such as *Pseudomonas* sp. is being inhibited by the conditioning treatment and this is potentially leading to an increased storage life of these types of lettuce due to a reduction in the counts of spoilage microorganisms (Beuchat & Ryu, 1997).

Figure 2.11 indicates how counts of psychrotrophic bacteria on asparagus remained above 10^6 cfu/g during the 72 hour storage time and were not significantly affected by any of the four treatments. This is most likely due to the fact that asparagus conditioning was different from the other three types of produce tested. Asparagus was only dipped into 1 to 1.5 inches of the conditioning solution compared to the other types of produce being fully immersed in the water. This means that the conditioning procedure only effectively washed the bottoms of the stems of the asparagus. This result for the psychrotrophic count indicates that the different treatments cannot significantly change the growth rate of psychrotrophic bacteria including potential spoilage microorganisms on asparagus. Results produced from celery for the counts of aerobic psychrotrophic bacteria were similar to asparagus, and no significant differences were seen. For asparagus and celery, these results indicate that these four differing treatments do not affect the counts of aerobic psychrotrophic bacteria and would most likely not affect the rate of spoilage of these types of produce during produce rack storage.

Aerobic mesophilic bacteria

Counts of aerobic mesophilic bacteria were analyzed because this is another important group of bacteria. Although this group's growth may be inhibited at refrigeration temperatures, they are still present and may be in high numbers due to growth while the produce was still in the field on the farm. Figure 2.4 indicates that the treatments that contained conditioning significantly reduced counts of aerobic mesophilic bacteria on red leaf lettuce compared to no conditioning with no conditioning. Similarly, conditioning with no misting produced the lowest counts of aerobic mesophilic bacteria on romaine lettuce compared to the other three treatments. These results for lettuce show that conditioning had a positive impact of reducing the counts of aerobic mesophilic bacteria during the course of a 72 hour simulated grocery store produce rack storage.

Aerobic mesophilic bacteria counts on asparagus were significantly reduced when conditioned with misting compared to both treatments that did not contain misting. There were no significantly differences for the counts of aerobic mesophilic bacteria on celery. This result for celery is the same as what was observed for the aerobic psychrotrophic bacteria count, indicating that regardless of treatment of celery, the counts of aerobic psychrotrophic and aerobic mesophilic bacteria were not affected.

Enterobacteriaceae

Counts of *Enterobacteriaceae* were analyzed because they are a preliminary safety indicator used by the food industry. *Enterobacteriaceae* contains pathogenic microorganisms including pathogenic species of *Escherichia* and *Salmonella* (Coburn B, 2007; Sivapalasingam, et al., 2004). Figure 2.3 indicates that counts of *Enterobacteriaceae* were not affected by the four different treatments on red leaf lettuce. Interestingly on romaine lettuce, conditioning with no misting reduced counts of *Enterobacteriaceae* compared to no conditioning with misting. This is a rare difference between how the two types of lettuce were affected by the treatments. Counts of *Enterobacteriaceae* on asparagus and celery were both not significantly affected by the four differing treatments. These results that on three of the four types of produce tested, *Enterobacteriaceae* counts were not significantly affected by the four differing treatments.

Fungi

Fungi populations were analyzed because they can be both a safety and spoilage indicator (Beuchat & Ryu, 1997). Figure 2.2 shows that fungi counts on red leaf lettuce were significantly reduced by treatments including conditioning compared to treatments that did not contain conditioning. The average log reduction of fungi on red leaf lettuce between conditioning and no conditioning was 1.1 log cfu/g. This reduction indicates that conditioning the red leaf lettuce every 24 hours was crucial to the removal of fungi from the produce. This reduction is potentially indicative of inactivation of both spoilage and pathogenic fungi on the red leaf lettuce. Results from romaine lettuce were the same as red leaf lettuce. Conditioning lettuce reduced counts of fungi compared to lettuce that was not conditioned. The same result can be seen on celery, conditioned celery had significantly lower counts of fungi compared to not conditioned celery.

Figure 2.12 shows how these conditioning treatments did not greatly affect the counts of fungi on asparagus. Conditioning with misting produced significantly lower counts of fungi on the asparagus during its 72 hour shelf life compared to asparagus that was treated with no conditioning and no misting. The difference was only 0.4 log cfu/g. These results generally indicate that on a microbial basis asparagus was not greatly affected by the conditioning treatment applied to it. This is in stark contrast to both types of lettuces that were tested, which were consistently affected by the type of treatment applied to them during their 72 hour storage in simulated grocery store conditions. This is most likely due to asparagus not being fully immersed in the conditioning water during conditioning. Asparagus is traditionally not fully immersed in water during conditioning due to the water quickly causing quality loss in the tips of the asparagus which are prone to microbial growth when they become too hydrated.

2.6 - Conclusion

Results from the analysis of aerobic psychrotrophic bacteria growing on asparagus, red leaf lettuce, celery and romaine lettuce during a 72 hour simulated grocery store produce rack storage indicate that differing treatments only affected the counts on the two lettuce's tested, but asparagus and celery were not affected. Results from red leaf and romaine lettuce indicated that conditioning with no misting was a better treatment for reducing psychrotrophic bacteria counts compared to no conditioning with no misting.

Results from the analysis of fungi counts indicated that conditioning celery, romaine lettuce and red leaf lettuce significantly reduced the counts of fungi found on the produce compared to produce that was not conditioned. Results for aerobic mesophilic bacteria counts indicated that in general, conditioning these fresh vegetables reduced the counts found on the produce compared to unconditioned treatments. Results for *Enterobacteriaceae* count indicated that regardless of treatment on three of the four types of produce, no significant differences in count were found. Sensory results indicate that on all four types of produce, conditioning with no misting produced the most crisp produce, with no conditioning with no misting producing the lowest average crispness.

These results indicate that conditioning produce during storage on a grocery store produce rack improved important traits of the produce including crispness and microbial load of important microorganisms. The effect of misting was harder to distinguish due the misters being set to spray every two minutes which was perhaps too frequent causing a loss of crispness in some cases when produce was also conditioned.

2.7 References

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Chapter 3 - Conditioning with electrolyzed water and contribution to the microbiological quality of fresh produce during simulated storage in retail establishments and consumer's home

3.1 – Abstract

The safety and quality of fresh produce sold at grocery stores is a point of competition between many grocery store chains. Companies that can offer the most fresh, safest and least-expensive fresh produce are well-equipped to increase their customer base. Grocery stores may use antimicrobial agents to improve the quality of fresh produce and one of these agents is electrolyzed water. Solutions of antimicrobial agents may be used during washing or misting of fresh produce while it is on the produce rack shelves. Washing the produce with electrolyzed water is also referred to as conditioning and is typically done daily while the produce is held on the produce rack. Misting is performed periodically with diluted electrolyzed water sprayed onto the produce on the produce rack. This research analyzed the effectiveness of electrolyzed water as the conditioning agent versus tap water, along with the impact of trimming.

Asparagus, red leaf lettuce and romaine lettuce were the fresh produce used for this research. The four conditioning treatments tested were (i) electrolyzed water with the produce stems intact, (ii) electrolyzed water with the stems trimmed, (iii) tap water with the stems intact, and (iv) tap water with the stems cut. Conditioning was performed every 24 hours during a 72 hour produce rack storage; misters periodically sprayed the produce throughout those 72

hours. The produce was then transferred to refrigerated storage for seven days to simulate consumer handling of purchased products.

Sensory results of the crispness of the produce also did not indicate any significant difference between using electrolyzed water versus tap water as the conditioning agent. Microbial results indicated that electrolyzed water was not more effective than tap water at reducing the counts of psychrotrophic bacteria on any of the types of produce during both the produce rack storage and the refrigerated storage. Results did indicate that electrolyzed water was effective at reducing counts of *Enterobacteriaceae* and fungi on all three types of produce during the produce rack storage time period and this result carried over for the most part to the refrigerator storage time period as well.

3.2 – Introduction

The sale of fresh produce in grocery stores accounts for billions of dollars every year in the United States of America (Bruhn, 2002). Hundreds of different kinds of fresh produce are available at grocery stores with their own unique characteristics, cooking applications and safety concerns (Cook, 2002). Maintaining the freshness and safety of the produce is of absolute importance to grocery stores around the world (Cook, 2011a).

Fresh produce retail brings unique challenges to grocery companies involved. The nature of selling fresh and minimally processed produce causes these products to be susceptible to microbial contamination that contribute to safety and spoilage problems (Cantwell & Suslow, 2002; Harris, et al., 2002). Pathogenic bacteria commonly inhabit fresh produce and are not effectively eliminated due to the lack of processing before the product is sold (Cantwell & Kasmire, 2002). Fresh produce can also be contaminated with spoilage microorganisms such as *Pseudomonas* sp. (Beuchat & Ryu, 1997). Electrolyzed water is applied to fresh produce in some grocery stores to improve the crispness of the produce and decrease microbial counts (Gomez-Lopez, 2012).

This research was initiated to analyze the impact of using electrolyzed water, rather than tap water, to condition and mist fresh produce while it is held in the grocery store. The impact of these treatments was determined by microbiological analysis to determine bacterial and fungal counts, and sensory analysis to measure product crispness. These tests were done during simulation of fresh produce storage for three days in retail establishment, followed by a seven-day refrigerated storage simulating consumer handling of these products. The impact of trimming lettuce heads and asparagus stalks during conditioning was also analyzed.

3.3 - Materials and methods

Experimental

In order to simulate the environment during holding fresh produce in retail establishments, an industrial produce rack equipped with water spraying misters and a refrigeration unit was installed in a pilot plant at Ohio State University. The produce rack was calibrated to maintain 3° C during experimentation. The misters sprayed the produce with electrolyzed water for seven seconds every 15 minutes. Electrolyzed water was produced on site via an electrolyzing water unit that produced a solution of 50 parts per million (ppm) chlorine, which was used for the conditioning treatments. This electrolyzed water was diluted to 4 ppm chlorine for use in the misting system. The produce rack was equipped with a vertical plastic barrier in the center in order to allow for testing of two different treatments at the same time. In order to simulate a consumer's home storage conditions, a walk-in refrigerator maintained at 4°C was used. Testing took place over the course of six consecutive weeks. Three replicates were completed for each of the four treatments, with two different treatments randomly tested each week.

Romaine lettuce, Red leaf lettuce and asparagus were acquired, within three days of harvest, from a regional distribution center and transported to Ohio State University where the produce was immediately transferred to a walk-in refrigerator set at 4° C. Using simulated grocery store procedures, the produce was prepared for storage on the grocery store produce rack. Produce was conditioned following set procedures depending on the type of produce. Romaine and red leaf lettuce were conditioned following the same procedure. Lettuce was first inspected for physical damage or molding on the outer leaves, any of these leaves that were deemed damaged enough to prevent someone from buying that piece of produce at the grocery store were removed. In the produce designated as “conditioned,” the browned cut-surface on lettuce head bottoms were cut down by one centimeter to reveal a fresh white bottom. This cutting step was skipped in the “soaking” treatment produce. The lettuce heads were then transferred to 10 gallon tubs containing the 50 ppm chlorine electrolyzed water solution. They were fully immersed and soaked for 15 minutes. Once the electrolyzed water soaking was complete, the lettuce was removed and gently shaken to remove water and placed on the produce rack in a standardized pattern.

The asparagus conditioning procedure was slightly different. The bottoms of the asparagus stalks were inspected and the bottoms were cut down by 1.5 centimeters for the batches designated “conditioning”. This step was skipped for the batches designated “soaking”. The asparagus was then transferred to a 10 gallon tub containing 50 ppm chlorine electrolyzed water at a depth of about 2 inches. The asparagus bunches were placed standing up in the water to prevent water from over hydrating the tips of the asparagus. The asparagus was soaked for 45 minutes before being returned to the produce rack and placed in a standardized pattern.

The produce was always loaded into the produce rack following the same pattern in order to ensure consistent results. Asparagus was loaded on the top shelf, with red leaf lettuce on the middle shelf and romaine lettuce on the bottom shelf. The produce was bunched together tightly on the shelf to simulate a grocery store environment. The produce was all oriented the same direction. Whenever produce was removed from the rack to be used for experimentation, extra produce that had been kept in the walk-in refrigerator was used to replace it. These replacement pieces were marked and any other pieces of produce that came in contact with them were not used for subsequent testing. The replacement pieces were used to ensure consistent density of the produce within the rack. Picture 1 illustrates the produce rack used to simulate a grocery store setting.

Picture 3.1: Example of the simulation of grocery store rack fresh produce storage[a]
[a] = celery was not present in this phase of testing, only the top three shelves were used

Once the 72 hour storage was complete, the pieces of produce were individually bagged in plastic grocery store bags, allowed to sit at room temperature for one hour, and then transferred to the 4° C refrigerator. This was done to simulate a grocery store customer purchasing the produce, transporting it to their home, and storing it in their home refrigerator. The bags of produce were not touched until they were individual taken for analysis after three, five and seven days of storage in the refrigerator.

Sample preparation for microbiological and sensory analysis

Produce was analyzed by microbiological and sensory methods at 0, 24, 48 and 72 hours of storage on the produce rack. The produce was then tested after three, five and seven days of storage in the walk-in refrigerator for a total of 10 days of storage with seven testing points for each batch of produce. The zero hour was defined as when the process of loading the produce rack began, before any conditioning treatments were applied. Aseptically handled pieces of produce were randomly selected and cut in half for testing. One half of the piece of produce was used for sensory analysis, while the other half was used for microbiological analysis. Sensory analysis was always performed within one hour after cutting occurred. Microbiological samples were aseptically stored at 4° C and testing was completed within three hours after cutting occurred.

Sensory analysis

A trained panel of eight people was used for sensory analysis. Between four and seven of these panelists attended each sensory analysis session. Panelists were trained to examine only the crispness of the produce. They were allowed to touch and manipulate the produce to help them assign a score, breaking of the pieces of produce was allowed. Scores were marked on an anchored non-number line scale. The marks the panelists made were then measured and converted to a score out of 100 for crispness of the produce on that individual day.

Microbiological analysis

Pieces of produce used for microbiological analysis were homogenized and serially diluted in buffered peptone water to be plated for enumeration on agar plates. The groups of bacteria that were tested for were aerobic psychrotrophic, aerobic mesophilic, *Enterobacteriaceae* and fungi. Aerobic mesophilic and psychrotrophic were plated on tryptic soy agar (Becton, Dickinson and Company, Franklin Lakes, New Jersey) and incubated at 37° C for 48 hours and 4° C for ten days respectively. Testing for *Enterobacteriaceae* was done using Violet Red Bile Agar (EMD Millipore, Billerica, Massachusetts) incubated at 37° C for 48 hours. Fungi testing was done using Dichloran Glycerol 18% Agar (DG18) (Oxoid, Hampshire, England) incubated at 23° C for five days.

Statistical analysis

Three replicates were completed for each of these four treatments. Statistical analysis was performed using the program SAS (SAS Institute, Inc., Cary, North Carolina) Version 9.3. An advanced generalized linear model was used that accounted for the random effected created by the six separate weeks of testing. The model used was:

Sensory score or microbiological count = treatment applied + day of analysis + weekly batch.

A Tukey's analysis was performed and the significant differences are represented in the figures below by differing letters associated with each treatment. A 95% confidence interval was used to calculate significant differences. Data from the 24, 48 and 72 hour time points during produce rack storage were grouped together to improve statistical power. Data from the three, five and seven day time points of refrigerator storage was also grouped together to improve statistical power. The day 0 counts were not included in the results seen below.

3.4 – Results

Asparagus

Figures 3.1 illustrates how aerobic psychrotrophic bacteria counts were affected by four different conditioning and misting treatments applied to the produce during its storage time on the produce rack. Results show that treating the asparagus with electrolyzed water with soaking produced higher counts of psychrotrophic bacteria compared to both treatments that used tap water. Figure 3.2 shows how these results changed during the asparagus's subsequent storage in a refrigerator simulating a consumer purchasing the asparagus and bringing it home. In all four treatments, the counts of psychrotrophic bacteria increased by one log or more. The results indicate that electrolyzed water with soaking produced significantly higher counts of psychrotrophic bacteria on asparagus than tap water with soaking during the seven days of refrigerator storage.

Aerobic mesophilic bacteria counts were not significantly affected by the differing treatments applied to asparagus during the 72 hours of produce rack storage. Considering data in Figure 3.3; there are no significant differences between the four treatments. Figure 3.4 shows that asparagus treated with electrolyzed water with conditioning produced significantly lower counts of aerobic mesophilic bacteria compared to tap water with conditioning. Counts during the seven day refrigerator storage were about 0.75 logs higher than counts from the produce rack storage time.

Fungi counts on asparagus were also not significantly affected by the different treatments applied to asparagus. This was observed for both the 72 hour produce rack storage and the seven day refrigerator storage. Figures 3.5 and 3.6 illustrate this result and show that average fungi counts started as low as 2.7 log cfu/g during produce rack storage and increased by less than a log during refrigerator storage.

Enterobacteriaceae counts were not significantly affected by the differing treatments applied to asparagus during the 72 hours of produce rack storage. Figure 3.7 shows that regardless of treatment *Enterobacteriaceae* counts remained approximately 4.2 log cfu/g. Figure 3.8 indicates that during the seven day refrigerator storage, a significant difference in *Enterobacteriaceae* counts was found. Asparagus treated with electrolyzed water with conditioning produced significantly lower *Enterobacteriaceae* counts compared to both treatments that used tap water instead of electrolyzed water.

Results from the sensory analysis to evaluate the crispness of the asparagus produced no significant differences between the treatments. Figures 3.9 and 3.10 indicate that average crispness scores for the asparagus during its 72 hour produce rack storage were around 78 out of 100, while scores from the subsequent refrigerator storage dipped to around 73 on average.

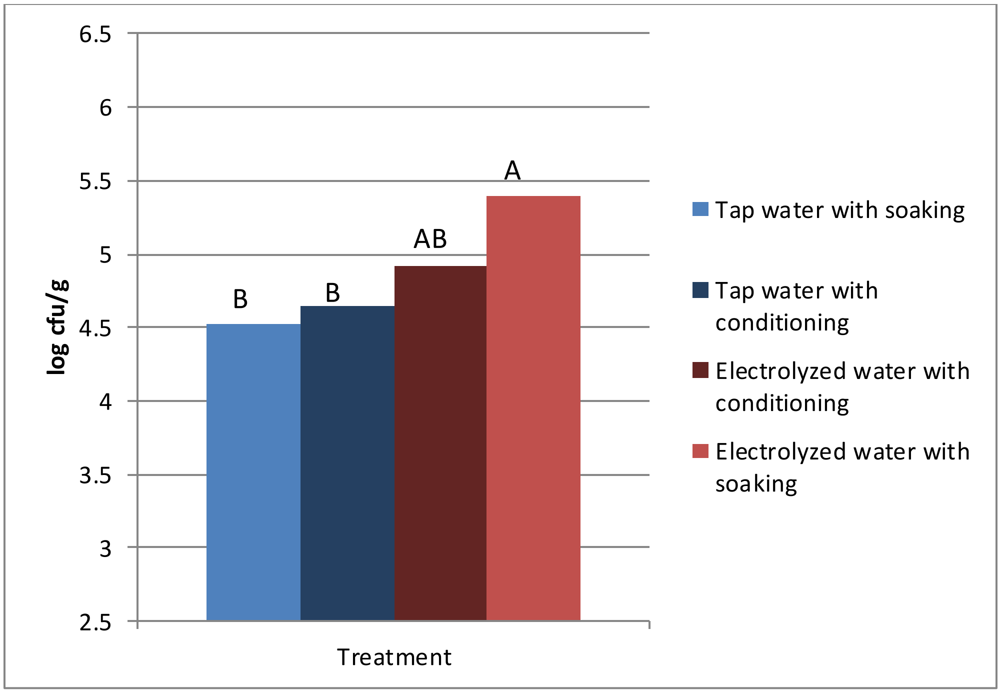

Figure 3.1: Average Log_{10} colony forming units per gram (cfu/g) of psychrotrophic bacteria found in asparagus during 72 hours of grocery store produce rack storage.

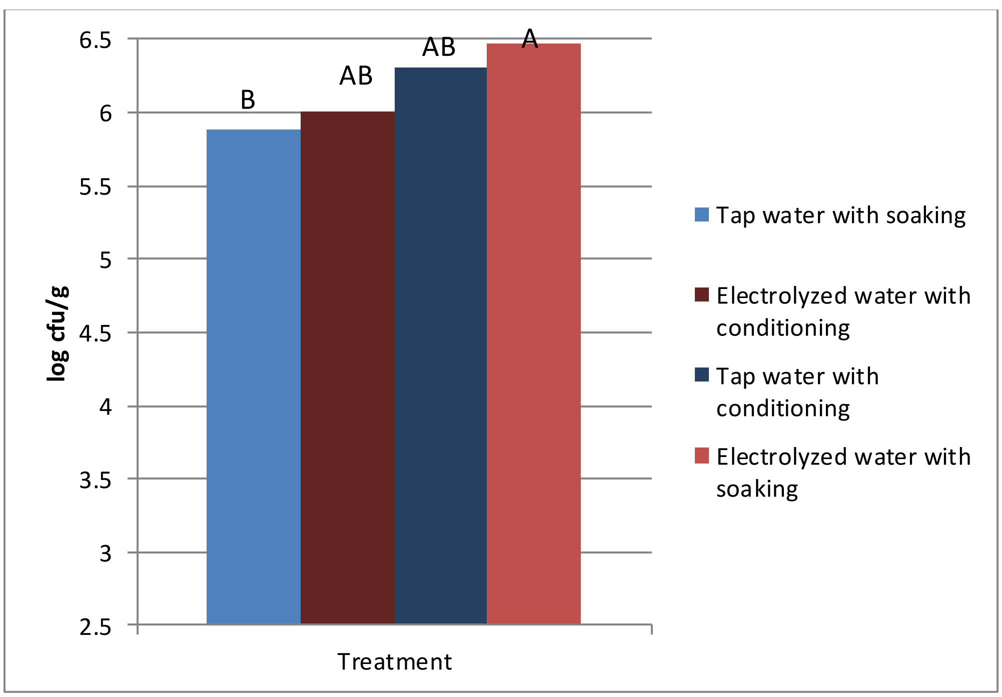

Figure 3.2: Average Log_{10} colony forming units per gram (cfu/g) of psychrotrophic bacteria found in asparagus during seven days of refrigerated storage following 72 hours of produce rack storage.

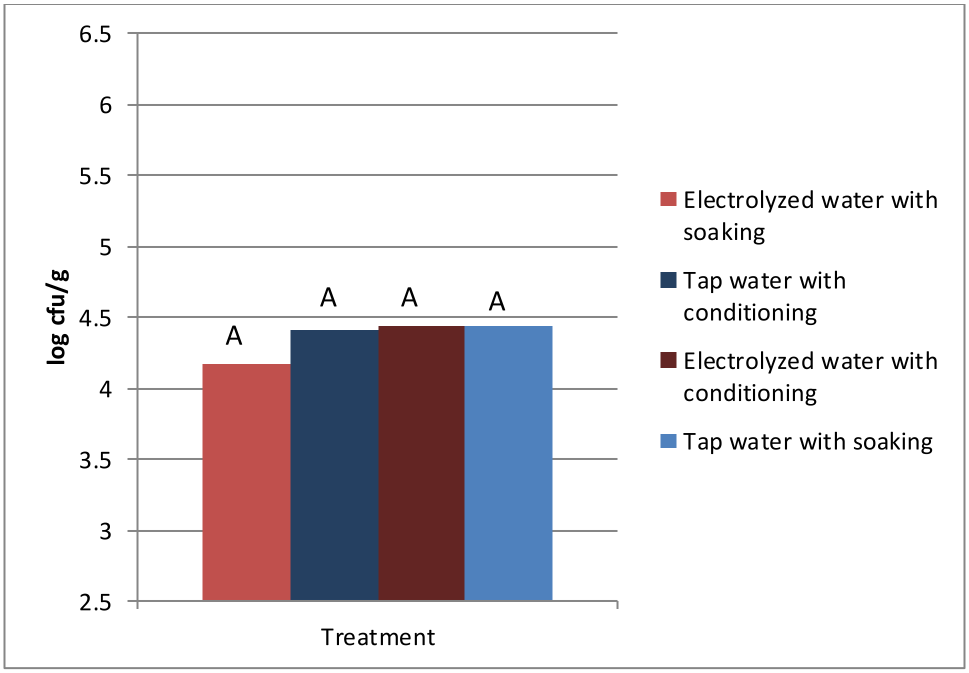

Figure 3.3: Average Log_{10} colony forming units per gram (cfu/g) of aerobic mesophilic bacteria found in asparagus during 72 hours of grocery store produce rack storage.

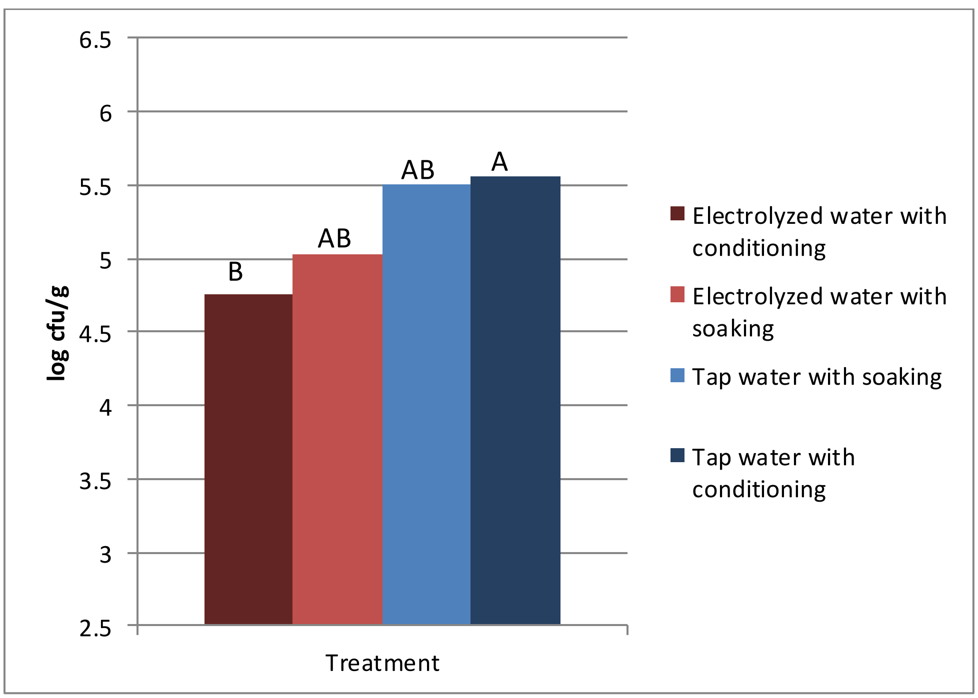

Figure 3.4: Average Log_{10} colony forming units per gram (cfu/g) of aerobic mesophilic bacteria found in asparagus during seven days of refrigerated storage following 72 hours of produce rack storage.

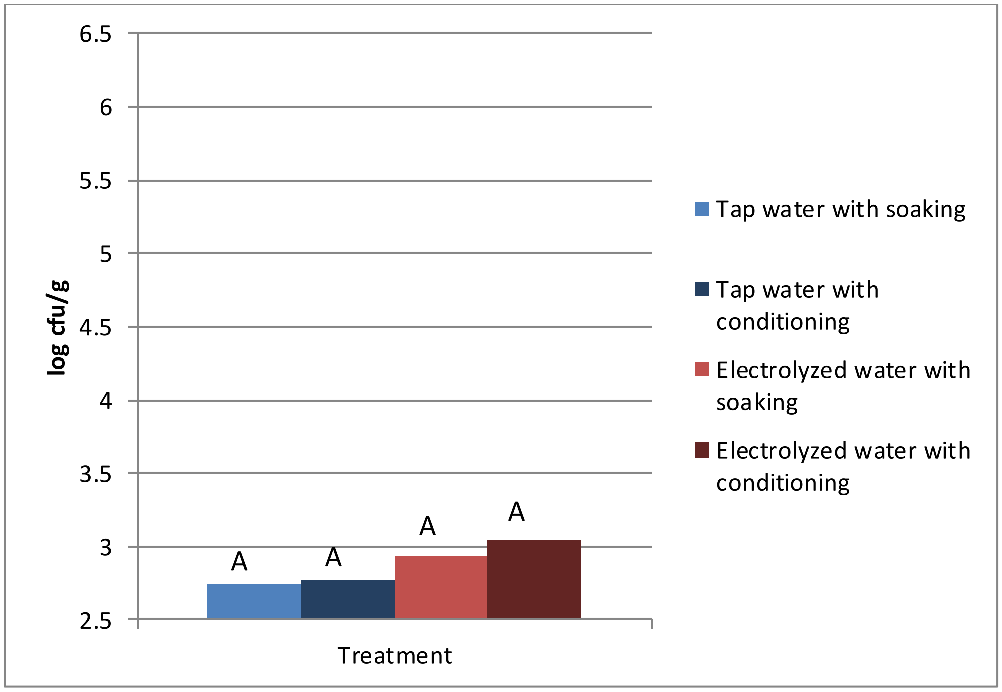

Figure 3.5: Average Log_{10} colony forming units per gram (cfu/g) of fungi found in asparagus during 72 hours of grocery store produce rack storage.

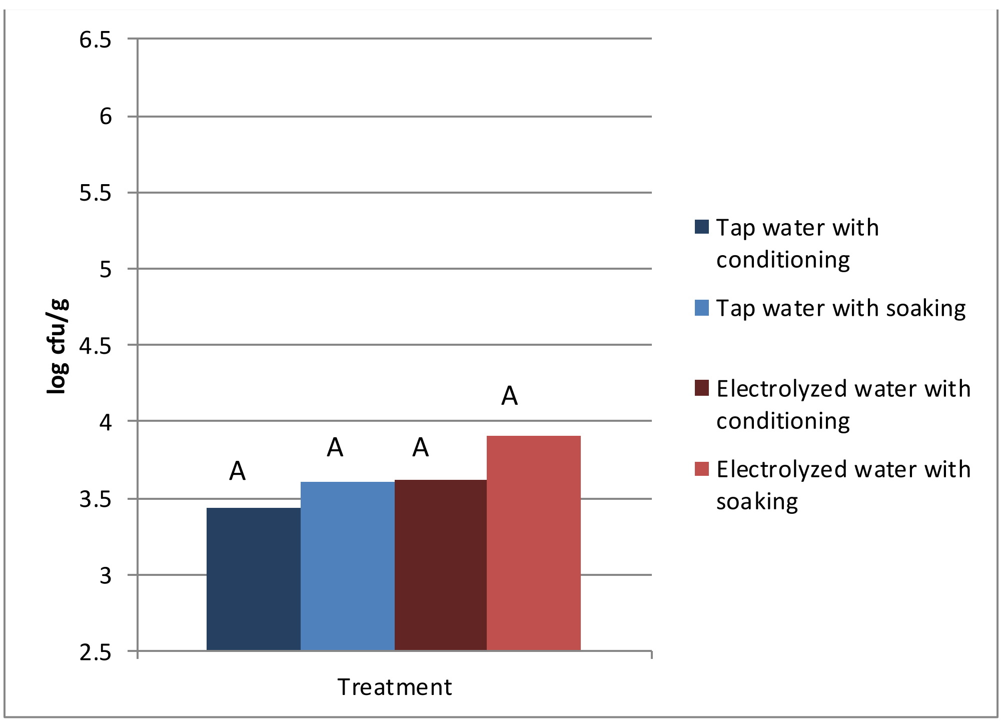

Figure 3.6: Average Log_{10} colony forming units per gram (cfu/g) of fungi found in asparagus during seven days of refrigerated storage following 72 hours of produce rack storage.

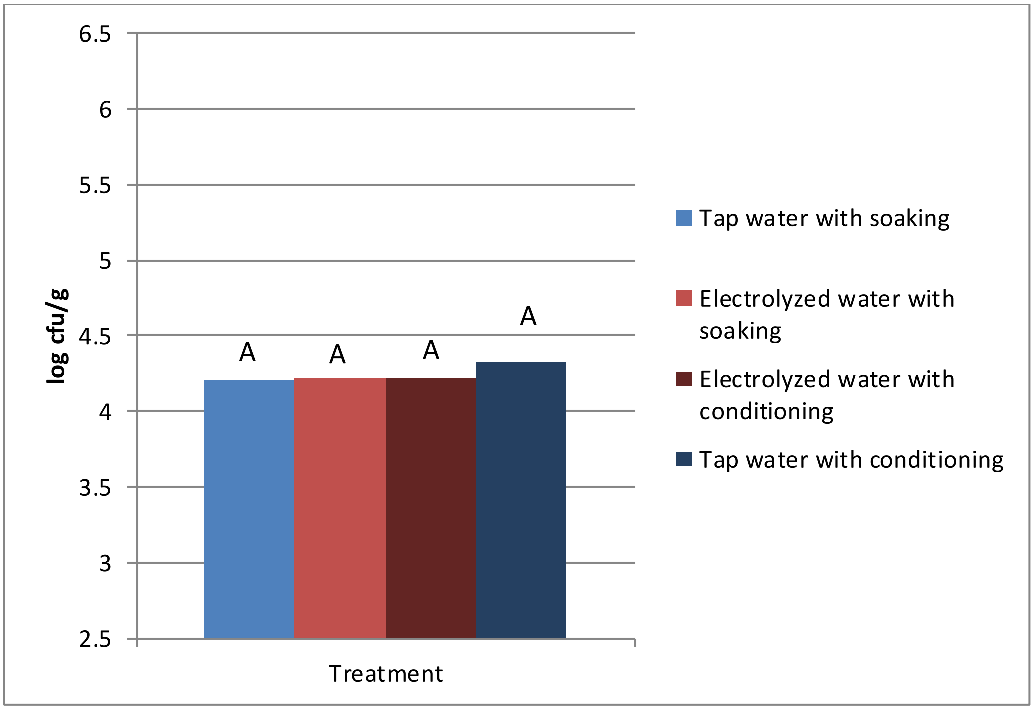

Figure 3.7: Average Log_{10} colony forming units per gram (cfu/g) of *Enterobacteriaceae* found in asparagus during 72 hours of grocery store produce rack storage.

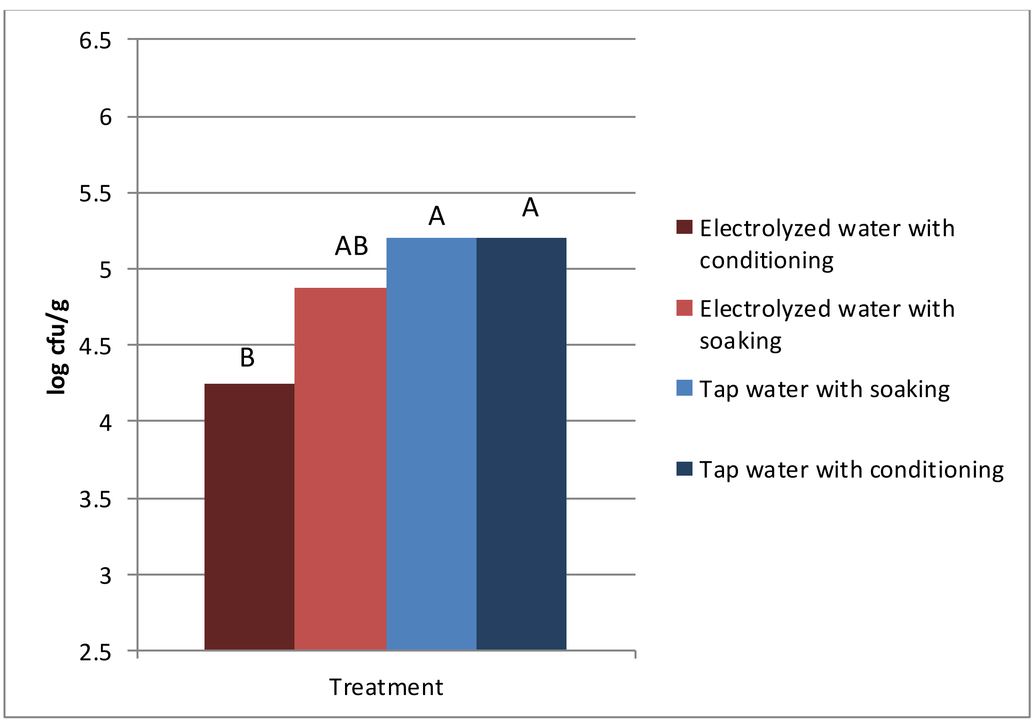

Figure 3.8: Average Log_{10} colony forming units per gram (cfu/g) of *Enterobacteriaceae* found in asparagus during seven days of refrigerated storage following 72 hours of produce rack storage.

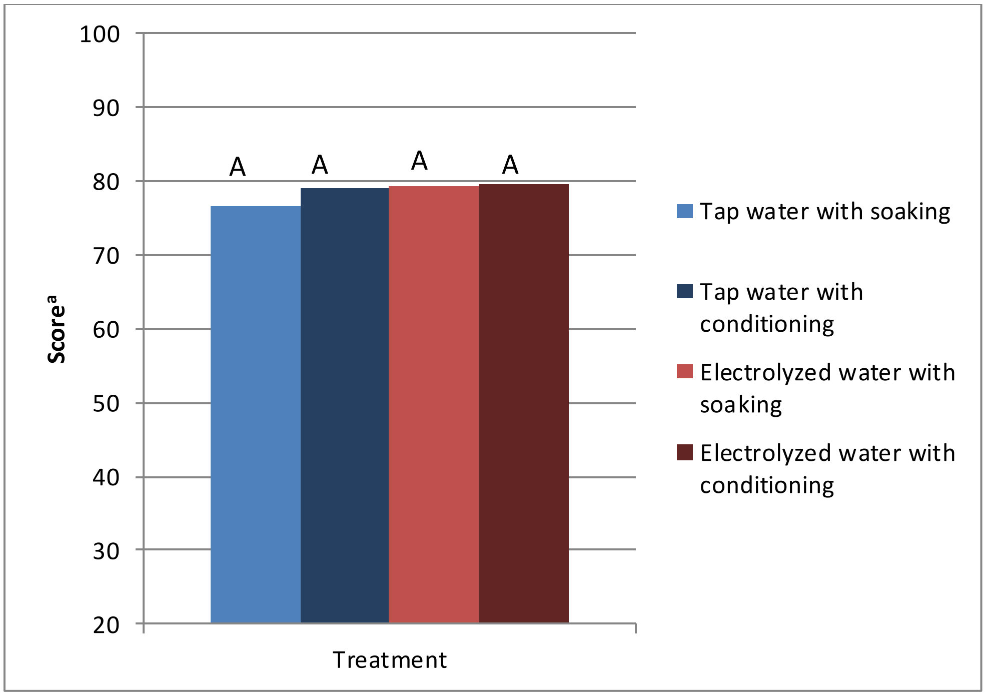

Figure 3.9: Average crispness sensory score of asparagus during 72 hours of grocery store produce rack storage.

a = sensory score as measured by a non-numeric anchored line scale with the score converted to a score out of 100

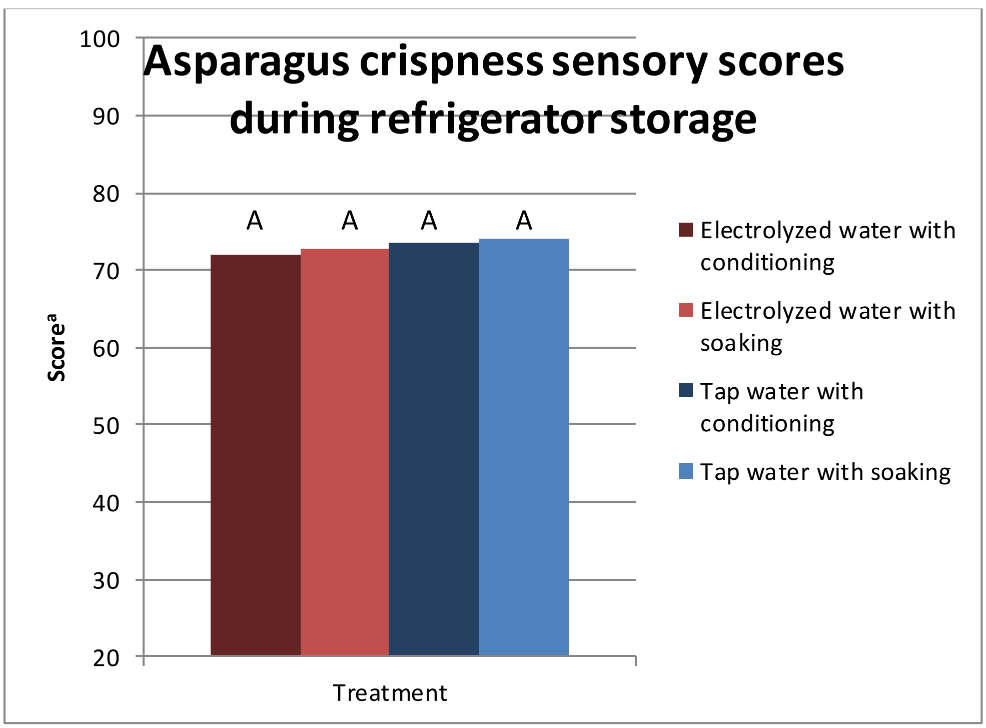

Figure 3.10: Average crispness score of asparagus during seven days of refrigerated storage following 72 hours of produce rack storage.

a = sensory score as measured by a non-numeric anchored line scale with the score converted to a score out of 100

Red leaf lettuce

Figures 3.11 and 3.12 indicate how the counts of aerobic psychrotrophic bacteria were affected by differing treatments that were applied to red leaf lettuce during a 72 hour produce rack storage followed by a subsequent seven day storage in the refrigerator. No significant differences in counts were noted during either the 72 hour produce rack storage or the seven day refrigerator storage. Average psychrotrophic counts did increase by 1.5 log cfu/g between the days on the produce rack against the days in the refrigerator.

Figure 3.13 shows that applying electrolyzed water treatments to the red leaf lettuce during its 72 hour produce rack storage significantly reduced aerobic mesophilic bacteria counts compared to red leaf lettuce treated with tap water. This significant different partially carried over to average aerobic mesophilic counts during the seven day refrigerator storage (Figure 3.14). Red leaf lettuce treated with electrolyzed water with soaking had significantly lower counts of aerobic mesophilic bacteria compared to both treatment groups involving tap water.

Fungi counts on red leaf lettuce were significantly affected by the type of water applied to red leaf lettuce during its produce rack storage. Figure 3.15 indicates that red leaf lettuce treated with electrolyzed water produced average fungi counts of about 2.4 log cfu/g, while the tap water treated red leaf lettuce produced average counts of 3.5 log cfu/g. This difference was also apparent during the subsequent seven day refrigerator storage. Figure 3.16 indicates that red leaf lettuce treated with electrolyzed water produced average fungi counts of 2.9 log cfu/g, compared to the tap water treated red leaf lettuce produced average counts of 3.7 log cfu/g.

Enterobacteriaceae counts on red leaf lettuce were affected in a pattern similar to what was observed in fungi counts during the 72 hour produce rack storage. Figure 3.17 indicates that red leaf lettuce treated with electrolyzed water produced significantly lower counts of

Enterobacteriaceae compared to the two treatment groups using tap water. Figure 3.18 indicates that this difference was partially carried over to *Enterobacteriaceae* counts on the red leaf lettuce during the seven day refrigerator storage, the electrolyzed water with conditioning treatment produced significantly lower counts compared to the tap water with soaking treatment.

Sensory analysis of the crispness of the red leaf lettuce indicated that tap water with conditioning produced significantly crisper produce compared to electrolyzed water with conditioning during the 72 hour produce rack storage (Fig. 3.19). Figure 3.20 illustrates that no significant differences were seen in the crispness of red leaf lettuce regardless of the treatment applied before the seven day refrigerator storage.

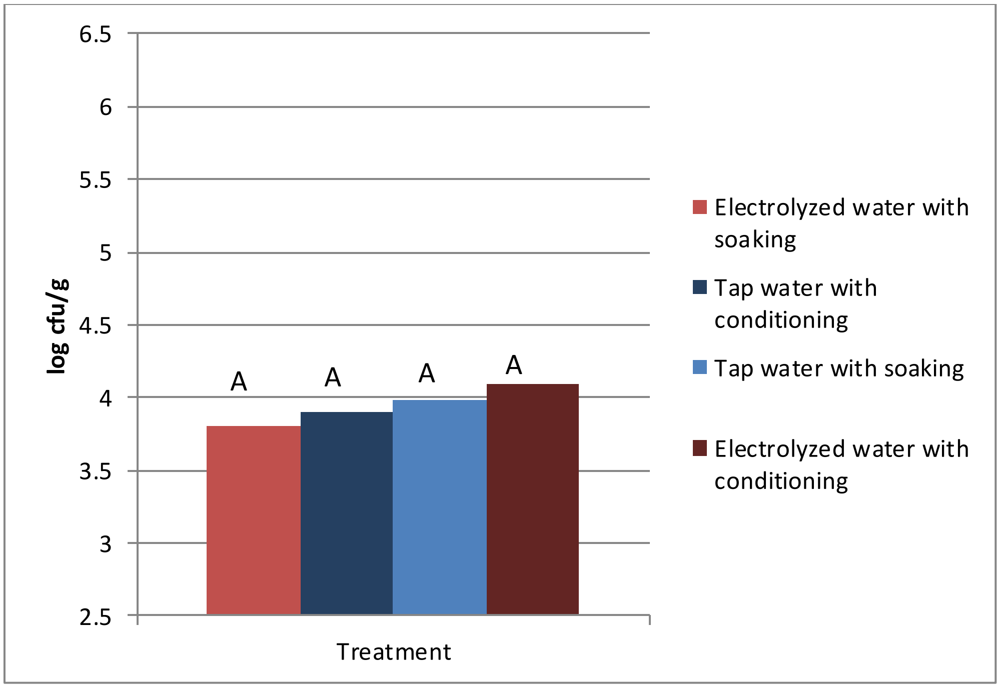

Figure 3.11: Average Log_{10} colony forming units per gram (cfu/g) of psychrotrophic bacteria found in red leaf lettuce during 72 hours of grocery store produce rack storage.

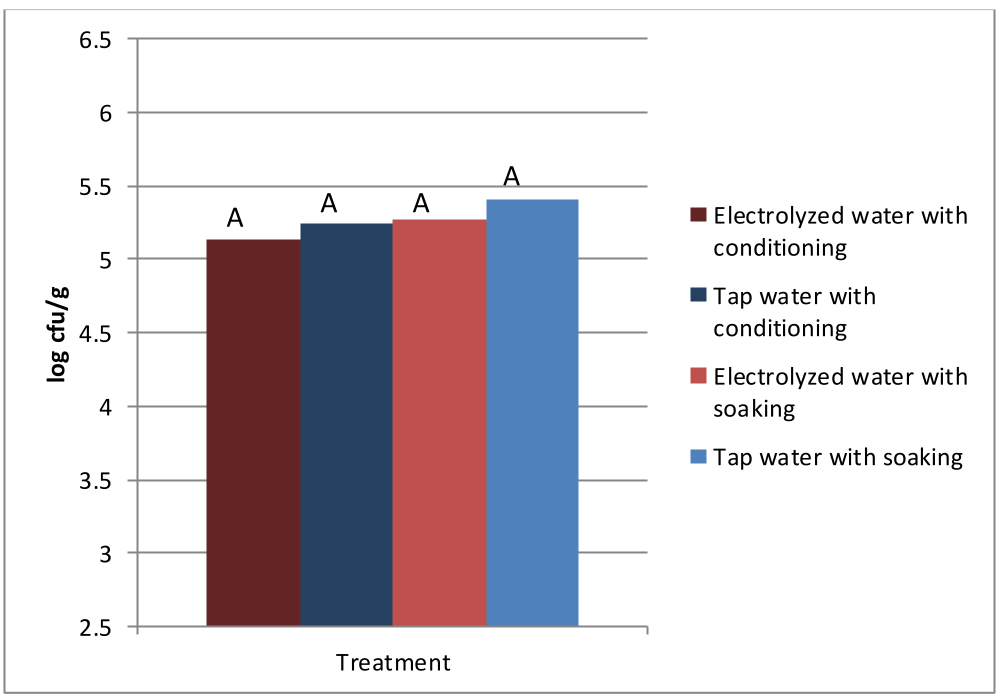

Figure 3.12: Average Log_{10} colony forming units per gram (cfu/g) of psychrotrophic bacteria found in red leaf lettuce during seven days of refrigerated storage following 72 hours of produce rack storage.

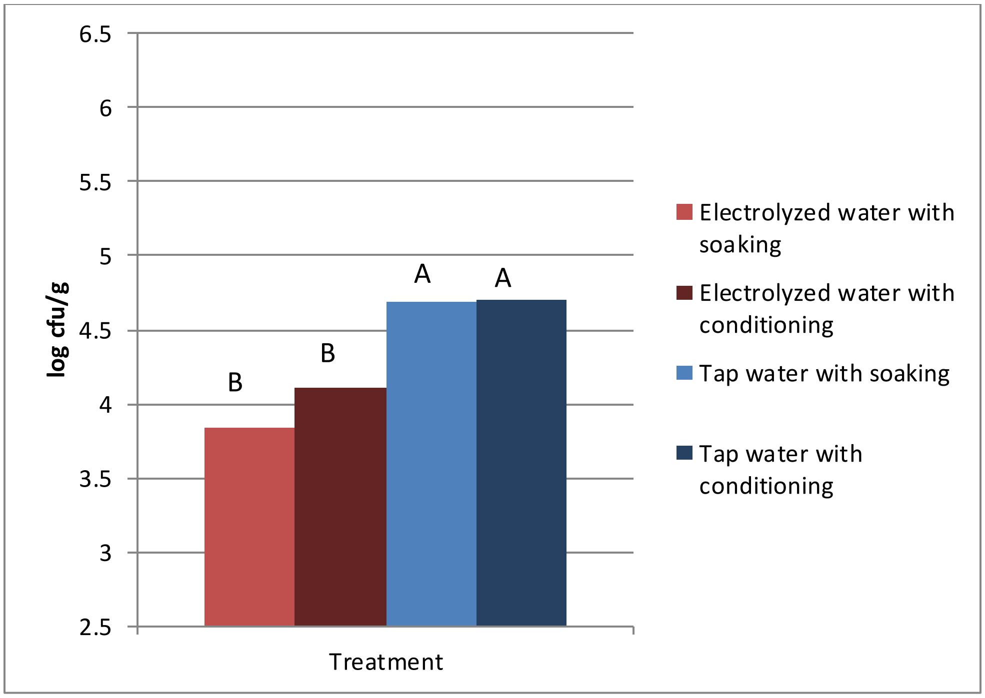

Figure 3.13: Average Log_{10} colony forming units per gram (cfu/g) of aerobic mesophilic bacteria found in red leaf lettuce during 72 hours of grocery store produce rack storage.

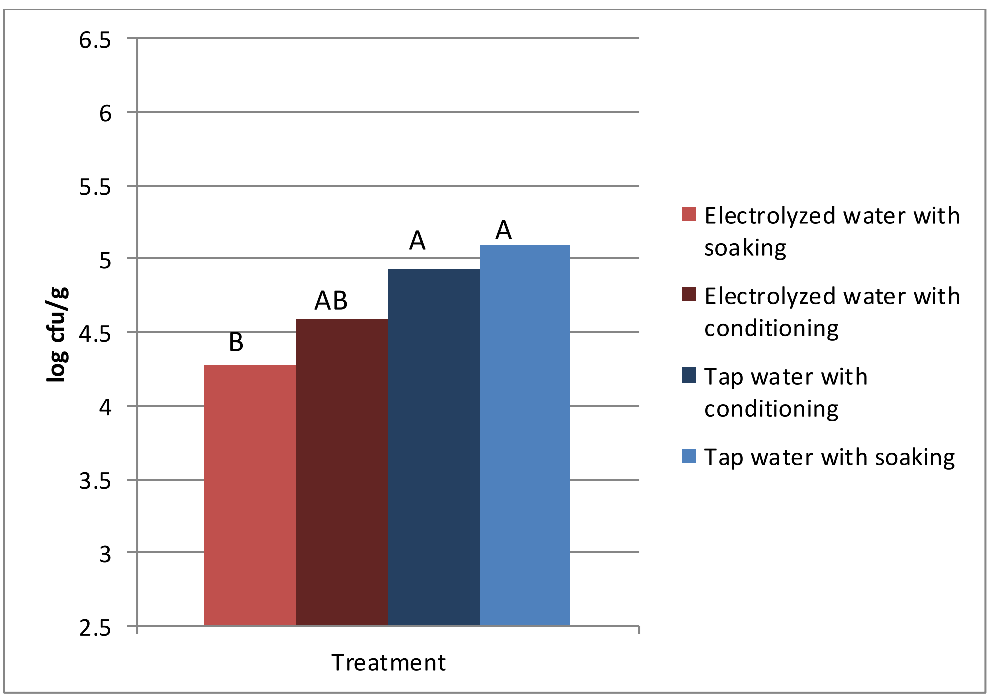

Figure 3.14: Average Log_{10} colony forming units per gram (cfu/g) of aerobic mesophilic bacteria found in red leaf lettuce during seven days of refrigerated storage following 72 hours of produce rack storage.

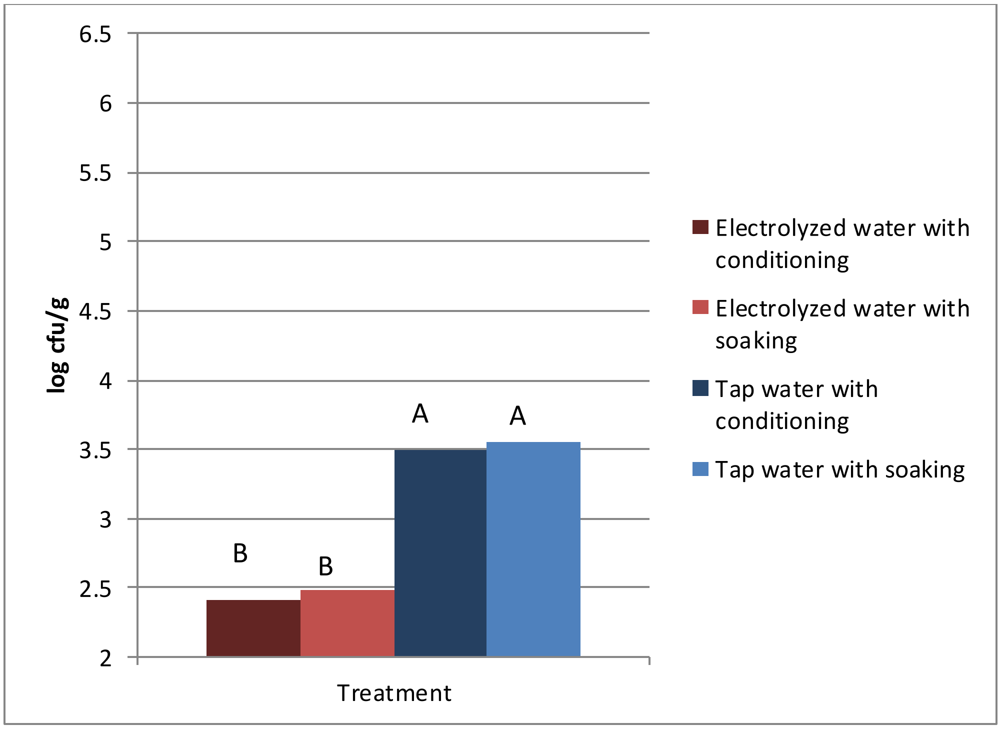

Figure 3.15: Average Log_{10} colony forming units per gram (cfu/g) of fungi found in red leaf lettuce during 72 hours of grocery store produce rack storage.

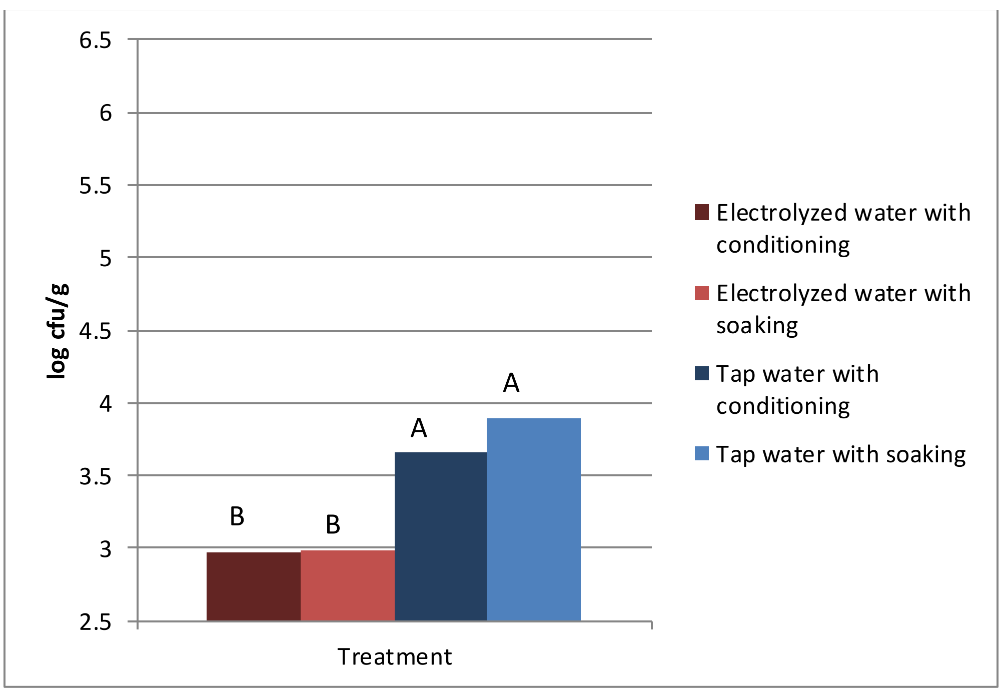

Figure 3.16: Average Log_{10} colony forming units per gram (cfu/g) of fungi found in red leaf lettuce during seven days of refrigerated storage following 72 hours of produce rack storage.

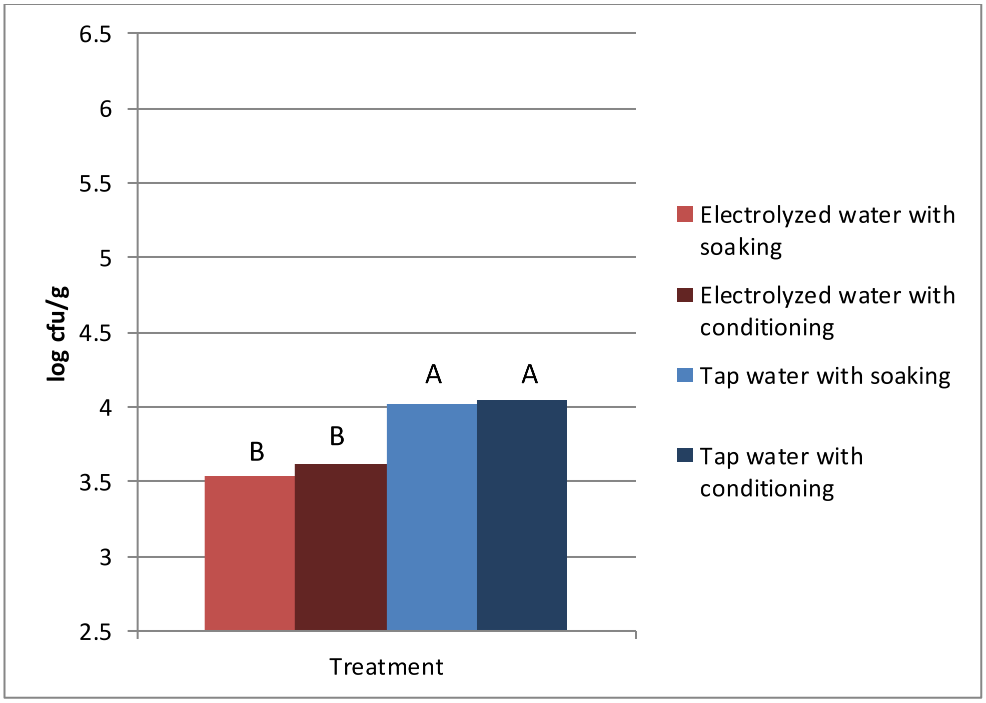

Figure 3.17: Average Log_{10} colony forming units per gram (cfu/g) of *Enterobacteriaceae* found in red leaf lettuce during 72 hours of grocery store produce rack storage.

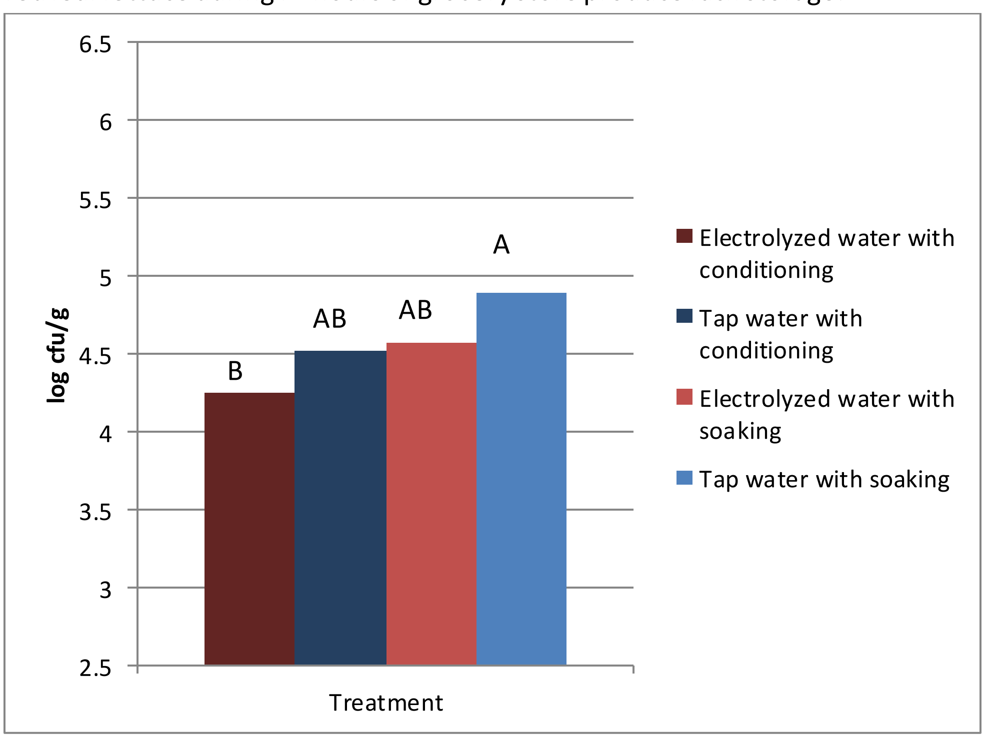

Figure 3.18: Average Log_{10} colony forming units per gram (cfu/g) of *Enterobacteriaceae* found in red leaf lettuce during seven days of refrigerated storage following 72 hours of produce rack storage.

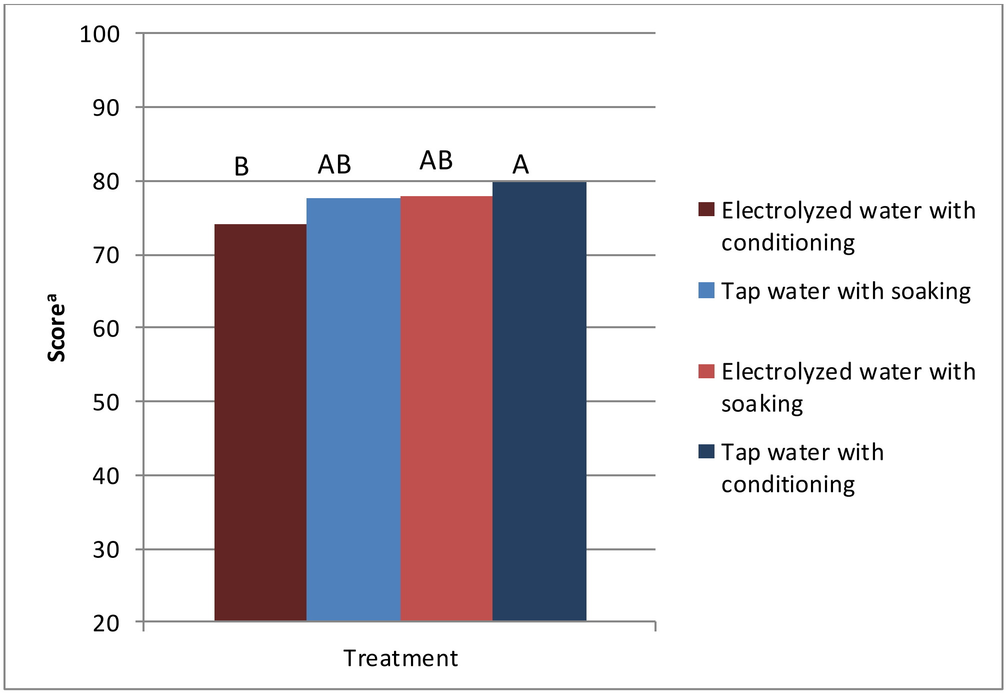

Figure 3.19: Average crispness sensory score of asparagus during 72 hours of grocery store produce rack storage.

a = sensory score as measured by a non-numeric anchored line scale with the score converted to a score out of 100

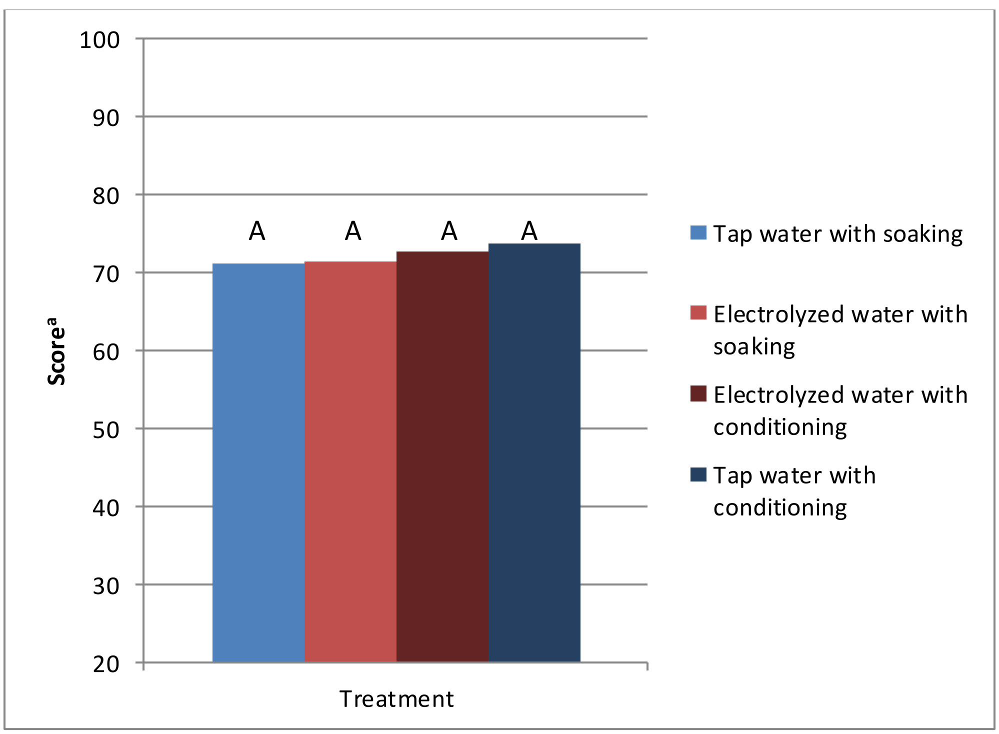

Figure 3.20: Average crispness score of asparagus during seven days of refrigerated storage following 72 hours of produce rack storage.

a = sensory score as measured by a non-numeric anchored line scale with the score converted to a score out of 100

Romaine

Figure 3.21 indicates that aerobic psychrotrophic bacteria counts on romaine lettuce were not significantly affected by the four differing treatments applied during the 72 hour produce rack storage. A significant difference in aerobic psychrotrophic bacteria counts was noted during the seven day refrigerator storage. Figure 3.22 illustrates that romaine lettuce treated with electrolyzed water with conditioning produced significantly lower average counts of aerobic psychrotrophic bacteria compared to the other three treatments.

Figures 3.23 and 3.24 indicate that during both the 72 hour produce rack storage and the seven day refrigerator storage, romaine lettuce treated with electrolyzed water as opposed to tap water, produced significantly lower average counts of aerobic mesophilic bacteria. The difference was approximately 0.75 log cfu/g throughout testing.

Fungi counts on romaine lettuce during produce rack storage were significantly affected by the type of treatment applied. Figure 3.25 indicates that romaine lettuce treated with electrolyzed water produce significantly lower counts of fungi compared to romaine lettuce treated with tap water during its 72 hour produce rack storage. Figure 3.26 shows that during the seven days of refrigerator storage the romaine lettuce that had been previously treated with electrolyzed water with conditioning produced significantly lower counts of fungi compared to the other three treatment groups.

Figure 3.27 illustrates how *Enterobacteriaceae* counts reacted similarly to fungi counts on romaine lettuce during the 72 hour produce rack storage. The romaine lettuce treated with electrolyzed water treatments produced significantly lower counts of *Enterobacteriaceae* compared to the tap treated romaine lettuce. This difference was partially transferred on to the seven day refrigerator storage. Figure 3.28 indicates that romaine lettuce previously treated with electrolyzed water with conditioning produced significantly lower *Enterobacteriaceae* counts compared to the two tap water treatment groups.

Figures 3.29 and 3.30 indicate that no significant differences in crispness between the four treatments were found. This was observed in both the 72 hour produce rack storage time and the seven day refrigerator storage. Crispness scores dropped by an average of seven points when scores are compared between the produce rack and refrigerator storage time periods.

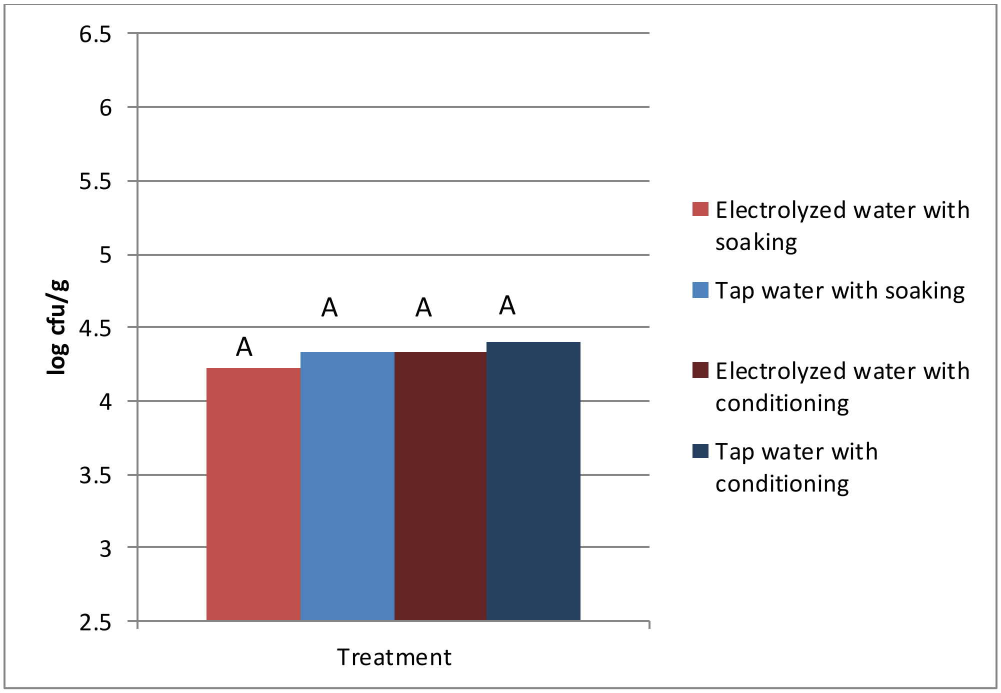

Figure 3.21: Average Log_{10} colony forming units per gram (cfu/g) of psychrotrophic bacteria found in romaine lettuce during 72 hours of grocery store produce rack storage.

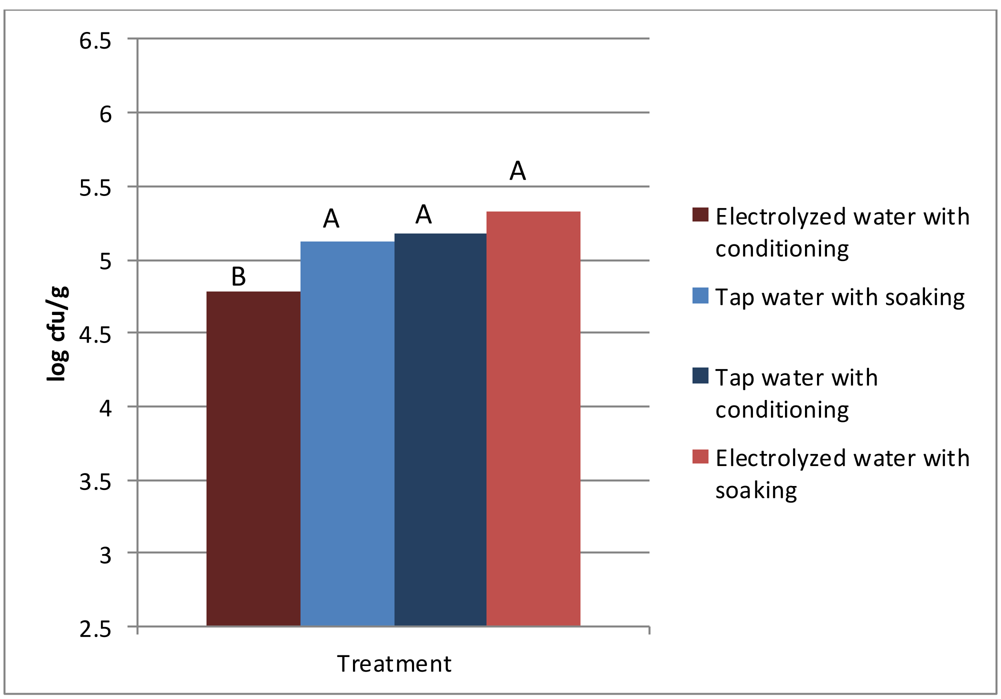

Figure 3.22: Average Log_{10} colony forming units per gram (cfu/g) of psychrotrophic bacteria found in romaine lettuce during seven days of refrigerated storage following 72 hours of produce rack storage.

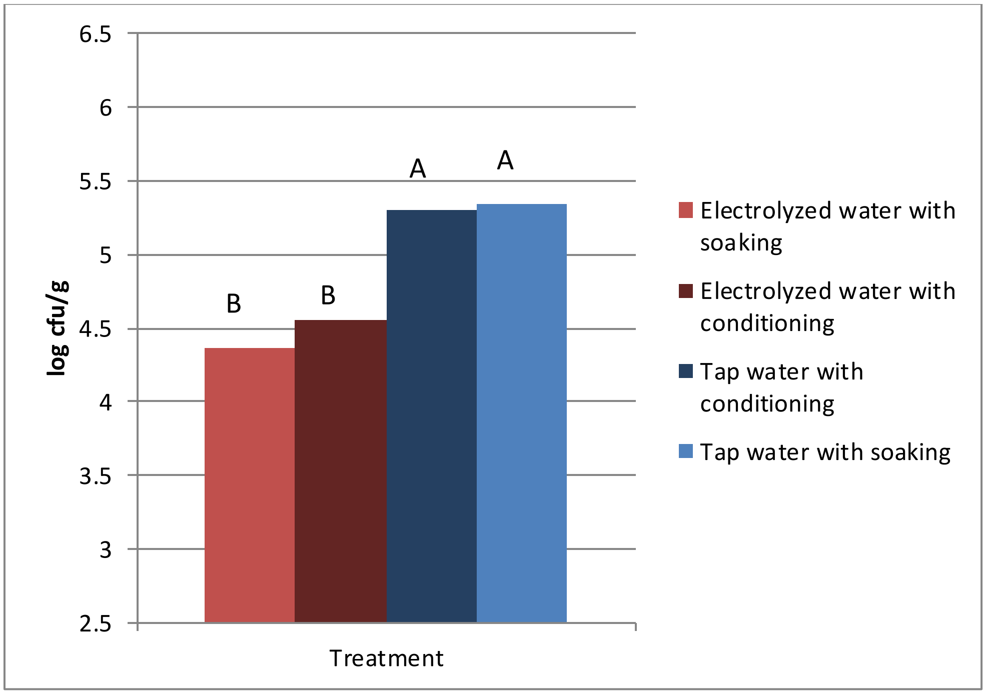

Figure 3.23: Average Log_{10} colony forming units per gram (cfu/g) of aerobic mesophilic bacteria found in romaine lettuce during 72 hours of grocery store produce rack storage.

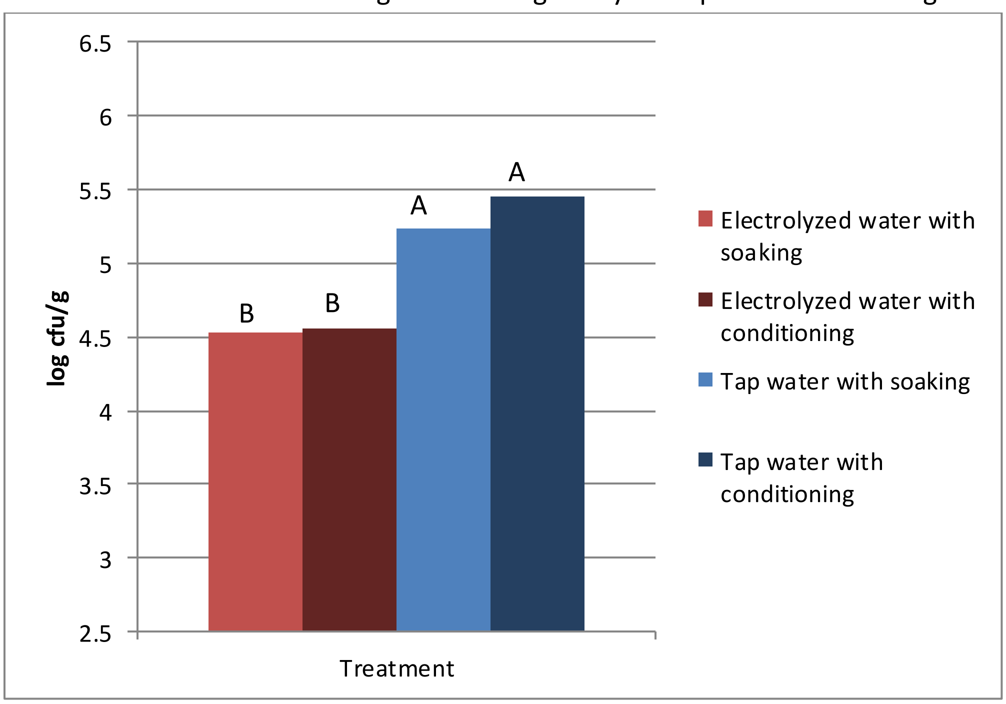

Figure 3.24: Average Log_{10} colony forming units per gram (cfu/g) of aerobic mesophilic bacteria found in romaine lettuce during seven days of refrigerated storage following 72 hours of produce rack storage.

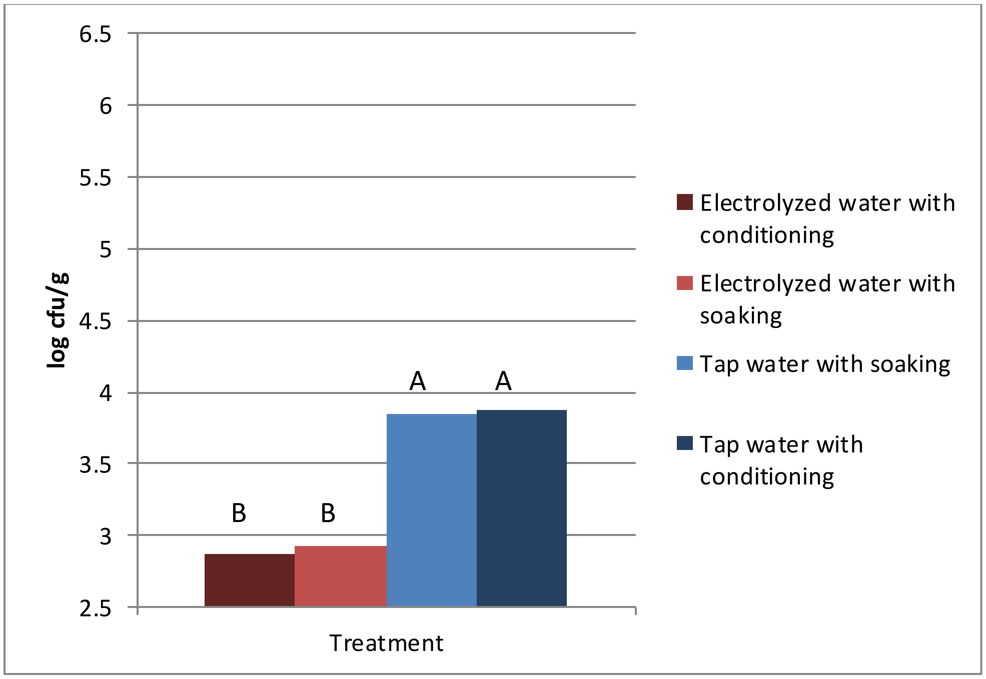

Figure 3.25: Average Log_{10} colony forming units per gram (cfu/g) of fungi found in romaine lettuce during 72 hours of grocery store produce rack storage.

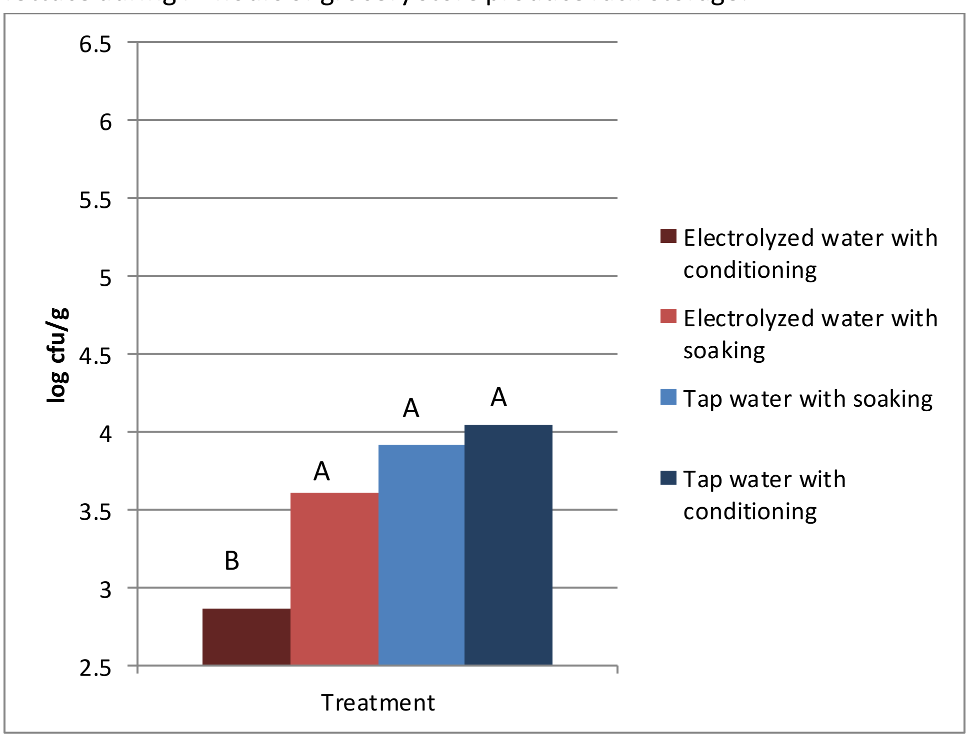

Figure 3.26: Average Log_{10} colony forming units per gram (cfu/g) of fungi found in romaine lettuce during seven days of refrigerated storage following 72 hours of produce rack storage.

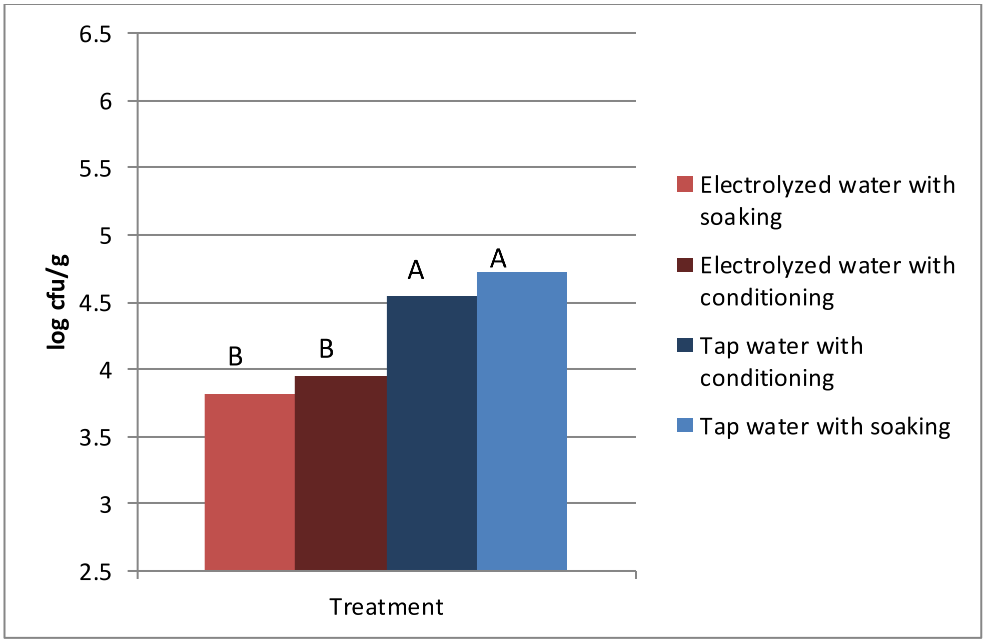

Figure 3.27: Average Log_{10} colony forming units per gram (cfu/g) of *Enterobacteriaceae* found in romaine lettuce during 72 hours of grocery store produce rack storage.

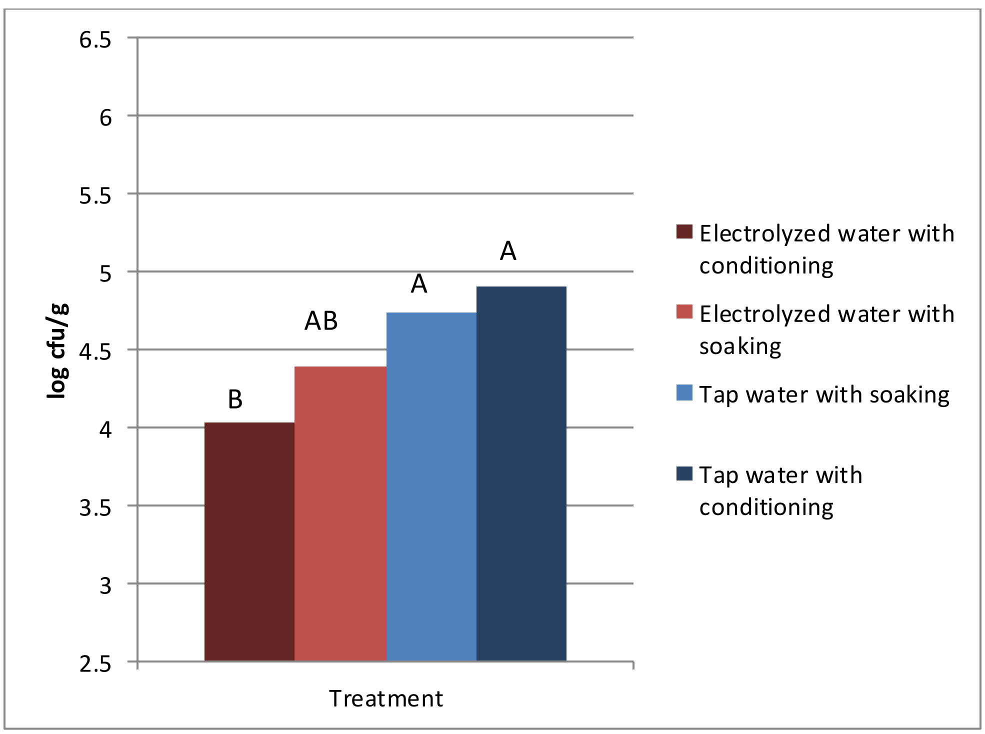

Figure 3.28: Average Log_{10} colony forming units per gram (cfu/g) of *Enterobacteriaceae* found in romaine lettuce during seven days of refrigerated storage following 72 hours of produce rack storage.

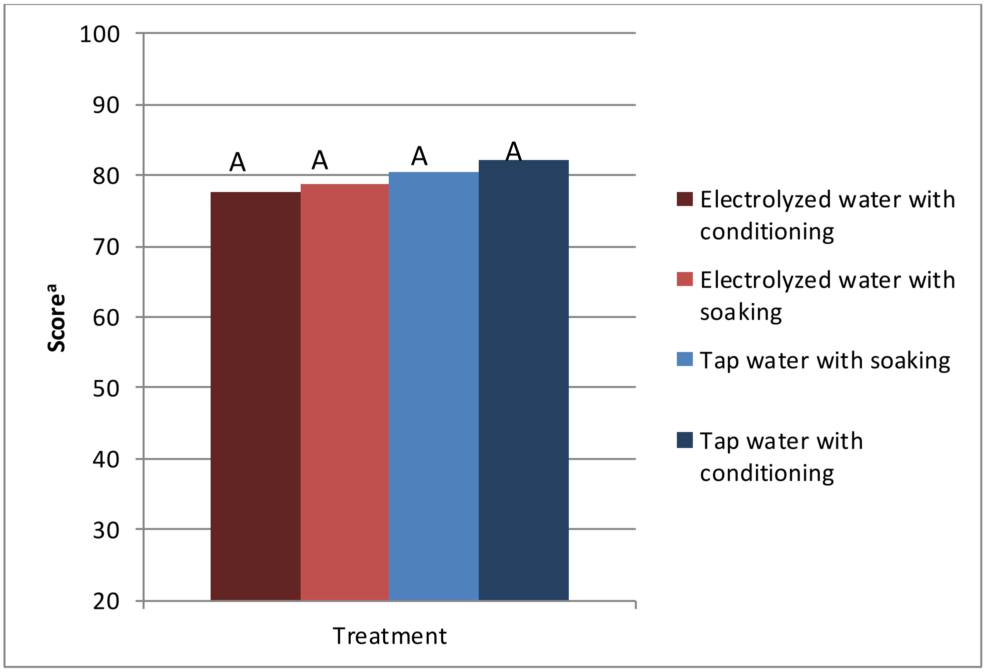

Figure 3.29: Average crispness sensory score of asparagus during 72 hours of grocery store produce rack storage.

a = sensory score as measured by a non-numeric anchored line scale with the score converted to a score out of 100

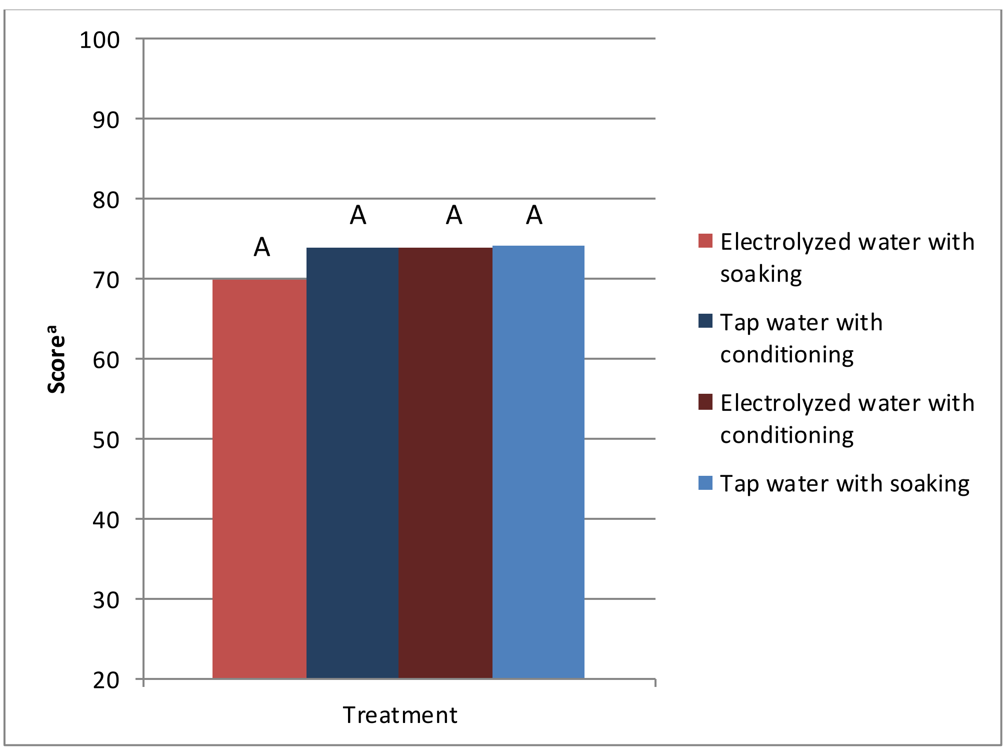

Figure 3.30: Average crispness score of asparagus during seven days of refrigerated storage following 72 hours of produce rack storage.

a = sensory score as measured by a non-numeric anchored line scale with the score converted to a score out of 100

3.5 - Discussion

Sensory analysis of crispness

Sensory results for the crispness of the asparagus during its produce rack storage and refrigerator produced no significant differences between the treatments. This result indicates that both the type of water used for conditioning and the act of cutting off the bottom of the stalks of asparagus do not noticeably affect the crispness of the asparagus at either the grocery store or in the consumer's home. The same result was seen for romaine lettuce, no significant differences in crispness were noted on the produce rack or during refrigerated storage. These results are significant because many grocery stores began using electrolyzed water on their produce because they believed it would improve the crispness of their produce.

Sensory results for the crispness of red leaf lettuce produced interesting results. During the produce rack storage time period, tap water with conditioning produced red leaf lettuce that was scored significantly higher for crispness compared to electrolyzed water with conditioning. This significant difference disappeared during the seven day refrigerator storage simulating storage in the consumer's home. Similarly to the results for asparagus, the results for red leaf lettuce indicate that applying electrolyzed water as the conditioning water does not significantly improve the crispness of the produce undergoing simulated grocery store procedures. These results actually indicated the opposite was partially true during the produce rack storage time period of red leaf lettuce.

Aerobic psychrotrophic bacteria count

Results from the application of four differing produce conditioning treatments applied to asparagus during a 72 hour produce rack storage followed by a seven day refrigerator storage produced interesting results. Conditioning the produce with tap water without trimming the bottoms of the asparagus stalks produced the lowest counts of aerobic psychrotrophic bacteria during both the produce rack and refrigerator storage. This result is significant because psychrotrophic bacteria are the cold loving bacteria capable of slow yet steady growth at refrigeration temperature which asparagus is subjected to throughout its lifetime in the grocery store and at the consumer's home. Comparing the results collected from the produce rack versus the refrigerator storage confirm this, average psychrotrophic bacteria counts in the refrigerator were more than 1 log higher than the average produce rack counts collected a week earlier. Psychrotrophic bacteria include spoilage causing bacteria and opportunistic pathogens such as species of *Pseudomonas* spp (Gómez-López, 2012).

Counts of psychrotrophic bacteria on red leaf lettuce grew 1.5 log cfu/g from the average day on the produce rack to the average day in the refrigerator. This result once again indicate that electrolyzed water is not more effective than tap water at reducing the counts of psychrotrophic bacteria on produce, as the same result was seen with asparagus. Similar results were seen on romaine lettuce, no significant differences in aerobic psychrotrophic bacteria were found on either romaine lettuce or red leaf lettuce when comparing electrolyzed water versus tap water. Other than improving crispness, grocery stores primarily use electrolyzed water because they believe it will cause their fresh produce to remain fresh and resist spoilage when treated with electrolyzed water (Al-Haw & Gomez-Lopez, 2012; Hricova D, 2008). In the case of both crispness and aerobic psychrotrophic count, neither of them saw a noted improvement in produce quality over tap water when treated with electrolyzed water.

Aerobic mesophilic bacteria and *Enterobacteriaceae*

During the 72 hour produce rack storage, the four treatments did not significantly affect the aerobic mesophilic bacteria and *Enterobacteriaceae* counts on asparagus. Interesting, during the subsequent refrigerator storage period, counts of aerobic mesophilic bacteria and *Enterobacteriaceae* was significantly slowed on asparagus treated with electrolyzed water with conditioning. It is interesting that the differences between the treatments did not become apparent until the asparagus was stored untouched in the refrigerator.

Red leaf lettuce treated with electrolyzed water produced significantly lower counts of aerobic mesophilic and *Enterobacteriaceae* bacteria counts compared to red leaf lettuce conditioned with tap water. This difference did not fully carry over to the refrigerator storage time period. Results for the counts of aerobic mesophilic bacteria and *Enterobacteriaceae* on romaine lettuce were also similar to red leaf lettuce. During the 72 hour produce rack storage time period, the romaine lettuce treated with electrolyzed water produced lower counts of the two aforementioned groups of bacteria compared to the tap water treatment groups. The differences mostly carried over to the refrigerator storage time period. Indicating that compared to red leaf lettuce, electrolyzed water applied to romaine lettuce was more effective at inhibiting the growth of aerobic mesophilic bacteria and *Enterobacteriaceae* during the refrigerator storage time period. In general, these four treatments affected counts of *Enterobacteriaceae* and aerobic mesophilic bacteria on all three types of produce in a similar fashion.

Reducing *Enterobacteriaceae* counts potentially indicates electrolyzed water's ability to inactive pathogenic bacteria in a simulated grocery store setting. Previous studies have shown electrolyzed water's effectiveness at inactivating pathogens on produce; however none of these studies attempted to simulate a grocery store's procedures and environment (Abadias M, 2008; Forghani, 2013). Further studies need to be done to confirm electrolyzed efficacy against a specific pathogenic bacteria while simulating grocery store procedures.

Fungi

Fungi counts were analyzed because they can be both a safety and spoilage indicator (Beuchat & Ryu, 1997). Fungi counts on asparagus were not significantly affected by any of the four treatments. This is explained by the fact that the conditioning procedure for asparagus involved only dipping them in about two inches of water. This meant that the fungi attached to any part of the upper portion of the asparagus stalks was unaffected by the treatment.

Fungi counts were significantly affected by the type of water used during conditioning of red leaf lettuce. Red leaf lettuce treated with electrolyzed water produced significantly lower counts of fungi during both the produce rack storage and the refrigerator storage. This difference indicates that the chemical oxidizing action of chlorine was effective at inactivating fungi on red leaf lettuce, and this difference carried over to the seven day dormant storage in the refrigerator simulating storage at a consumer's home. Romaine lettuce produced very similar results to red leaf lettuce when the counts of fungi on them were analyzed. Romaine lettuce treated with electrolyzed water produced significantly lower counts of fungi compared to romaine lettuce treated with tap water during the produce rack storage time period. This result indicates that the chlorine in electrolyzed water is effective at inactivating fungi on fresh produce. Dissimilarly to red leaf lettuce, while the romaine lettuce was stored in the refrigerator only the electrolyzed water with conditioning treatment group continued to produce significantly lower counts of fungi compared to the other three treatments. Overall these results indicate that electrolyzed water conditioning was more effective at reducing counts of fungi on lettuce than tap water.

3.6 Conclusion

The populations of aerobic psychrotrophic bacteria on these three types of produce were largely not affected by the type of conditioning water applied to it. On asparagus, tap water was actually better at reducing the count of psychrotrophic bacteria compared to electrolyzed water.

On both types of lettuce, fungi counts were significantly reduced by using electrolyzed water instead of tap water as the conditioning washing solution. This difference was apparent on both the produce rack and in the extended refrigerator storage time period. Similar results were also seen looking at the counts of aerobic mesophilic bacteria and bacteria belonging to the group *Enterobacteriaceae*. Although the significant differences resulting from the differences did not carry over to the refrigerator storage time period in some cases.

Sensory results indicated that using electrolyzed water instead of tap water did not bring about an increase in the crispness of any of the types of produce during their time on the produce rack or the refrigerator. Cutting off or leaving the bottoms or stems of the asparagus and lettuce also did not affect the crispness of the produce.

Overall results indicating that electrolyzed water was advantageous in reducing counts of aerobic mesophilic bacteria, bacteria belonging to the group *Enterobacteriaceae* and fungi on the three types of fresh produce tested. Electrolyzed water showed no benefit compared to tap when attempting to improve the crispness of the produce or the aerobic psychrotrophic bacteria count. In general, the removal of the stem of the produce during conditioning did not affect any of the tested variables.

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