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The microbiological effect of ozone and chlorine treatments on minimally processed lettuce

Andres Garcia Garcia

To the Graduate Council:

I am submitting herewith a thesis written by Andres Garcia Garcia entitled "The microbiological effect of ozone and chlorine treatments on minimally processed lettuce." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

John Mount, Major Professor

We have read this thesis and recommend its acceptance:

P. M. Davidson, R. Yoder

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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The Microbiological Effect of Ozone and Chlorine Treatments on Minimally Processed Lettuce

A Thesis
Presented for the Master of Science
Degree
The University of Tennessee

Andrés García García
May 2001

Dedication

This thesis is dedicated to my parents, my sister and my two brothers

Isaias García Terrazas

Elvira García de García

Gabriela García Holland

Isaias García García

Flavio J. García García

Who have given me love and encourage me through my education

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Abstract

Chlorine is an effective sanitizer against many foodborne microorganisms. However, it may cause the formation of carcinogenic trihalomethane compounds. Ozone is an effective disinfectant having greater oxidation potential than chlorine. Limited studies have been done to determine the optimum concentration and contact time for ozone and if there is a synergistic interaction with chlorine when treating minimally processed produce.

Our objective was to determine the sanitizing efficacy of ozone and chlorine, alone or in combination on microbial reduction on fresh-cut lettuce and to develop data that was of used for the ready-to-eat salad industry based on the sensory characteristics and shelf-life of these products.

Iceberg lettuce was cut into 2 by 5 cm strips and inoculated with log 8 CFU/g of a mixture of natural microflora strains isolated from cut lettuce stored at 10°C. 100 g samples were treated with 1 L distilled water solutions containing combinations of 0, 100, 150 or 200 ppm chlorine and 0, 2.5, 5.0 or 7.5 ppm ozone for a total of 16 treatments. Lettuce-water solutions were stirred constantly for 10 min and then lettuce was sampled for Aerobic Plate Counts (APC) and Psychrotrophic Plate Counts (PPC), four repetitions were used in this study. Commercially processed salads treated with chlorine, ozone or an ozone-chlorine mixture were evaluated for shelf-life using visual inspection by an untrained panel (n=30). Water samples were also analyzed for UV-Vis and total solids to determine the effect of the treatments in the processing water.

Lettuce treated with only chlorine had reductions in APC up to 1.38 Logs and 1.71 Logs for PYS. Samples treated with ozone decreased in APC up to 1.12 Logs and 2.00 Logs for PPC. Lettuce treated with the combinations of chlorine and ozone had the greatest reduction in APC up to 2.5 Logs and 1.91 Logs for PPC. Sensory evaluation showed that the commercially processed samples treated with chlorine were least desirable having the shortest shelf-life with product decay after 16 days of treatment. Samples treated with ozone alone had a shelf-life of at least 20 days. Lettuce treated with the combination had the longest shelf-life retaining good visual sensory characteristics until at least 25 days after treatment.

Results suggest that washing fresh-cut salads with an ozone-chlorine sanitation treatment can improve and extend the shelf-life of these products compared to either ozone or chlorine solutions individually.

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Chapter 1

Introduction

The increasing popularity of minimally processed fruits and vegetables has been attributed to the health benefits associated with fresh produce; combined with the ongoing trend toward consumer eating ready-to-eat foods. In 1998, the sales volume of minimally fruits and vegetables in the United States was estimated to be around $6 billion, and it is expected to increase to about $20 billion in the next 3-5 years (Reyes 1996). The increasing demand of these minimally processed products represents a challenge for researchers and processors to make them more stable and safe. The concerns associated to ready-to-eat fruits and vegetables are chemical, physical and microbiological. The sources of contamination for produce involve the incoming raw materials, plant workers, processing environment and proper sanitation of the equipment. When vegetables are peeled, chopped and shredded, they release plant cellular fluids that provide a nutritive medium for microbial growth followed by toxin production. High moisture content, lack of lethal process to eliminate microbial pathogens, and the potential of temperature abuse during preparation, distribution and handling increase the risk of food-borne illnesses. Traditionally processors have used water with or without sanitizing agents to wash fresh-cut and minimally processed produce. Chlorine has been the most widely used sanitizer. However it has a limited effect in reducing microorganisms on fruits and vegetables surfaces and some health concerns have been raised about the residual by-products that can be generated such as trihalomethanes, chloroform and other chemical residues formed in the wastewater. In 1997, ozone was

self-affirmed as Generally Recognized As Safe as a disinfectant for food. Research and commercial applications have indicated that ozone can replace chlorine (Graham 1997).

Limited studies have been done to determine the ozone effect as a disinfectant in the fresh-cut produce industry. None of the studies have tested the synergistic effect of using a combination of ozone and chlorine to treat fresh-cut produce in order to reduce the number of surviving microorganisms.

The objectives of this study were to determine the sanitizing efficacy of ozone and chlorine, alone or in combination on microbial reduction on fresh-cut salads, and to determine the effects of ozone, chlorine, and ozone-chlorine commercial treatments on the sensory characteristics of these products.

Chapter 2

Literature Review

Lettuce

Characteristics

Lettuce (*Lactuca sativa*) is among the most popular salad plants in the world. The name "lettuce" comes from a milky juice produced by the plant. This juice is called "*lac,"* meaning "milk," the root of the Latin name. The name "lettuce" also comes from "*laities*," plural of the archaic French word for "milky" (Anonymous 1970).

Lettuce heads fall under two classes, which differ in seasonal adaptability, availability and disease resistance. The *butter-head* types tend to be soft, with thick, oily leaves, whereas the *crisp-heads* have brittle-textured leaves, and are very hard and compact under ideal temperature conditions. Table 1 contains the composition of iceberg lettuce based on a 100g sample.

Grade standards for lettuce were developed in order to identify the degrees of quality in a commodity that are the basis of its usability and value. The United States Department of Agriculture (USDA) lettuce grade standards consider turgidity, color, maturity (firmness), trimming (number of wrapper leaves), freedom from tip burn, other physiological disorders, mechanical damage, seedstems, other defects, and decay; and are used by many private and government procurement agencies when purchasing fresh fruits and vegetables. California is one of the few states that has its own quality standards. These standards are mandatory for all the horticulture crops produced within the state. The California grade standards for lettuce require that crisp-heads are free from insect

Table 1. Composition of iceberg lettuce per 100 grams

Nutrient	
Water (g)	95.89
Calories	13.00
Protein, g	1.01
Fat g	0.19
CHO: total, g	2.09
CHO: fiber, dietary g	0.53
Ash, mg	0.48
Calcium, mg	19.00
Phosphorus, mg	20.00
Iron, mg	0.50
Sodium, mg	9.00
Potassium, mg	158.00
Vitamin A, IU	330.00
Thiamine, mg	0.046
Riboflavin, mg	0.03
Niacin, mg	0.187
Ascorbic Acid, mg	3.90

(USDA, 1984.)

damage, decay, seedstems, tip burn, freezing injury, broken midribs, and bruising. The standards for sectioned, chopped, or shredded lettuce are the same as for intact heads plus the standards also include freedom from discoloration and excessive moisture (Kader 1992a).

History

Ancient forms of lettuce were probably cultivated and enjoyed some 4,500 years ago. Evidence of this comes from illustrations in hieroglyphics of the ancient Egyptians. Hippocrates wrote of Greeks cultivating lettuce in 430 B.C. The Moors carried lettuce into Spain from North Africa and have been credited with developing the Romaine variety.

In 1494, Christopher Columbus introduced lettuce to the New World. Lettuce was first grown on Isabela Island in the Caribbean. Cultivation of lettuce spread from the Bahamas to Haiti to the South American mainland. Until about 1848, there were a limited number of varieties available. After that, many new varieties were bred and distributed, including the more modern crisp-head varieties such as the New York variety. Varieties with the characteristics now associated with iceberg lettuce were not developed until the 1940s. (Roach 2000)

The first California growers to plant lettuce were in Los Angeles and Imperial Counties. In 1910, 595 acres of lettuce were planted in Southern California. By 1918 the number of acres under cultivation had increased to 6300 acres. The Great Lakes cultivars dominated the western lettuce industry into the 1970s. In 1975, the cultivar, Salinas, was

introduced. The Salinas-Vanguard group of cultivars are most commonly grown today in California (Anonymous 2000).

Fresh-cut produce

Fresh-cuts are traditional produce products that have been washed, cleaned, cut, packaged, and refrigerated and are ready to sell to customers seeking a great food value with a minimum investment of time. The U.S. Department of Agriculture defines fresh-cuts as fruits and vegetables that have been physically altered after harvest but are presented fresh to the customer.

Before 1990, the majority of the fresh-cut produce was sold in food service channels. Advances in processing, preservation, distribution and marketing have been a key factor in meeting the growing consumer demand for healthy and convenient foods. They also have enabled the food industry to supply fresh products of high quality to consumers all year round.

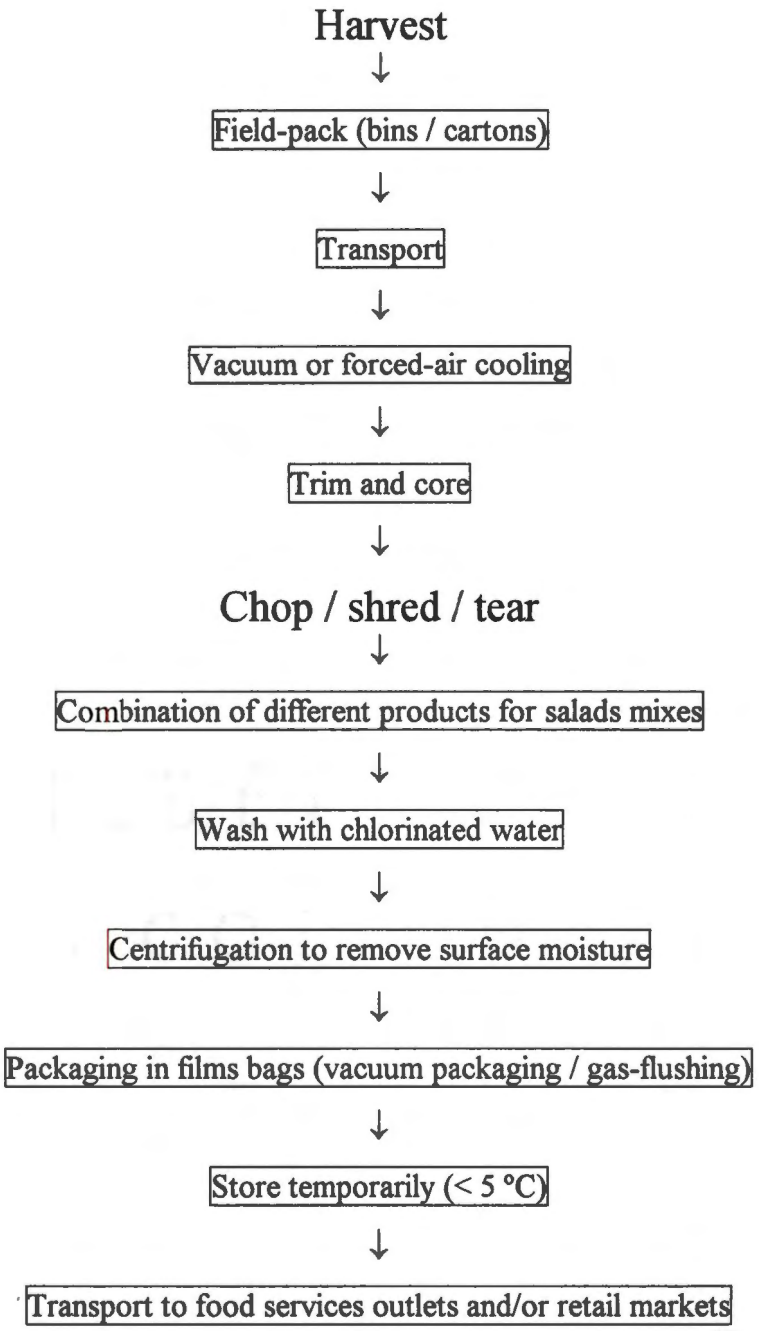
Consumer health concerns and awareness of the nutritional benefit form eating fresh-cut produce have benefited the marketability of both fresh and processed fruits and vegetables. Other factors that have contributed to the increase in consumption of these commodities are improved quality and greater variety of produce, introduction of convenient fresh-cut forms, and development of year-round availability.

Processing of fresh-cut produce

Processed fruits and vegetables have contributed to a growing trend of new product development in the food industry. In 1995, fifty-seven percent of total fruit and vegetable consumption in the USA was in the processed form. Two hundred new products were introduced in this category in 1989, 552 in 1996 and 251 in 1997. While the proliferation of minimally processed fresh-cut items, such as bagged or packaged salads, shredded broccoli, microwave-ready fresh vegetables, and washed baby carrots has fueled new product growth, many specialty items have also been a factor (Cook 1998). In 1996, Reyes reported that the market for minimally processed produce in the USA was valued at about $6 billion and that it was predicted to grow to $20 billion by 2001.

Processing of fresh-cut produce can be in a "direct chain" of preparation and handling Figure 1, in which the product is grown, prepared, distributed, and then marketed or utilized; or they can be processed in an "interrupted chain" in which the product may be stored before and after processing, or it may be processed to different degrees in different locations (Kader 1992b).

One of the main concerns in the fresh-cut industry has been whether it is preferable to process at the shipping point, where the product is at its freshest, or at the destination. At the latter stage the product may be reworked (corrective measurements can be performed on the product in the processing steps by controlling temperature or pH, removal of spoiled raw material, etc). Either option requires optimal temperature management through the distribution system to maximize yield. At the present time, there exist many regional processing plants.



(Kader 1992b)

Figure 1. Flow diagram of minimally processed lettuce products

These are necessary because of their proximity to markets and the demand for "just-in-time" deliveries. Improvements in equipment, modified atmospheres packaging, packaging film technology, and management of storage temperatures have helped the fresh-cut industry overcome marketing and distribution problems, extending the shelf life of these products. Consumers and food services users are willing to pay for the costs of making minimally processed products (Cook 1998).

Microbiology of fresh-cut produce

The quality, shelf-life, and safety of fresh-cut produce depend primarily on the biochemical changes that occur after harvest. Damaged tissues may be caused by minimal processing which results in leakage of cellular fluids that contain nutrients, creating a favorable environment for microbial growth and spoilage (Heard 1999). Some spoilage characteristics include visible mold growth, fermentation, browning, and development of off-odors. To minimize this problem, producers have optimized their manufacture processes, making them more efficient causing less damage to the tissue of there raw materials, and by these means increasing the quality of their finish product.

Consumption of microorganisms present on minimally processed vegetables, including lettuce, has been associated with spoilage and food-borne illnesses. These microorganisms are yeasts, molds, lactic acid bacteria, fecal coliforms, and pectinolytic bacteria (Simons and Sanguansri 1994). The growth of these spoilage microorganisms is affected by several factors including temperature, pH, biological structure, processing, and packaging.

Temperatures at which vegetables are harvested, transported, processed and packaged greatly influence the number and the type of microorganisms present and the rate at which they grow. Storage at low temperatures selects for the growth of psychrotrophic organisms. Spoilage of fresh-cut produce stored at low temperatures is not always the result of psychrotrophic bacteria. Mesophilic microorganisms may continue growing under low temperature storage but at reduced or slower rates. The presence of lactic acid in salads can indicate the growth of lactic acid bacteria and it can be used as an indicator of temperature abuse (Manvell and Ackaland 1986).

The pH of a vegetable generally decreases during storage time. This is attributed to an increase in bacterial population, but the development of microflora is not simply related to the pH and the presence of organic acids. The combined effect of pH and temperature can inhibit their growth (Heard 1999).

The cuticle of the lettuce is a biological structure that provides protection. Biochemical and physiological changes that occur in salad greens during processing and storage result in cuticle damage. Therefore the cuticle is no longer a barrier between the nutrients and the microorganisms, leading to leakage of nutrients, which microorganisms may use for growth.

Produce may become contaminated when being grown or harvested or they can also become contaminated through contact with processing equipment or the environment of the processing facility. For example, shredders and slicers are considered to be a major source of contamination. Grag and others (1990) found that the number of microorganisms on lettuce increased 1.9 log CFU/g after it had been shredded. Improper cleaning and sanitation of the equipment can result in a build up of organic matter that

encourages microbial growth and the formation of biofilms. The use of recycled wash water may also contribute to microbial spoilage as the buildup of organic residues in the water, increases the potential for microbial contamination of the produce (Heard 1999). This may be over come by the proper clean up and sanitation of the processing equipment and with the use of ozone or other oxidizing agent to treat the recycled wash water (Strickland 2000).

Minimally processed vegetables are packaged sealed, in the presence of air, or under modified atmospheres. If a package is sealed in an impermeable film wrapped package under air, a modified atmosphere results from the respiration of the product. Packages are also filled with gas mixtures containing, 5-10% CO_2 and 2-5% O_2 that is used to extend the shelf-life of whole vegetables (King and Bolin 1989). "Carbon monoxide concentrations of 5 to 10 percent under low oxygen (<5 percent) conditions retard browning and reduce microbial growth, lengthening shelf-life in lettuce and other produce" (Kader 1992a). Other factors influencing the growth of spoilage microorganisms are the packaging material used, relative humidity, temperature of storage, type of produce, and the microbiological load present (Nguyen and Carlin 1994).

Quality of fresh-cut produce

Minimally processed produce must be visually acceptable and appealing. Products must have a fresh appearance, be of a consistent quality and free of defects. In fresh-cut produce, the quality of the total product is only as good that of the most perishable component. Quality and shelf-life of fresh-cut produce depends on factors that may be categorized as follows (Heard 1999):

- Properties of the food - pH, water content, nutrients, and protecting biological structures such as skin and cuticle.
- Processing factors - washing, blanching, cutting, shredding, packaging, temperature of process, and the addition of preservatives.
- Properties and characteristics of microorganisms - growth rate and tolerance of temperature and pH.
- Other factors - storage temperature, use of modified atmospheres, etc.

Ozone

Characteristics

Ozone, the triatomic form of oxygen (O_3), was first discovered in the 1840s (Liangji 1999). Due to its characteristic odor it was named "ozone" a word that is derived from the Greek word "ozein" which means, "to smell" (Ankeney 2000). It has a molecular weight of 48, a boiling point of –119 °C and a melting point of –192 °C at 1 atm and weighs ca 200.1 g/m^3 (Jin-Gab 1999). Ozone is a triangular-shape molecule with a bond angle of 127 degrees. Ozone is a gas at ambient temperatures and is partially soluble in water. It is a very strong oxidizing agent with a redox potential of (-2.07V) for the following reaction:

$$O_3 + 2H^+ + 2e^- \longrightarrow H_2O + O_3$$

Ozone has limited solubility in water (~1g / L at 25 °C) and like most gases it increases in water solubility as temperature decreases (Korycka-Dahl and Richardson 1978). As the pH of the solution containing dissolved ozone increases, the rate of

decomposition of molecular ozone to hydroxyl groups also increases such that at approximately pH 10, ozone decomposes instantaneously (Graham 1997).

In aqueous solution, the half-life depends almost entirely on the amount of dissolved or suspended elements (organic or inorganic) that have the potential of being oxidized (ozone-demanding material). Cleaner water results in lower quantities of ozone-demanding material and a longer half-life of ozone. In practice, the half-life of ozone can be as short as one second in dirty, high ozone-demanding material content, water or as long as several hours in clean water used to wash and process foods. Since ozone does not remain in water for a very long period of time, there are no concerns about consumption of residual ozone in food products.

Ozone generation

Ozone is a trace constituent of the stratosphere at 0.05 mg / L. It is produced by the action of ultra-violet irradiation from the sun on oxygen (0.05mg/L):

$$3\ O_2 \rightleftharpoons 2\ O_3 + \text{heat and light}$$

Small amounts of ozone are also produced in the troposphere as a result of photochemical reactions from car exhausts, industry, forest, and volcanic activity. Ozone gas production is very unstable and it degrades to oxygen in the air.

To be used as a sanitizer in the food industry, ozone has to be produced on site. One of the most common methods to produce ozone in the industry is the Corona discharge method Figure 2. This method consists of applying a high-voltage alternating current across a discharge gap in the presence of oxygen, this excites the oxygen molecules, breaking or splitting some of them apart. Split atoms combine with other

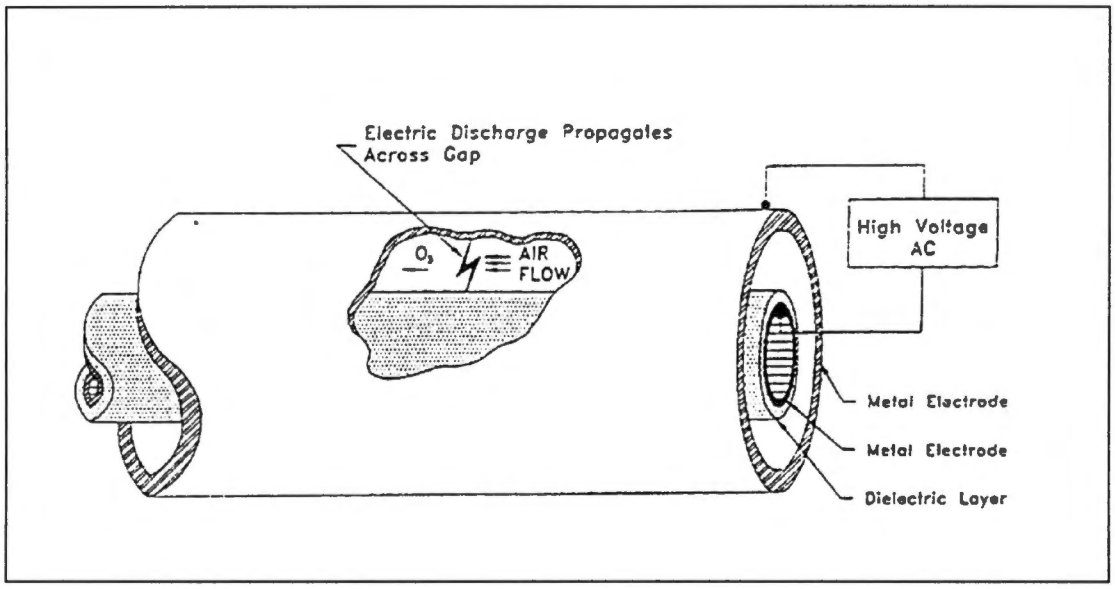


Figure 2. Electrical discharge ozone generator

oxygen molecules to form ozone. Ozone can also be produced by the radiation of oxygen at a wavelength of 185 nm (Law and Kiss 1992).

Although ozone treatment is more expensive to install than other treatments like chlorination or the use of quaternary salts, improvements in ozone generation systems (pressurized, filtration systems and grater contact surfaces) and better controls (temperature, flow rate, pressure) have made ozone easier and more economical to generate on-site, eliminating transportation and storage costs. A good example for reducing cost is illustrated at a major poultry processing facility in Georgia, where an ozone based water-reuse system was installed. This system has a potential to save the poultry processing industry more than 15 billion gallons of water and 70 million dollars each year (Anonymous 1999). As concern increases about the hazards of storing large supplies of toxic chlorine gas, the handling and disposal of corrosive chemicals required

for on-site generation of chlorine, and the production of organic chlorine byproducts, ozone has been recognized as a good alternative disinfectant.

Ozone as a sanitizer

Ozone has characteristics that make it an effective sanitizer in food processing. On the basis of oxidizing power, ozone is a more effective disinfectant (sanitizer) than chlorine having an oxidation potential of (-2.07 V) compared to that of hypochlorous acid (-1.49 V) or chlorine (-1.39 V) that are the most common sanitizers today (Jin-Gab 1999). Ozone is an unstable compound that decomposes spontaneously or in contact with oxidizeable surfaces, producing hydroxyl radicals and other free radicals.

Traditional technologies use water and water plus sanitizing agent to wash and sanitize fruits and vegetables. Chlorine is the most commonly used sanitizer in the minimally processed produce industry. However, many studies have indicated that it has a limited effect in killing bacteria on fruit and vegetables surfaces. At the concentrations that are used (200mg/L), it can only reduce the microbial load by ca. 2 log CFU/g (Sapers 1998). Environmental and health organizations have expressed concerns about using such high quantities of chlorine with respect to the formation of chlorinated by-products, like trihalomethanes (THM's) that have been recognized as carcinogenic substances. Simpson and others (2000) stated "A focus on chlorine dioxide: The "ideal biocide"" that, the chlorination of potable water has been proven to be linked to an increasing cancer mortality rate. This association has been made because of the increasing levels of THM's, primarily chloroform, in potable water.

Research and commercial applications have shown that ozone can be a good replacement for chlorine. Ozone is 1.5 times more effective as an oxidizing agent than chlorine (Liangji 1999), and since it is relatively non selective, it will react with many substances making it more effective over a much wider spectrum of microorganisms (Simpson and others 2000). Ozone, after reacting, decomposes into simple oxygen with no safety concerns about consumption of residual ozone in the treated produce. Ozone can destroy chemical residues, pesticides, chlorinated by-products, and toxic organic compounds (Liangji 1999). Gas ozone can be used as a sanitizer for foods during shipping or storage to prevent bacteria, mold, yeast growth, and to control insects (Rice and others 1982). It can also be used to sanitize package materials or to prevent products from rotting and over ripening during storage (Lamarre 1997).

Many applications in the food industry appear to be appropriate for the use of ozone. These include, increasing the yield of certain crops, protecting raw agricultural commodities during storage and transit, and sanitizing water used for washing food processing equipment, food, and packaging material (Graham 1997).

Ozone has also been used for the treatment of water, soft-drink bottling plants, and chilled water baths or as a surface disinfectant. Ozone has been used for decades as a safe disinfectant agent in water treatment plants in Europe. A municipal water purification plant utilizing ozone was first built in 1906 in France. In 1940, ozone was first used in the USA in a water drinking plant. Today there are over 200 plants in the USA using ozone to disinfect drinking water. In the USA, ozone has been approved as a safe treatment for bottled water and as a sanitizer for process equipment in bottled water

plants. Ozone is considered to be safer than other sanitizers commonly used (Graham 1997).

Ozone against microorganisms

Restaino and others 1995, investigated the antimicrobial efficacy of ozonated/deionized water, with and without added organic material, against various heterotrophic bacteria and fungi (pathogens, spoilage organisms, and fecal contaminants) that are of concern in the food industry. For this experiment, the average output level of dissolved ozone in deionized water was 0.188 mg/mL. Cells of *Salmonella* Typhimurium and *E. coli* were killed very rapidly in the ozonated water with a > 5 log CFU/g reduction being obtained. For *Candida albicans* and *Zygosaccharomyces bailii,* a > 4.5 logs CFU/g reduction was also seen, whereas less than 1 log CFU/g of *Aspergillus niger* spores were killed after 5 minutes of exposure. Finch and others (1988) reported up to 6.5 log CFU/g reduction of *E. coli* population using ozone at a concentration of 4.4 to 800 µg/L with a contact time of 30 to 120 seconds.

In the meat industry, Kaess and Weidemann (1968) tested ozone to control surface microflora (*Pseudomonas, Salmonella, Staphylococcus,* and molds) in the process of tenderizing meat. For this experiment, an ozone gas treatment was used at up to 0.01mg / L, with a relative humidity of 60-90% to inactivate bacteria. A higher concentration of ozone was needed to inhibit molds. Ozone treatment also improved storage quality and decreased microbial counts in meat-transportation vehicles. Ozone treatment of 10 to 20 ppb (µg/liter) inhibited microbial growth on beef that was kept at 0.4 °C and extended the normal storage period by 30 to 40 % (Kolodyaznaya and

Suponina 1975). Dondo and coworkers (1992) reported that ozone reduced the growth of surface microorganisms, improved the sensory quality, and decreased the formation of total volatile N-compounds of beef under refrigeration (4 °C). In contrast, Fournaud and Laurent (1972) found reductions of microbial load of no significance on beef surfaces. In this experiment investigators treated beef carcasses with 100 mg / L of ozone gas for 30 min. They concluded that low activity and effects, such as discoloration, and odor development, render ozone use unacceptable.

In the poultry industry, ozone has been evaluated for disinfecting hatching eggs, water chillers, poultry carcasses, and contaminated eggs. In 1979, Yang and Chen, investigated the germicidal properties of ozone on poultry meat. In the study they stated that the germicidal effect of ozone was affected by contact time, temperature, pH, and the presence of inorganic and organic material in the solution. Longer contact times, lower pHs, and lower temperatures resulted in greater anti-bacterial effects. Sheldon and Brow (1986) chilled carcasses in ozonated water (3 – 4.5 mg/L) for 45 minutes and found that the microbial counts during storage at 4 °C, were consistently lower than the non-treated carcasses. The residual ozone achieved a microbial destruction greater than 2 logs CFU/g and an increase in the light transmission (500 nm) of the treated water was also seen. In 1989, Whistler and Sheldon treated hatching eggs with a mist of ozonated water for 2 hr to determine the sanitizing effect of ozone. Microbial counts decreased up to 2.5 log CFU/g.

In the fishery industry, ozone has been used to disinfect fishery products, to remove odor and color, and to improve sensory qualities. Treatment of the fish skin with ozone and NaCl decreased the load of *Vibrio cholera, E. coli, Salmonella* Typhimurium,

V. parahaemolyticus and *S. aureus* by 2 to 3 log CFU/g, and increased the storage life by 20 – 60% (Haraguchi and others 1969). Ozone treatments of shrimp decreased *E. coli* by 98.5%. Ozone also has been proven to remove odor and color from fish flesh, and to improve the sensory quality of fish by decreasing the formation of trimethylamine. However an oxidation of fish oils occurred as well as a decrease in pH of the fish (Dondo and others1992). Ozone has also been tested in the washing process that is applied during the manufacture of dark-flesh fish surimi. Research shows that ozone reduces surimi-washing times and improves color. (Jin-Gab 1999)

In the water and the fluid food industries, research has been done to develop an ozone treatment for fruit juices and liquid dairy products that minimizes quality deterioration. Pressurized ozone has shown to be effective in decreasing psychrotrophic counts by 2.4 log CFU/mL in skim milk and in whey and apple juice a microbial reduction was also achieved (Rojek and others 1995). Ozone has proven effective with more than a 99% reduction of biofilms of milk spoilage bacteria on a stainless steel plates with a treatment of 5 mg/L for 10 minutes (Greene and others 1993). The purification of contaminated spring water for use in the food industry has also been study. A treatment of 0.1 to 3.2 mg/L for a period of 8 minutes kills coliforms and spore-forming bacteria. This experiment also showed that ozone demand increased with increases in the suspended matter (organic material, oxidizing compounds, etc.) and pH. In this industry ozone treatments can also be applied to water before ice manufacture, in the brewery industry, for the washing of yeast (at low ozone concentrations), selective removal of bacteria and rinses of bottles, cans, fillers, pipelines, tanks as well as an aging agent in fermented products. (Jin-Gab 1999)

In the fruit and vegetable industry, ozone may be used to improve the safety of fresh produce (Hamson and Fiori. 1997). Ozone treatments have been shown to increase the shelf-life of various fruits including, grapes, apples, and blackberries, by considerably reducing bacteria, fungi, and yeast. Spotts and Cervantes. 1992, investigated the effect of ozonated water on post harvest pathogens of pears. In their study they concluded that mold spore inhibition was directly correlated with ozone concentration in a 1 to 5 min. exposure. They calculated that the LD95 values for *Botrytis cinerea*, *Mucor piriforms*, and *Penicillium expansum* were 0.99, 0.69 and 0.39 mg / L of ozone in water, respectively. In 1999 Perez and coworkers studied the ozone effect on postharvest quality of strawberries. For this experiment the fruit was stored at 2 °C in an environment containing 0.35 mg/L of ozone for 3 days. The changes in fungal decay measured by color, sugar, and acid distribution, and the aroma were evaluated. Ozone was ineffective in preventing fungal decay, reducing volatile esters emission by 40%. However, a significant difference in acid and sugar content was also seen with ascorbic acid content being three times higher on ozonated fruits. On vegetables, ozone decreases the chemiluminescence (changes in color), oxygen uptake, catalase, and peroxidase activity, and has a strong inhibitory effect on the growth of surface microorganisms (Jin-Gab 1999). In 1994, Liew and Prange, studied the effect of ozone on postharvest diseases and physiology of carrots. Pathogen-inoculated and uninoculated whole carrots were exposed to ozone gas concentrations of 0, 7.5, 15, 30 and 60 ppb for an 8-hour period and stored for 28 days. A 50% reduction of *Botrytis cinerea* Pers. and *Sclerotinia sclerotiorum* de Bary was achieved at the highest ozone concentration. Carrot respiration and color

differences also increased with the increase in ozone concentrations. Ozone treated carrots were lighter and less intense in color that the untreated ones.

Previous Studies on Lettuce Treated with Ozone

Jin-Gab and coworkers (1998) studied the effect of bubbling ozone at a concentration of 62 mg/L, in a mixture of shredded lettuce and water, for different time periods. When treating the mixture for three minutes, counts of mesophilic and psychrotophic bacteria decreased 1.4 and 1.8 log CFU/g respectively. When bubbled for 5 minutes, a decrease of 3.9 and 4.6 log CFU/g was achieved. The effect of the ozone delivery method was also studied. Bubbling ozone in the water-lettuce mixture while stirring (high and low speed), sonication, or stomaching, decreased the microbial count by 1.4, 1.9 and 1.9 log CFU/g, respectively. They concluded that bubbling ozone in water is the most effective ozonation method, and that for effective ozone delivery to microorganisms on lettuce requires a combination of ozone bubbling and high-speed stirring.

In 1999 Byeong-Sam studied the effect of using different treatments (chlorinated water, ozonated water, ultrasonic wave and vortex, and spray washing) on the surface sterilization of leafy lettuce. In this study, he showed that samples treated with chlorine concentrations of 100 mg/L, 150 mg/L, and 200 mg/L for 20 minutes decreased the microbial load 0.49 log CFU/g, 1.78 log CFU/g and 2.11 log CFU/g, respectively. Samples treated with ozone at concentrations of 1.0 mg/L, and 1.5 mg/L for 10 min at 4° C decreased the microbial load by 0.68 log CFU/g, and 1.05 log CFU/g, respectively.

Ozone treatment for 60 minutes achieved a 99.99% reduction of coliforms. Ultrasonic wave, vortex, and spray washing techniques decreased microbial load, however none achieved greater than a one log CFU/g reduction

Chapter 3

Material and Methods

Lettuce Preparation

Iceberg lettuce (*Lactuca sativa*) was purchased at a local supermarket, delivered to the pilot plant of the Department of Food Science and Technology, University of Tennessee, and held at 6 °C until processed. The steps required for the processing of lettuce are illustrated on Figure 3. The lettuce was cored and cut into 2 by 3 cm strips with a sanitized, sharpened knife. The strips were placed in a Ziploc® plastic freezer bags (26.8 cm x 27.8 cm, 1-gallon capacity) and held at 6 °C for no longer than 10 minutes prior to inoculation.

Inoculation of lettuce

Preparation of Inoculum

For inoculation studies, a mixture of natural microflora was isolated from aseptically cut iceberg lettuce by chopping the lettuce into small pieces, with a previous sanitized sharp knife, to release the juices. The mixture of lettuce and juices was placed in a freezer bag and incubated at 10 °C until visible spoilage occurred. This was evidenced by structurall breakdown resulting in secretion of juices from the lettuce and the lettuce becoming discolored with a brown appearance.

The chopped lettuce was stored at below 10 °C to stimulate psychrotrophic microorganism growth. Five mL of the spoiled lettuce excretions were transferred to a 9-mL tryptic soy broth (TSB) (Becton Dickinson, DIFCO-211825, Sparks, MD). The

Lettuce

↓

Cut into strips (2 cm x 3 cm)

↓

Inoculated with a 8 log CFU/ml solution / drain after 1 hr

↓

Incubate for 24 hr for cell attachment

↓

Spin dry for 10 seconds.

↓

100-g of lettuce were weighed and 900 mL of treated distilled water (Cl^-, O_3, Cl^- and O_3) were applied for 10 minutes (1:10) ratio

↓

Spin dry 10 seconds

↓

25-g of the treated lettuce were weighed and 225 mL of peptone water were added (1:10) ratio in a sterile stomacher bag / stomach at high speed for 10 minutes

↓

Dilute with peptone water from 10^2 to 10^8

↓

Plate in PCA and incubate at 37 °C for 48 hrs for Aerobic Plate Count / at 4 °C for 168 hrs (7 days) for psychrotrophic microorganisms

↓

Count CFU /g in plates

↓

Report

Figure 3. Flow diagram, steps required for processing and microbial analysis of lettuce.

mixture of juices and broth was then incubated at 10 °C. After 3 days, a loopful of the culture was transferred into 9 mL of TSB and incubated at 10 °C. The culture was transferred every three days until used. The population of spoilage microorganisms in the broth was 9.8 log CFU/mL as determined by aerobic plate count.

Inoculation of samples

Lettuce had to be inoculated in order reach the microbiological load of 8 log CFU/g. After three days of incubation, 9 mL of inoculated TSB was mixed thoroughly with 491 mL of distilled water. The inoculum mixture was poured over 500 g of chopped lettuce placed in a 1-gallon plastic bag and the mixture was agitated. After one hour, the excess water was drained from the lettuce. Inoculated samples were stored at room temperature (~20° C) for 24 hrs to ensuring the attachment of cells to the lettuce surface. The lettuce was dried for 10 sec in a spin drier (Delux Flow-Thru SALAD SPINNER, Progressive International Corp., Kent, WA) prior to ozone, chlorine and ozone-chlorine treatments.

Production of Aqueous Ozone

Ozonated water was generated by bubbling gaseous ozone, into 1 L of distilled water (Corning, Mega-Pure™ System MP-1, Corning, NY). Ozone gas mixture was produced using an active oxygen generator machine with two UV lamps (Active Oxygen Generator, Golden Buffalo, Orange, CA.) The gas was then pumped into the system using an aquatic air pump (Tetratec, deep water, DW 96-2) at a flow rate of 4 L/min. with an internal pressure in the system of 215.46 Pa Figure 4 shows a diagram of how ozone.

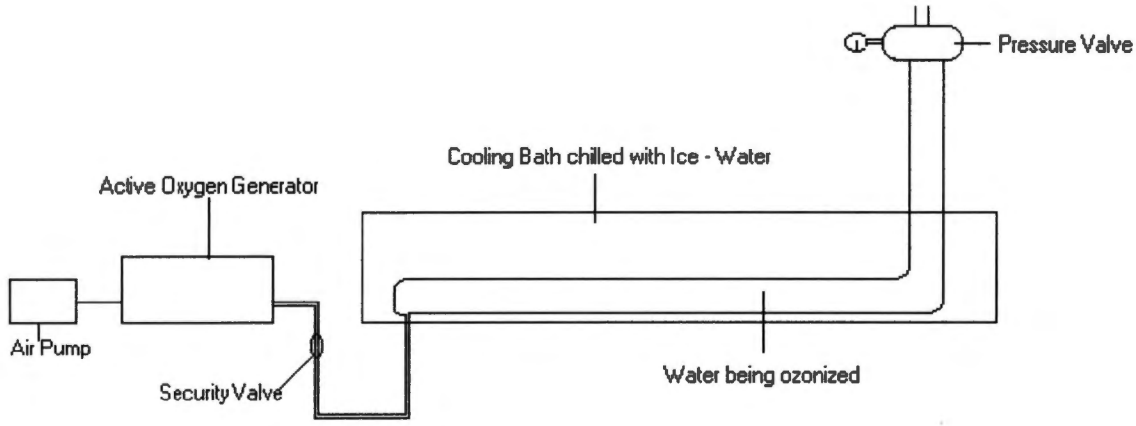


Figure 4. Diagram of ozonated water system used in experiment

was generated for this study. Ozonated water was held in a sealed container at 4 °C until stable and consistent concentrations of ozone were established prior to diluting to produce acceptable treatment levels

Measurement of Ozone Levels

The ozone concentration was determined using a commercially available ozone test kit (CHEMetrics, Vacu-vials, Ozone K-7403, Calverton, VA.). The method is based on the indigo method that involves the oxidation of iodine by ozone, which is then estimated iodimetrically, based on the reaction:

$$O_3 + 2I^- + H_2O \longrightarrow I_2 + O_2 + 2OH^-$$

The instructions for the determination of ozone concentrations using the kit are in Appendix A-1. A decrease in % transmittance (%T) at 565 nm is linear with increasing concentration of ozone. The ozone test vials containing the samples of water mixed with

the analysis chemicals were read using a spectrophotometer 20 (Spectronic Instruments, Spectronic® 20, Genesys™, Rochester, NY.)

After 24 hr of ozonation the dissolved ozone in 2 L of water was approximately 16 mg / L. The water was sampled and tested prior to each treatment and dilutions made, as needed, to obtain the ozone levels of 2.5, 5.0, and 7.5 mg / L ($\pm$ 0.2 mg / L) All dilutions were made with distilled water.

Preparation of chlorine solutions

Aqueous solutions of chlorine at 100, 150, 200 mg / L ($\pm$ 0.2 mg / L) were prepared by adding a predetermined volume of 5.25% sodium hypochlorite solution (Kroger, Cincinnati, OH) to distilled water. After 10 min of treating the lettuce with the chlorines solutions the remaining free chlorine was 79, 115, and 155 mg/L respectably. The chlorine concentrations were verified using a commercially available chlorine test kit (CHEMetrics, Vacu-vials Chlorine K-2513 kit, Calverton, VA.), based on the indigo method. In principle, this method is a colorimetric version of the DPD (N,N-diethyl-p-phenylenediamine) method. DPD is used as an indicator, and free chlorine reacts instantly with DPD producing a red color. Decrease in %T at 515 nm is linear with increasing concentration of chlorine. The directions for chlorine analysis from the test kit are shown in appendix A-3. All chlorine levels were measured prior to treatments. Dilutions were made in order to be able to use the test kit.

Production of aqueous ozone-chlorine solution

Ozone-chlorine solutions were prepared by adding 5.25 % sodium hypochlorite to prepared concentrations of analyzed ozonated water. The concentrations of the ozone – chlorine were 0-0, 2.5-100, 2.5-150, 2.5-200, 5-100, 5-150, 5-200, 7.5-100, 7.5-150 and 7.5-200 mg ozone/L – mg chlorine / L respectively. Ozone concentration was measured after ozonation and prior to addition of specific concentrations of chlorine to give the desired concentration. Final concentrations of both compounds in the solutions could not be determined because the two components interfere with the measurement of each other during the measurement.

Treatments of fresh-cut lettuce

The treatment order for the 16 combinations of chlorine – ozone solutions was randomized within each replication. Randomization of treatments was determined using a spreadsheet program (MS Excel 2000).

Inoculated spin-dried fresh-cut lettuce (100 g) was placed into a 4-L glass beaker and 1 L of each treatment solution was added to the beaker (1:10 w/w). A stainless steel cover was placed over the lettuce to keep it submerged in the solution. The lettuce-solution was then stirred (Fisher Scientific, Stirring hot plate, Sparks, MD.) for 10 minutes at a setting of 6 rpm. The lettuce-solution was then removed, and the lettuce spun dry for 10 seconds. A 25-g sample of the dried lettuce was used for microbiological analysis.

Microbiological Analysis

The 25-g sample was placed in a stomacher bag (Seward, Stomacher '400' bags, size 17.8 cm x 30.5 cm, London UK) with 225 mL of sterile 0.1% peptone water (Becton Dickinson, DIFCO-211825, Sparks, MD). The mixture was blended (Seward, Laboratory Blender, stomacher 400, London UK.) for 10 minutes at high speed.

Serial of dilutions were made using 0.1% peptone water and surface plated in duplicates onto Plate Count Agar (Dickinson, DIFCO-0479-17, Sparks, MD.). One set of plates was incubated for 24 h at 37 °C to obtain an Aerobic Plate Count (APC). The other set of plates was incubated at 7 °C for 10 days to obtain Psychrotrophic Plate Counts (PPC). All microbial counts are reported in log CFU/g. Microbial plate counts were performed in duplicates.

Sensory Analysis

A visual sensory evaluation study was performed to determine the shelf-life and appearance of ready-to-eat salads. For this study, lettuce was treated in a commercial facility with chlorine, ozone, or a combination of both. Lettuce from each treatment was packaged on the same processing line and under the same conditions at a large produce manufacturer in Nashville, TN. Packages were held at 4 °C until sensory evaluation was performed after 4, 16, 21, and 25 days.

A random, untrained panel was selected for this study. Panelists were asked to evaluate the lettuce packages visually to indicate their likelihood of purchasing the package of fresh-cut salad and to comment on the visual sensory qualities color, freshness and structure (scoresheet, Appendix A). A total of 30 panelists were used for each

evaluation. Each panelist viewed three different packages in a random order. Samples for sensory evaluation were held at 6 °C for a total of 25 days. Sensory results were evaluated based on the percentage of panelists who would purchase the products.

Total Solids

Total solids were determined on samples of water collected from the commercial salad processing operation. Every hour, a 200-mL sample of water was taken from the surge tank in the production line at a large produce manufacture in Nashville, TN. Samples were coded and place under chilled conditions and transported to the Department of Food Science and Technology for chemical analysis. One milliliter of water was sampled and placed on previously dried and weighed fiberglass sample screen pads (CEM Corporation, reorder part #200150, Matthews, NC.). Screens where placed in a microwave drying oven (Lab Wave 9000, CEM Innovators in microwave technology, Matthews, NC.) and dried to a constant weight at full power. Total solids were then recorded.

Turbidity Analysis

Turbidity analyses were performed on the water samples as described above, to determine if the different treatments had an effect on the water quality. The turbidity of the water samples in a quartz curvet was determined at wavelengths ranging from 180 nm to 840 nm, using a HP spec-20 spectrophotometer (Hewlett Packard 8452A, Diode Array spectrophotometer, San Jose, CA.).

Statistical Analysis

For the microbiological part of the study statistical analyses were performed for results generated by APC and PPC (dependent variables) using a randomized block design with three replications ($P< 0.05$), in a factorial treatment (SAS 8.1, 2000). Blocking on replicas to account for the variation of the initial lettuce quality and the initial microbial load. The independent variables in the study were chlorine (0, 100, 150, 200 mg / L), ozone (0, 2.5, 5, 7.5 mg / L) and the combination of both giving a total of sixteen treatments. The treatment order was randomly assigned through the use of a random number table generated by MS Excel 2000. Surface response analyses were also performed for each of the microbial data to be able to estimate the optimal treatment response.

For the sensory part of the study a Chi-square analysis was used to predict the distribution of the preference of the panelist within each replica; followed by an analysis of variance in a complete randomized design to determine significant differences among treatments. The interaction between shelf - life and treatment was determined using a complete randomized design, with a factorial treatment.

Chapter 4

Results and Discussion

Microbiological

The effectiveness of using ozone, chlorine and ozone-chlorine solutions as a sanitizing agent on the fresh-cut lettuce was determined on lettuce that was inoculated to a microbial load of 8 log CFU/g from APC. The treatments of 0, 2.5, 5 and 7.5 mg / L concentrations of ozone resulted in increased reduction in aerobic bacteria when ozone was added to the rinse solution compared to the distilled water solution ($p<0.5$) (Figure 5). However, there was no significant difference among the three ozone levels. The use of chlorine of at least 100 mg/L also had a significant increase in the reduction of aerobic bacteria (Figure 6). Chlorine levels of 100, 150 and 200 mg / L were not significantly different from each other.

There were significant interactions between the chlorine, ozone, and chlorine-ozone combination treatments on the lettuce so the LSMeans analysis was done for all sixteen treatments. The APC results from the chemical treatment affects on microbial reduction on the lettuce were significantly different ($P<0.05$) among the treatments (Figure 7). The greatest measured reduction was achieved by combining 7.5 mg/L ozone and 150 mg/L This ozone/chlorine treatment reduced the microbial load by 1.37 CFU/g; while in the lettuce washed with distilled water, only a log reduction of 0.30 CFU/g was achieved. All treatments enhanced APC microbial reduction when compared to distilled water ($p<0.5$). Treatments containing only ozone had significantly lower amounts of

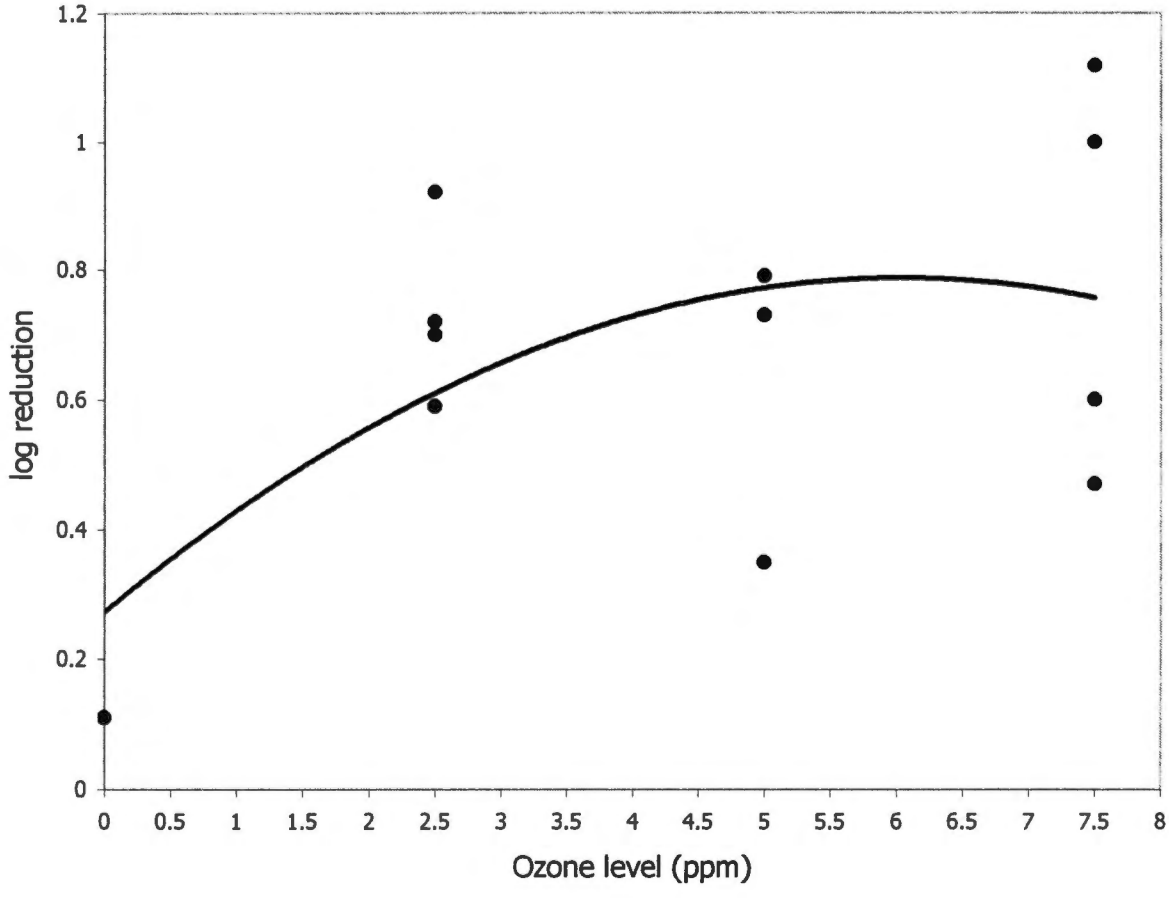


Figure 5. Microbial reduction in ozone treated inoculated lettuce samples

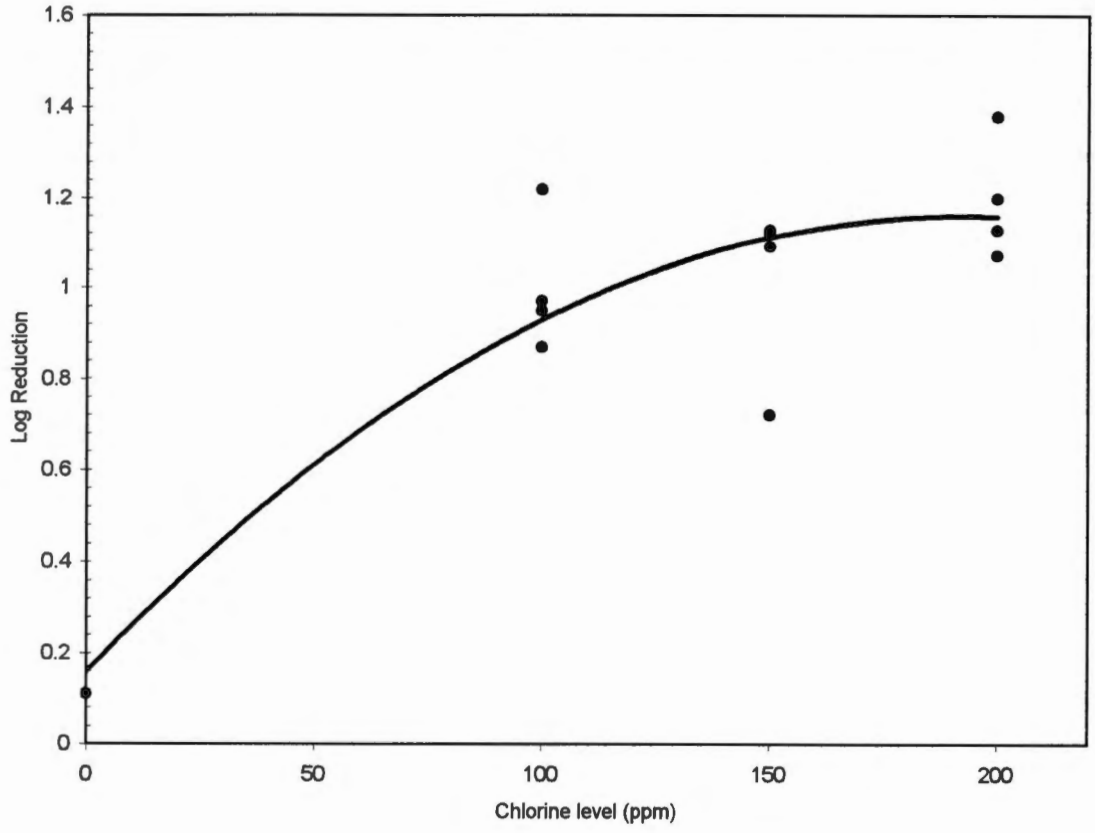
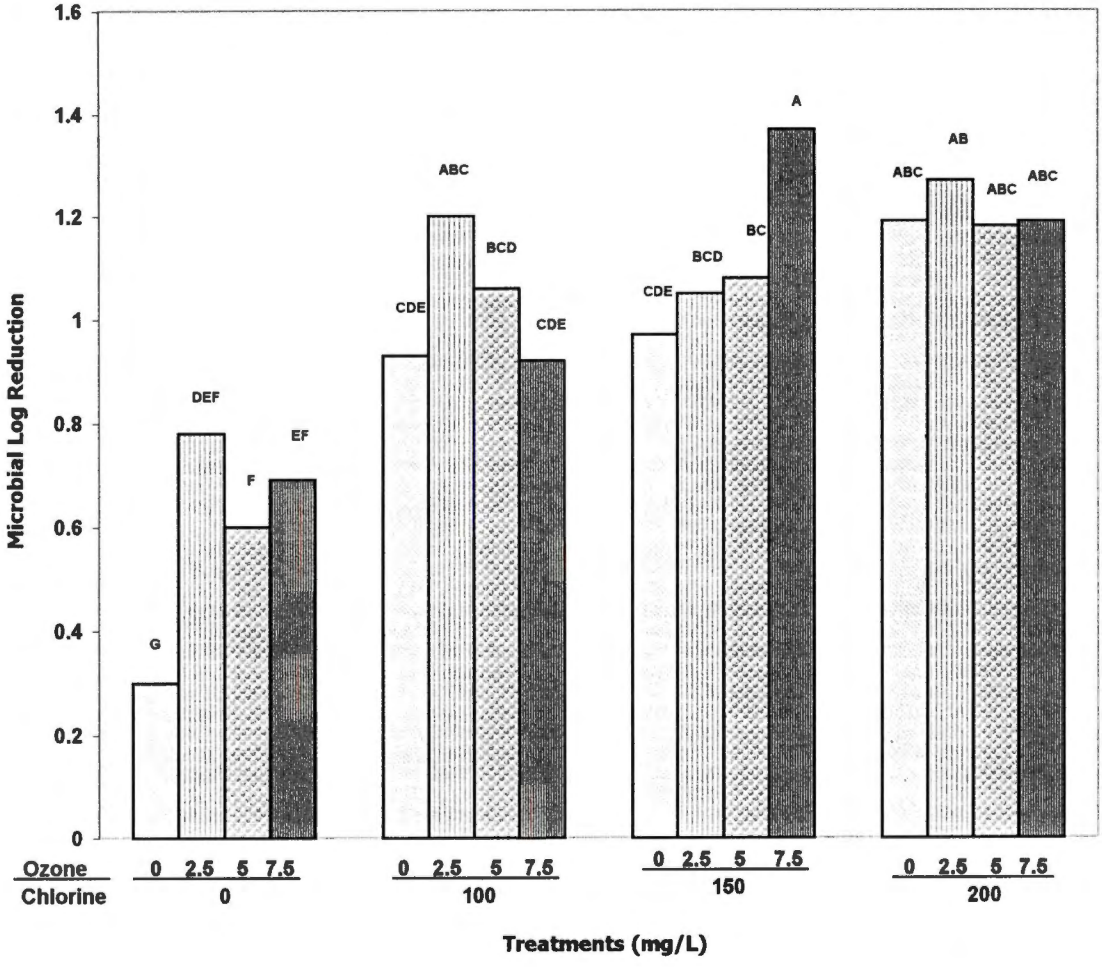


Figure 6. Microbial reduction in chlorine treated inoculated lettuce samples



Log reductions with like letters are not statistically different (P<0.5)

Figure 7. LSMeans for the microbial reduction of inoculated lettuce samples treated with ozone, chlorine and ozone-chlorine from the APC data

microbial reduction compared to any of the treatments containing 200 mg/L chlorine, 5 and 7.5 mg/L ozone and 150 mg/L chlorine, or 2.5 mg/L ozone- 100 mg/L chlorine.

Chlorine is recognized as an efficient sanitizing agent in reducing the microbial loads on lettuce and is the current standard practice. The level of chlorine can be a quality or safety factor. Therefore it is preferable to reduce the amount of chlorine to the lowest necessary level of use. The use of only 100 mg/L chlorine in combination with ozone was not significantly different ($p<0.5$) than any of the 150 or 200 mg/L chlorine containing solutions combined with any amount of ozone. Therefore a treatment with a lower chlorine concentration in combination with ozone could be recommended. The production of produce with lower chlorine levels would produce more acceptable sensory characteristics and also reduce risk of the formation of carcinogenic compounds such as trihalomethane compounds (Simpson and others 2000).

A response surface analysis was performed on replications 2, 3 and 4 (same source of raw material) to design a quadratic regression that would fit the data and predict microbial log reductions (APC) of ozone-chlorine treated fresh-cut lettuce. The quadratic regression obtained can be used to explain 57.39% of the variation in the study (Figure 8). The fitted data suggests that a concentration of 4.09 mg/L ozone in combination with 225 mg/L chlorine would be required to achieve the greatest APC log reduction or microbial kill. It should be noted that the critical chlorine value obtained from this analysis is above best use practices of 200 mg/L chlorine.

The analysis of the pyschrotrophic bacteria growth after the ozone treatments found no significant differences among 0, 2.5, 5 and 7.5 mg/L ozone treatments for reduction in microbial numbers on the fresh-cut lettuce. The chlorine treatments were not

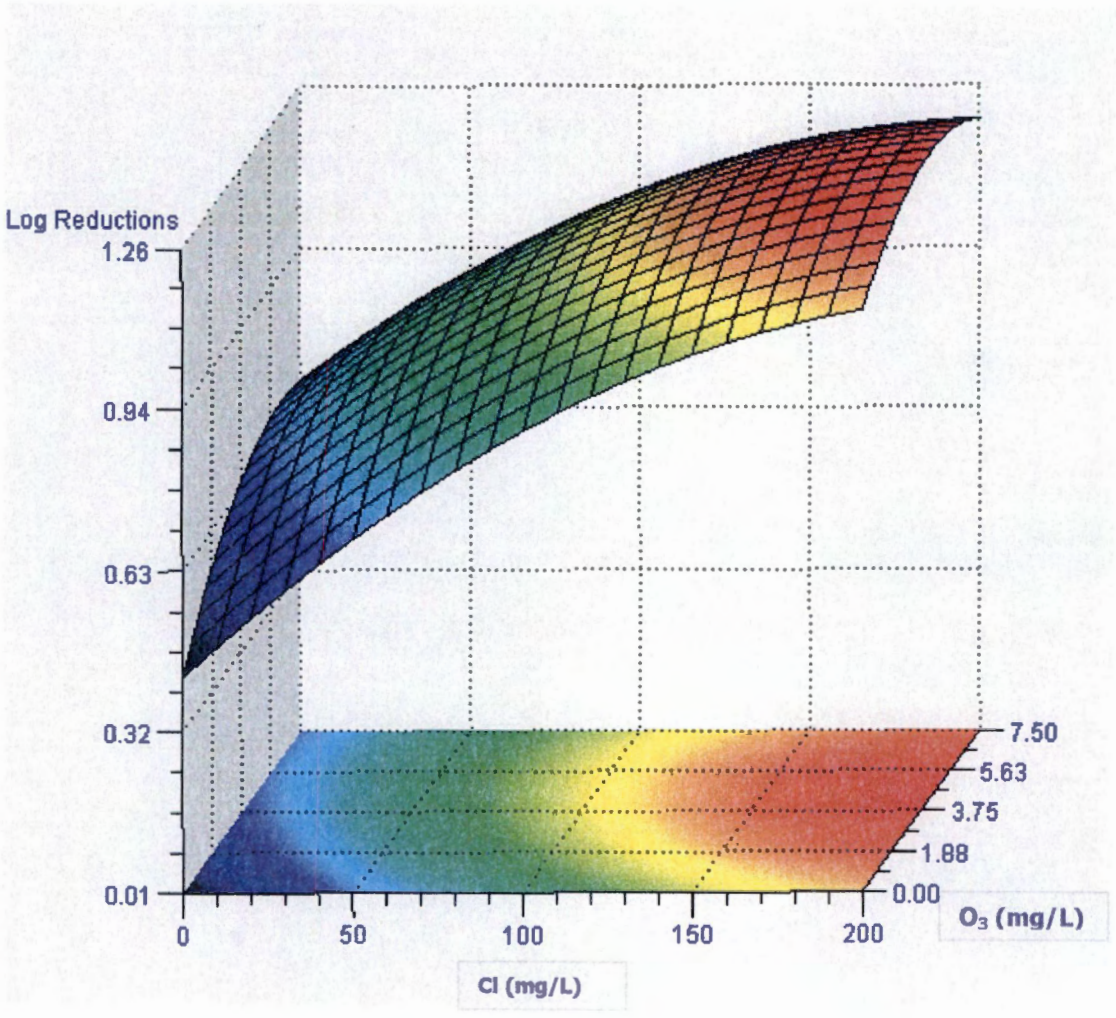


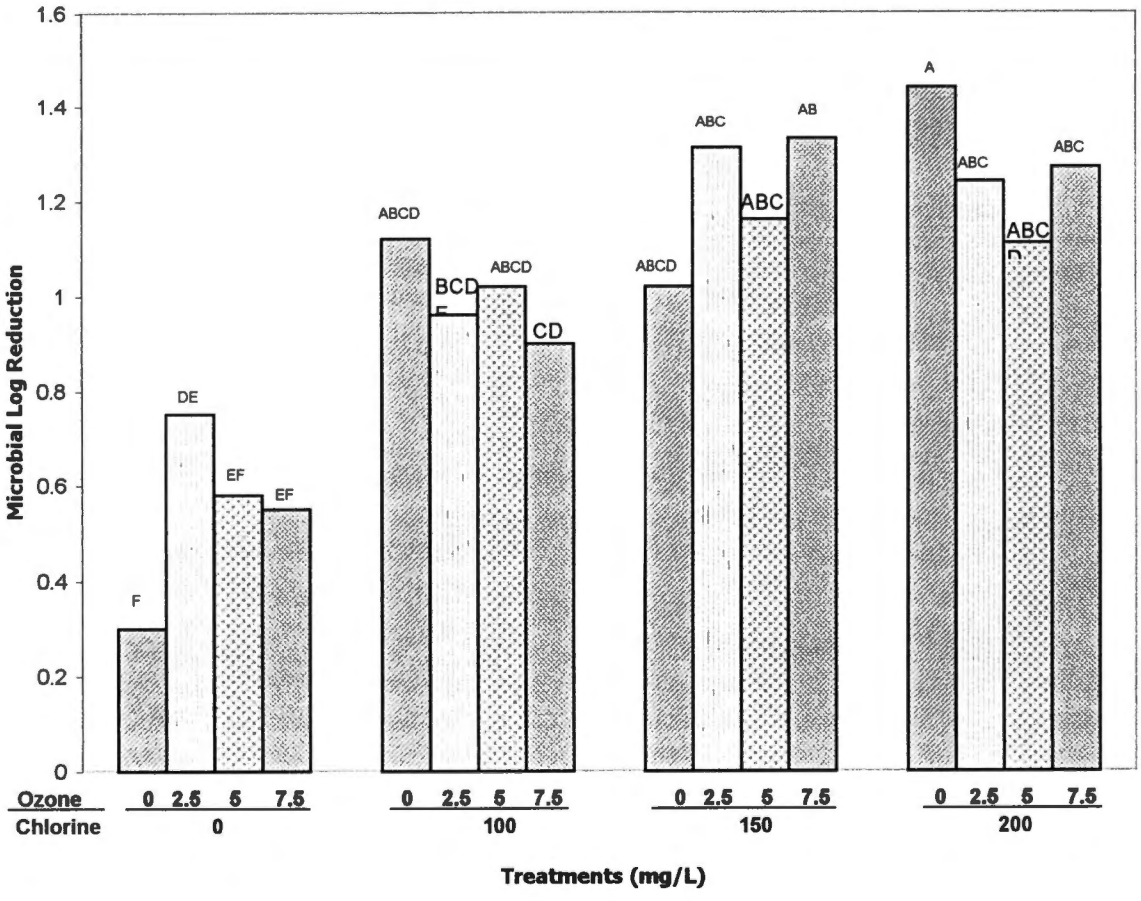
Figure 8. Quadratic regression model for the surface response for APC data, microbial log reduction versus ozone, chlorine and ozone-chlorine treatments

significantly different among themselves but were highly significantly in reduction of psychrotrophic microorganisms on fresh-cut lettuce than just the distilled water treatment ($P<0.01$).

None of the ozone-chlorine treatments were significantly better than using just chlorine at the same level as the combination. The addition of ozone to the treatment solutions would therefore not be necessary for controlling psychrotrophic bacteria when using at least 100 mg/L chlorine (Figure 9). Analysis of least square common means illustrates that the combination of 5 mg / L ozone and 100 mg / L chlorine does not significantly differ from using 200 mg / L chlorine alone which resulted in the highest measured reduction in psycrotorophic bacteria on fresh-cut lettuce.

Surface response analysis performed on replications 2, 3 and 4 (raw material obtained from the same batch) had a quadratic regression which only explained 39.0% of the variation of the experiment (Figure 10). The results suggest that a combination of 6.5 mg / L ozone and 233 mg / L chlorine is best in order to obtain the greatest microbial reduction. More data needs to be collected to develop a response curve that better explains the treatment variation.

Microbial control is important in the RTE produce industry. The microbial load present in the raw material is one of the major factors that will determine the shelf-life of the finish product. APC analyses are important because they give the produces an idea of the microbial load of the raw that is coming in their plant. PPC analysis will help to determine the load that can survive the treatments and that are more likely to grow under storage conditions. But microbial control is not the only factor that controls the



Log reductions with like letters are not statistically different (P<0.5)

Figure 9. LSMeans for the microbial reduction of inoculated lettuce samples treated with ozone, chlorine and ozone-chlorine from the PPC data

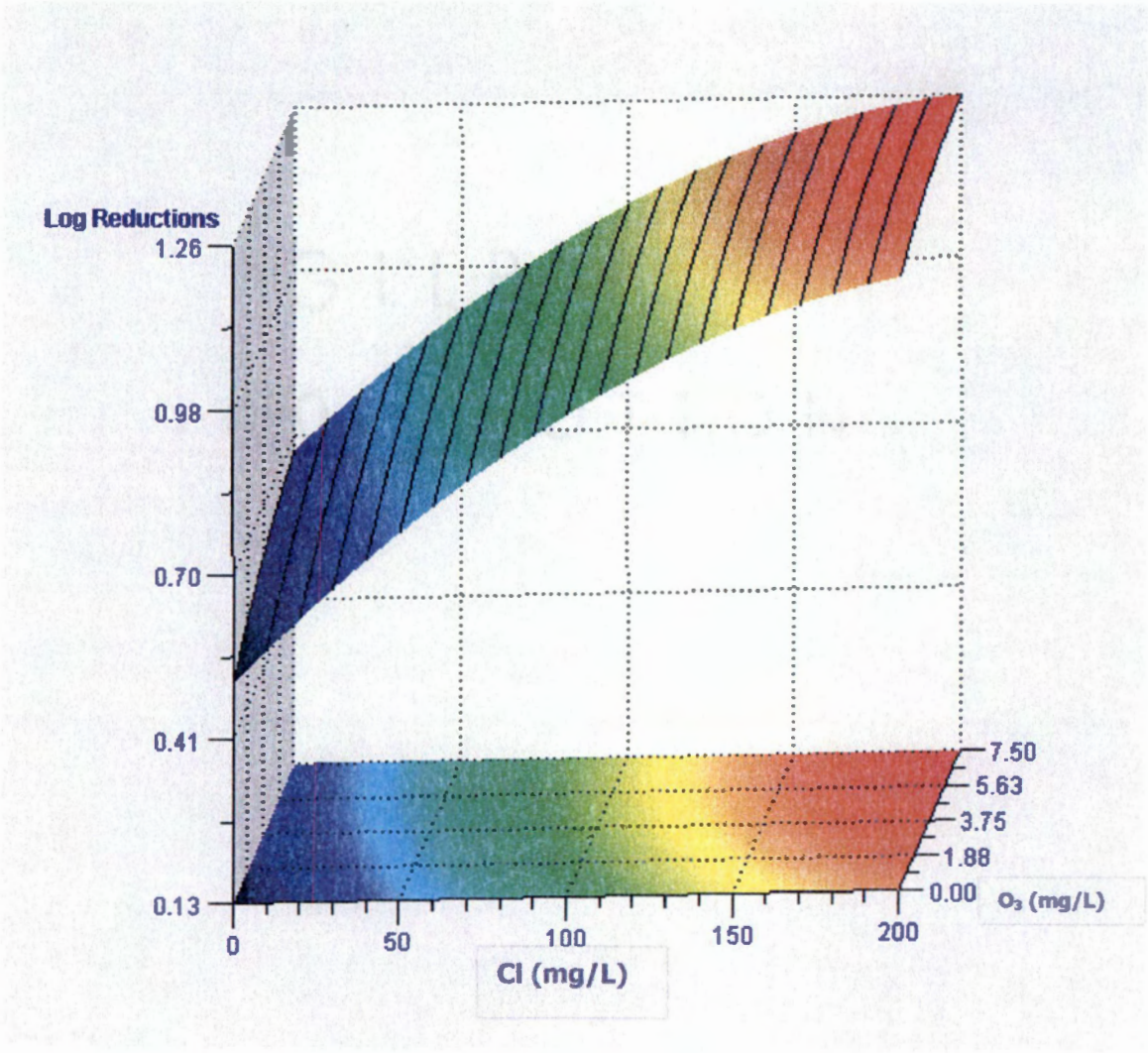


Figure 10. Quadratic regression model for the surface response PPC data, microbial log reduction versus ozone, chlorine and ozone-chlorine treatments

marketability of RTE produce; other factors like sensory analysis (freshness, color, firmness, humidity, etc.) are also important in marketing these products.

Sensory

Shelf – life extension of fresh-cut produce and ready-to-eat salads (RTE) is an important parameter for producers. The use of sanitizing agents to reduce microbial loads may enhance and extend the shelf – life of these products thus, providing the consumer with a higher quality product. Commercial samples of RTE salads that were treated with an aqueous ozone, chlorine and the mixture of ozone – chlorine were evaluated by an untrained panel (N=30) to determine whether or not they would purchase the samples..

The three treatments combinations on the RTE salads were important ($P<0.05$) in determining if the panelist were "willing to purchase" the salads. There was a significant interaction ($P<0.05$) within the combination of ozone and chlorine, mainly due to the effects of the different treatments over storage time on the salads (Figure 11). A significant difference among treatments ($P<0.05$) was identified by day 4, with the panelist scores for the ozone and the chlorine treated RTE salads not significantly different from each other but different for the ozone-chlorine treated product. The panelists were willing to "definitely purchase" the ozone-chlorine treated salads. This was still found at day 16 of storage. By day 21, the ozone and ozone-chlorine treated salads were significantly different from each other and were at the "definitely" to "probably" purchase level, but were different from the chlorine treated salads. By day 25, the RTE salads from all the treatments were significantly different from each other. The

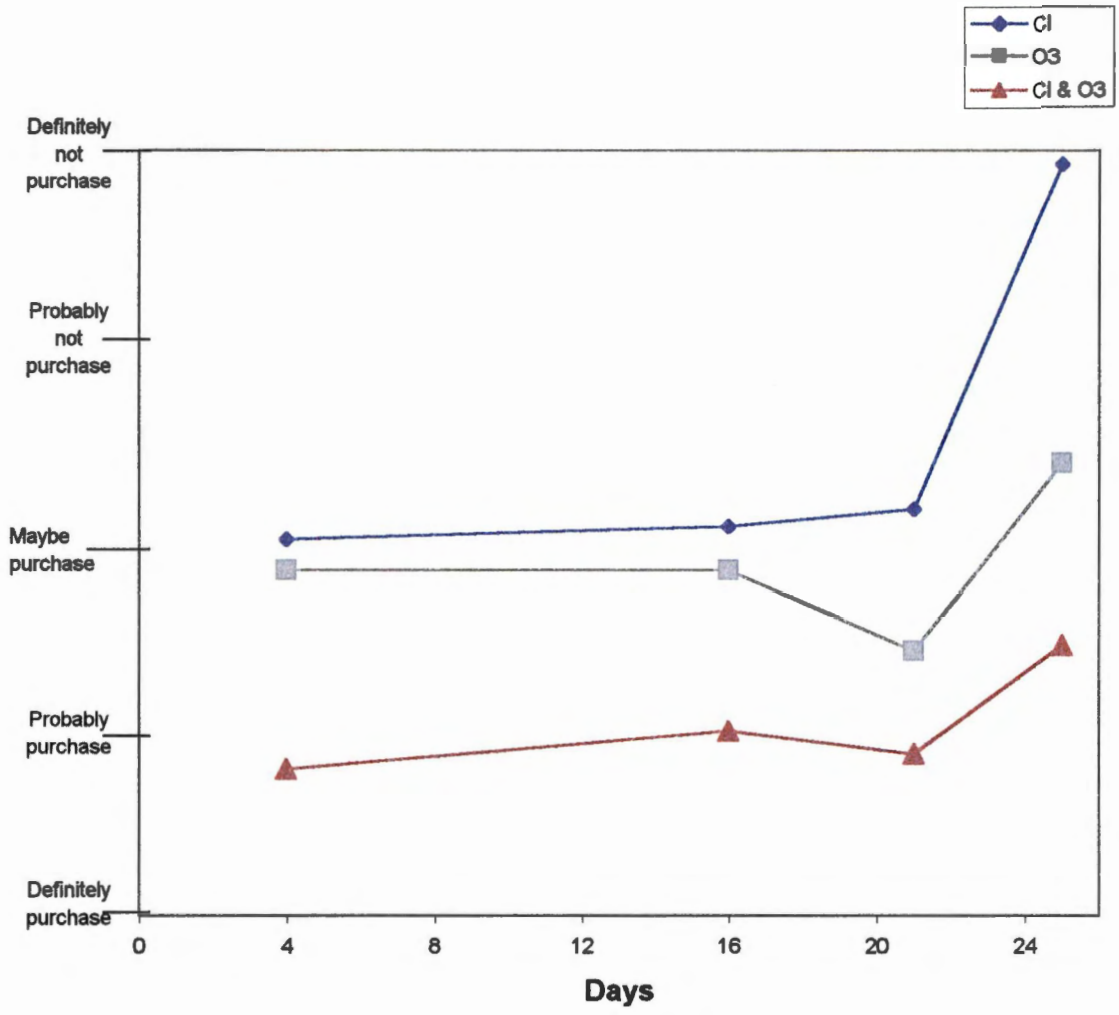


Figure 11. The estimate of the three treatments is graph against time in days.

ozone-chlorine treated salad was the most desirable and the panelists were willing to "probably purchase" with a LSMeans estimate of 2.4.

By day 25, packages treated with a combination of ozone and chlorine, started to shown a slight browning at the edges of the lettuce leaves, and structural break down, but 64.5% of the panelists would either "definitely purchase" or "probably purchase" the ozone-chlorine treated salads (Table 2). Therefore, the majority of panelist would still be willing to purchase the sample after 25 days, while none of the panelists would purchase the chlorine treated samples. The analyses of all the individual panels can be seen in Appendix A.

The effect of the three treatments on shelf-life of the RTE salads is shown in Figure 12. When the samples were only treated with a chlorine solution, the lettuce decay was very rapid. Panelist indicated browning, structural break down, color changes, water segregation and overall poor appearance occurred by day 16 indicating a shelf – life of at less than 16 days. When using an aqueous ozone solution treatment the decay of the salad was slower, extending the shelf – life of the RTE salads to approximately 21 days. The ozone and chlorine combination gave the best results achieving a shelf – life of greater than 25 days.

Turbidity analysis

Water samples were taken each hour for 5 hours from a processing line surge tank of a large produce manufacturing facility in Nashville, TN. for each treatment (chlorine wash, ozone wash and chlorine- ozone wash) system. Percentage of transmittance (%T) was measured at wavelengths that ranged from 180 – 840 nm as an indicator of water quality.

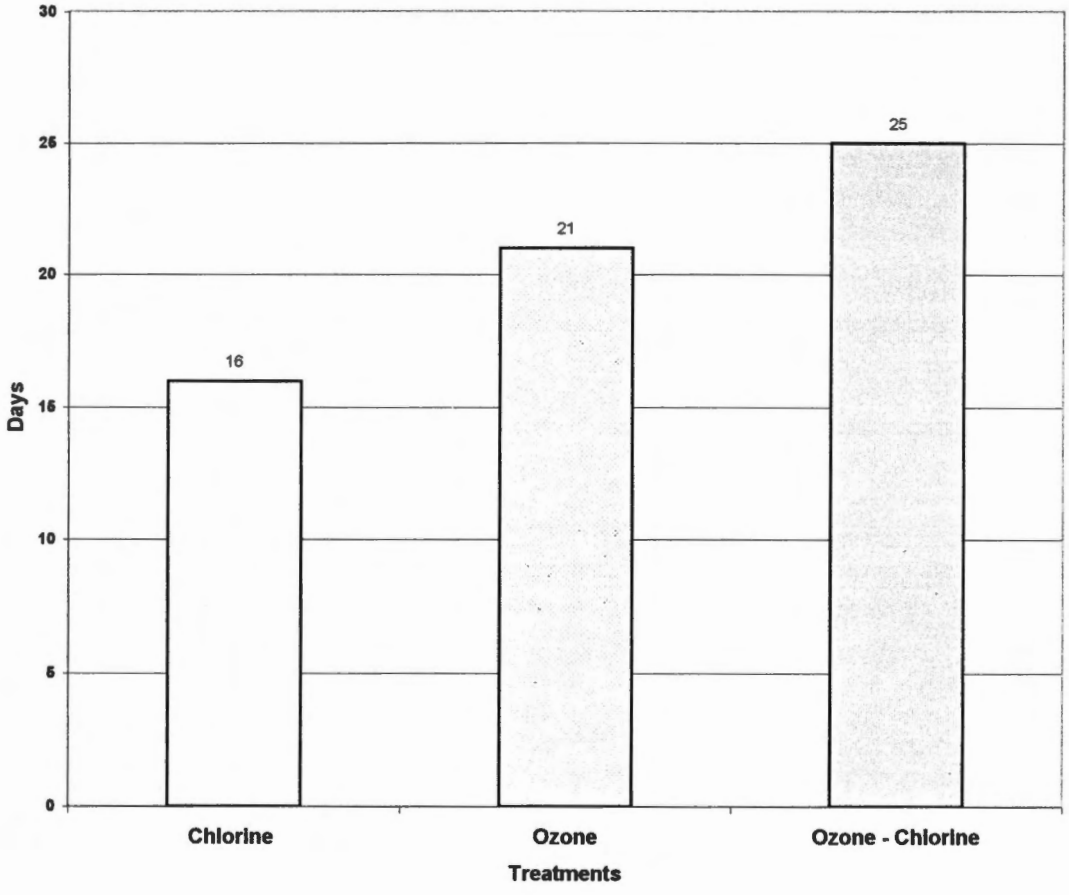


Figure 12. "Shelf – life Study" Effect of the ozone, chlorine and ozone-chlorine treatments on shelf- life is potted against time.

Table 2. Percentage of panelists that would purchase the salad sample 25 days after production

	Sensory Scale				
	1	2	3	4	5
Treatment % Acceptance	Definitely Purchase	Probably Purchase	Maybe Purchase	Probably not Purchase	Definitely not Purchase
Chlorine	0.00 %	0.00 %	0.00 %	6.90 %	93.10 %
Ozone	10.00 %	20.00 %	23.33 %	16.67 %	30.00 %
Ozone & Chlorine	22.58 %	41.94 %	19.35 %	3.23 %	12.90 %

The UV-Vis transmittance in the samples decreased as processing time increased, indicating that more particles, dissolved solids, organic material, and other soil is present in the water. When the lettuce was washed with a chlorine solution (Figure 13), the water remains clear (translucent) for at least 1 hour. After 2 to 3 hours of processing time the solution became cloudier. Thus, when adding chlorine the pigments and the organic material will react and formed THM's. When using an ozone-chlorine wash (Figure 14) the percentage of transmittance has a minor variation. The quality of the water remains constant for longer periods of time, making its reusable for consecutive batches of processing. The following hypothesis can be made by looking at the difference between the %T between the chlorine wash and the ozone-chlorine wash: Organic compounds that result from ozonation are more biodegradable than chlorinated organic compounds and since ozone has a higher oxidation potential than chlorine with a wider spectrum; it oxidizes more rapidly and more efficiently the organic matter that is suspended or dissolved in the water. Attacking the dissolved pigments in the water (α and β chlorophylls, phenols, carotenoids, etc.) and therefore keeping a low variation in the % of transmission of the treatment water. Also ozone does not attach to organic molecules in the water, like chlorine does; increasing levels of ozone are not needed to achieve the same level of microbial reduction in a batch.

Total solids were also measured in water samples; to determine if there was a relationship between the % transmittance and the total solids in the water. Results gave no indication of differences or variances between the two treatments having less than

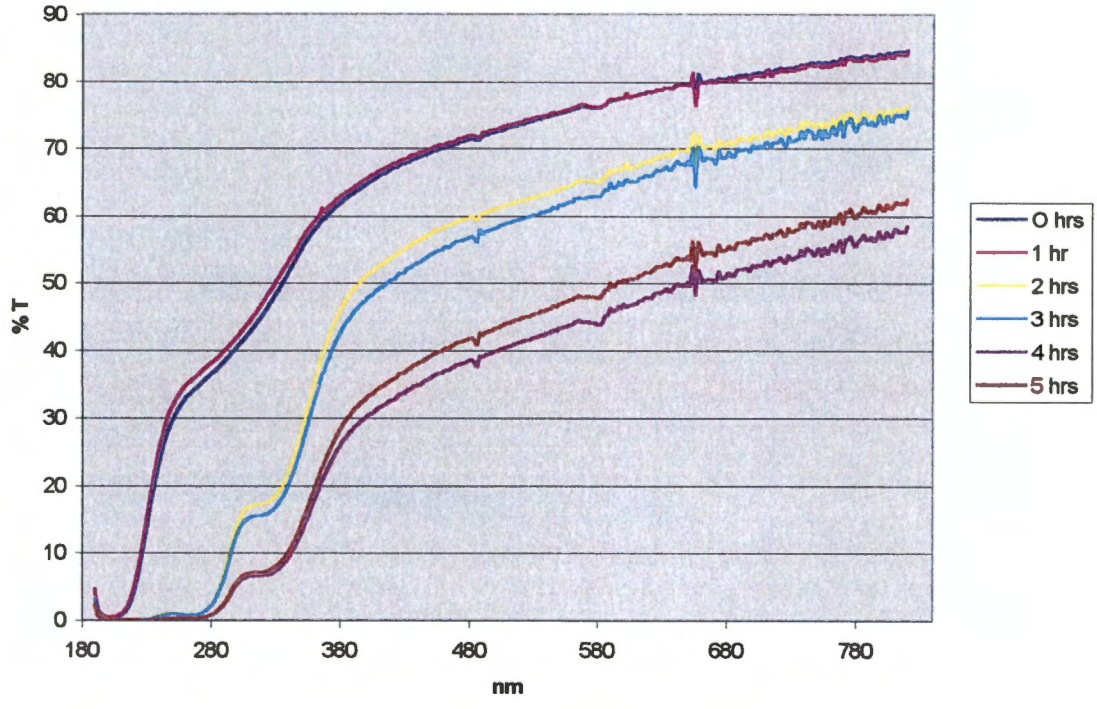


Figure 13. % Transmittance from chlorinated wash samples measure at wavelengths between 180 – 840 nm

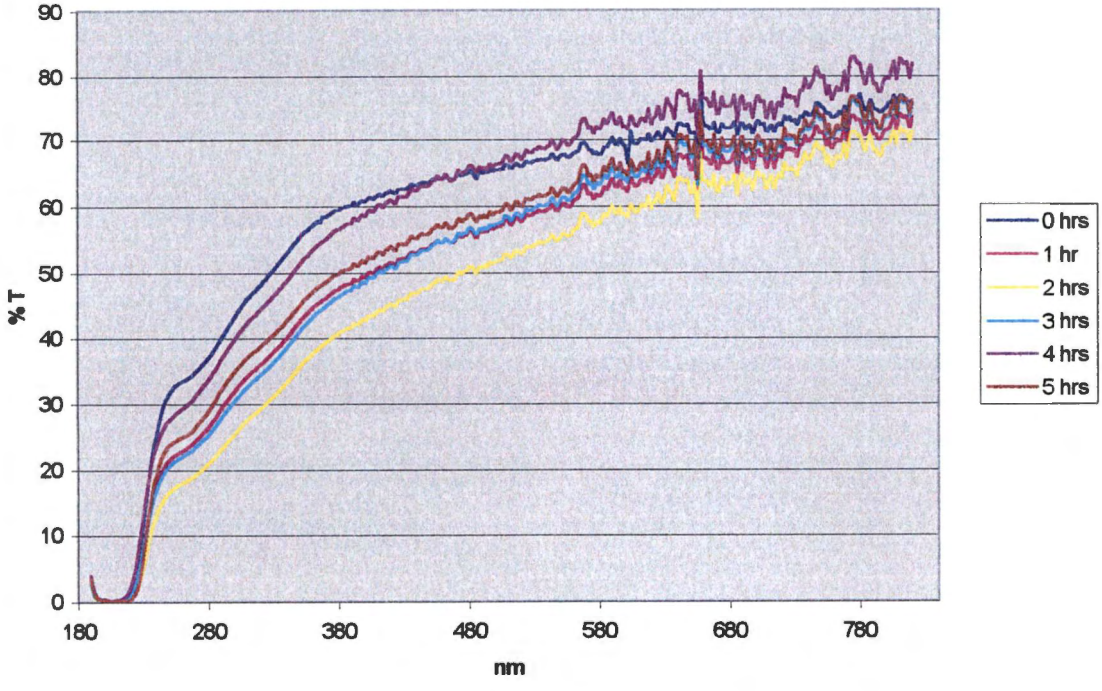


Figure 14. % Transmittance from ozone-chlorine wash samples measure at wavelengths between 180 – 840 nm

0.16% of total solids in the samples. Therefore total solids cannot be used as an indicator of water quality in this study. More test and analysis are needed to probe if this method can be used in the minimally processed vegetable industry as an indicator of water quality.

Chapter 5

Conclusions

Microbiological results show that by using an ozone concentration of 2.5 mg/L in combination with 100 mg/L chlorine will achieve a microbial Log reduction for APC and PPC that it is not statistically different from a high chlorine treatment (200ppm). The use of a low chlorine treatment has advantages. For example, the panelists indicated beneficial sensory characteristics for the ready-to-eat salads such as preserved color and reduced structural breakdown. Low chlorine levels reduced off-odors in treated salads. Using a low chlorine concentration treatment can also reduce the risk of the formation of THM's compounds, which have been proven to be carcinogenic. Using a combination of 2.5 mg / L ozone and 100 mg / L chlorine is recommended for the washing process of fresh-cut lettuce. These treatment levels were shown to be beneficial and effective in reducing the microflora of lettuce and might extend the shelf-life and improve the water quality in the processing line.

Sensory evaluation showed that by using an ozone-chlorine treatment an extension of the shelf-life of the ready-to-eat salads can be achieved, going from 16 days using a chlorine treatment, to 25 days using the combination of ozone and chlorine. By the 25^{th} day, 65% of the panelist would still purchases the ozone-chlorine treated salads while none would buy the chlorine treated ones. By the 14^{th} day, the chlorinated salads started showing a brownish color at the edges (probably generated by oxidation of the chlorophyll or by enzymatic reactions within the cells), structural break down, volume

reduction, secretion of juices, and color deterioration in general; while the salads treated with ozone-chlorine started to show similar characteristics after 25 days.

Spectrophotometry shows that the water quality, from a translucent point of view, remains cleaner for a longer period of time when using ozone in combination with chlorine to treat the salads. Spectophotometry from chlorinated water treatment shows a decrease in % transmittances over the time of processing, whereas the ozone-chlorine water treatment shows no differences in % transmittances over processing time, and consequently, preserves the water quality for longer periods of time.

A level of 2.5 mg/L free (available for microbial purposes) ozone with 150 mg/L of free chlorine is recommended when processing ready-to-eat salads. A higher amount of ozone might be needed for a large manufacturing plant because the amount of organics, dissolved solids and ozone-demanding material in the water is greater. Using a higher concentration of ozone will oxidize the organics in the water making the remaining free chlorine more efficient against microorganisms, achieving a greater Log reduction of microorganisms and extending the shelf-life of the fresh-cut produce. These amounts of ozone still need to be determined; therefore, further data collection is needed from the processing facilities to establish a model that can predict these amounts.

This study clearly shows that ozone at these levels cannot totally replace chlorine. Chlorine is needed for the purpose of achieving a greater microbial reduction in RTE salads. Chlorine levels can be reduced when used in combination with ozone, but further research is needed to determine if at higher ozone levels the characteristics of the RTE salads is not affected and the shelf-life is not reduced.

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Appendix

Ozone test kit directions

For the determination of ozone concentrations the kit directions were fallowed and the procedure involved adding a

- 25 mL sample of ozonated water into the sample cup
- 5 drops of activator solution were added to the solution under agitation.
- After a 1-minute waiting period, a vacu-vial ampoule was placed in the sample cup. The tip of the vial was broken to allow the solution to get into the ampoule. The ampoule was inverted several times to ensure proper mixing and then held for 1 minute until the full color was developed.
- Transmittance (T) of the reagent was then measured at a wavelength of 565 nm in a UV-VIS spectrophotometer (Spectronic Instruments, Spectronic® 20, Genesys ™, Rochester, NY.)

Table 3 Illustrates the correction from % T to ppm (grams/Liter)

Table 3. Correction from % T to ppm (mg / L) for ozone concentrations

CONCENTRATION vs TRANSMITTANCE for SPECTRONIC 21

Cat. No. K-2513 515 nm

CHLORINE 2, PPM (mg / Liter)

%T	%T UNITS									
TENS	0	1	2	3	4	5	6	7	8	9
10	5.03	4.82	4.62	4.44	4.27	4.12	3.97	3.84	3.71	3.59
20	3.47	3.36	3.26	3.16	3.06	2.97	2.88	2.8	2.71	2.63
30	2.56	2.48	2.41	2.34	2.26	2.21	2.15	2.09	2.03	1.97
40	1.91	1.86	1.8	1.75	1.7	1.65	1.6	1.55	1.5	1.45
50	1.41	1.36	1.32	1.28	1.24	1.19	1.15	1.11	1.08	1.04
60	1	0.96	0.93	0.89	0.85	0.82	0.78	0.75	0.72	0.68
70	0.65	0.62	0.59	0.56	0.53	0.5	0.47	0.44	0.41	0.38
80	0.35	0.32	0.3	0.27	0.24	0.22	0.19	0.16	0.14	0.11
90	0.09	0.06	0.04	0.01						

PPM (mg / Liter) = 5.18 (abs) - 0.15

CHEMetrics, Incorporated
Route 28, Calverton, VA 20138
Phone (540) 788-9026

2485-3

Chlorine test kit directions

For the determination of ozone concentrations the kit directions were fallowed and the procedure involved adding a

- 25 mL sample of chlorinated water was placed into the sample cup
- 5 drops of activator solution were added to the solution under agitation.
- After a 1-minute waiting period, a vacu-vial ampoule was placed in the sample cup. The tip was broken to allow the solution to get into the ampoule. The ampoule was inverted several times to ensure proper mixing and then held for 1 minute to allow the color reaction to proceed.
- Transmittance (T) of the reagent was measured at a wave length of 515 nm in a UV-VIS spectrophotometer (Spectronic Instruments, Spectronic® 20, Genesys™ , Rochester, NY.)
- Table 4 was used to convert the % T to ppm (grams/Liter)

Table 4. Correction from % T to ppm (mg / L) for chlorine concentrations

CONCENTRATION vs TRANSMITTANCE for SPECTRONIC 21

Cat. No. K-7403 565 nm

OZONE, PPM (mg / Liter)

%T	%T UNITS									
TENS	0	1	2	3	4	5	6	7	8	9
10	2.38	2.28	2.19	2.11	2.03	1.96	1.9	1.83	1.78	1.72
20	1.67	1.62	1.57	1.52	1.48	1.44	1.4	1.36	1.32	1.28
30	1.25	1.22	1.18	1.15	1.12	1.09	1.06	1.03	1.01	0.98
40	0.95	0.93	0.9	0.88	0.86	0.83	0.81	0.79	0.77	0.74
50	0.72	0.7	0.68	0.66	0.64	0.63	0.61	0.59	0.57	0.55
60	0.52	0.52	0.5	0.49	0.47	0.45	0.44	0.42	0.41	0.39
70	0.38	0.36	0.35	0.33	0.32	0.31	0.29	0.28	0.27	0.25
80	0.24	0.23	0.21	0.2	0.19	0.18	0.17	0.15	0.14	0.13
90	0.12	0.11	0.1	0.08	0.07	0.06	0.05	0.04	0.03	0.02

PPM (mg / Liter) = 5.18 (abs) - 0.15

CHEMetrics, Incorporated
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2251-6

Table 5. Sensory scorecard

Judge _____ Packaged salad mixes

You will view three samples of packaged lettuce salad. Please view in the order listed below and indicate whether or not you might bight the sample on the scale below. Please indicate in the comment column why you would or would purchase the sample.

SAMPLE	How likely would you be to purchase this sample of lettuce salad in the supermarket?	COMMENTS
449	_____ definitely purchase _____ probably purchase _____ maybe purchase _____ probably not purchase _____ definitely not purchase	
210	_____ definitely purchase _____ probably purchase _____ maybe purchase _____ probably not purchase _____ definitely not purchase	
725	_____ definitely purchase _____ probably purchase _____ maybe purchase _____ probably not purchase _____ definitely not purchase	

PLEASE TURN THE PAGE OVER

Lettuce Panel Judge _____

In order to evaluate the data, we would like to have some information about you. Please provide the information below. Your name will not be associated with the data in anyway.

Gender
_____ Male
_____ Female

Age
_____ younger than 20
_____ 20-29
_____ 30-39
_____ 40-49
_____ 50-59
_____ 60-69
_____ 70 and older

How often do you buy packaged salads to eat at home?
_____ Rarely or never
_____ 1-12 times per year
_____ 13-24 times per year
_____ 1 time per week
_____ several times per week

Thank you for participating in our panel today!

Table 6. Percentage of panelist that would purchase the salads samples after 4 days of production

Lettuce Panel One 4 days

The FREQ Procedure

Frequency
Row Pct

Table of sample by Purchasability

Sample	Purchasability					Total
	Definitely Purchase	Probably Purchase	Maybe Purchase	Probably not Purchase	Definitely not Purchase	
Chlorine	4 13.33	9 30.00	4 13.33	10 33.33	3 10.00	30
Ozone	6 20.00	6 20.00	9 30.00	6 20.00	3 10.00	30
Both	12 40.00	15 50.00	1 3.33	2 6.67	0 0.00	30
Total	22	30	14	18	6	90

Statistics for Table of sample by Purchasability

Statistic	DF	Value	Prob
Chi-Square	8	24.2606	0.0021

---------- ADJUSTMENT=LSD(.05) BYGROUP=1 Effect=sample ----------
----

Obs	sample	Estimate	Standard Error	DF	t Value	Pr > |t|	Let Grp
1	1 Cl	2.9667	0.2082	87	14.25	<.0001	A
2	2 O3	2.8000	0.2082	87	13.45	<.0001	A
3	3 Cl & O3	1.7667	0.2082	87	8.49	<.0001	B

Table 7. Percentage of panelist that would purchase the salads samples after 16 days of production

Lettuce Panel Two 16 days

The FREQ Procedure

Frequency
Row Pct

Table of sample by Purchasability

Sample	Purchasability					Total
	Definitely Purchase	Probably Purchase	Maybe Purchase	Probably not Purchase	Definitely not Purchase	
Chlorine	4 12.90	5 16.13	9 29.03	10 32.26	3 9.68	31
Ozone	4 13.33	10 33.33	6 20.00	5 16.67	5 16.67	30
Both	12 41.38	10 34.48	4 13.79	3 10.34	0 0.00	29
Total	20	25	19	18	8	90

Statistics for Table of sample by Purchasability

Statistic	DF	Value	Prob
Chi-Square	8	19.5475	0.0122

Effective Sample Size = 90

---------- ADJUSTMENT=LSD(.05) BYGROUP=1 Effect=sample ----------
----

Obs	sample	Estimate	Standard Error	DF	t Value	Pr > |t|	Let Grp
1	1 Cl	3.0968	0.2121	87	14.60	<.0001	A
2	2 O3	2.9000	0.2156	87	13.45	<.0001	A
3	3 Cl & O3	1.9310	0.2192	87	8.81	<.0001	B

Table 8. Percentage of panelist that would purchase the salads samples after 21 days of production

Lettuce Panel Three 21days

The FREQ Procedure

Frequency
Row Pct

Table of sample by Purchasability

Sample	Purchasability					Total
	Definitely Purchase	Probably Purchase	Maybe Purchase	Probably not Purchase	Definitely not Purchase	
Chlorine	3 10.00	8 26.67	7 23.33	8 26.67	4 13.33	30
Ozone	7 23.33	11 36.67	7 23.33	4 13.33	1 3.33	30
Both	13 41.94	11 35.48	5 16.13	2 6.45	0 0.00	31
Total	23	30	19	14	5	91

Statistics for Table of sample by Purchasability

Statistic	DF	Value	Prob
Chi-Square	8	16.7575	0.0327

----- ADJUSTMENT=LSD(.05) BYGROUP=1 Effect=sample -----

Obs	sample	Estimate	Standard Error	DF	t Value	Pr > |t|	Let Grp
1	1 O3	3.0690	0.2033	87	15.09	<.0001	A
2	2 Cl	2.3667	0.1999	87	11.84	<.0001	B
3	3 Cl & O3	1.8710	0.1967	87	9.51	<.0001	B

Table 9. LSMeans for APC

Chlorine	Ozone	Estimated Mean	LSD Common Means
200	0	1.4475	A
150	7.5	1.3350	AB
150	2.5	1.3150	ABC
200	7.5	1.2725	ABC
200	2.5	1.2475	ABC
150	5	1.1600	ABCD
100	0	1.1200	ABCD
200	5	1.1150	ABCD
100	5	1.0250	ABCD
150	0	1.0200	ABCD
100	2.5	0.9675	BCDE
100	7.5	0.9025	CDE
0	2.5	0.7500	DE
0	5	0.5825	EF
0	7.5	0.5550	EF
0	0	0.3025	F

Table 10. LSMeans for PPC

Chlorine	Ozone	Estimated Mean	LSD Common Means
150	7.5	1.4000	A
200	0	1.3600	A
200	7.5	1.3567	A
150	2.5	1.2500	A
200	2.5	1.1533	A
150	5	1.1033	AB
100	0	1.0533	AB
100	2.5	1.0433	AB
200	5	1.0333	AB
100	5	1.0267	AB
100	7.5	0.9567	AB
150	0	0.9067	AB
0	2.5	0.8767	AB
0	5	0.6133	BC
0	7.5	0.6033	BC
0	0	0.3033	C

Vita

Andrés García was born in Mexico D.F. on November 21, 1973 to Elvira H. García Fernadez and Isaias García Terrazas. He graduated from Instituto Queretano San Javier high school in May 1992. In august 1993 he entered the Instituto Technológico y de Estudios Superiores de Monterrey campus Querétaro where he completed Bachelors in Sciences in Biochemical Engineer Administrator in Food Processing, December 1998. In January 1999, he entered the Food Science and Technology Master's program at the University of Tennessee, Knoxville, where his degree was awarded on May 2000.