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To the Graduate Council:

I am submitting herewith a thesis written by Sarah Malone entitled "Effect of Gaseous Ozone on Antioxidant Content and Color of Sliced Tomatoes." 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 R. Mount, Major Professor

We have read this thesis and recommend its acceptance:

Svetlana Zivanovic, Carl E Sams

Accepted for the Council:

Dixie L. Thompson

Vice Provost and Dean of the Graduate School

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

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Carl E Sams

Accepted for the Council:

Anne Mayhew

Vice Provost and
Dean of Graduate Studies

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

Effect of gaseous ozone
on antioxidant content and color of sliced tomatoes

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

Sarah Catherine Malone
May 2003

DEDICATION

This thesis is dedicated to my family
whose love and support made this work possible.

ACKNOWLEDGMENTS

My sincere thanks goes to my major professor, Dr. John Mount, for all of his guidance, inventiveness, and patience. I would also like to thank my other committee members, Dr. Svetlana Zivonovic and Dr. Carl Sams, for their input on my research. I would like to thank the University of Tennessee, Department of Food Science and Technology, Agricultural Experiment Station, and the Food Safety Center of Excellence for allowing me to obtain a quality education. Great thanks goes to my friends and colleagues in the graduate department-Vivian Ann Rash, Kim Stanley, Nancy DeTrana, Charlene Belles, Charity Lakins, and Dr. Rob Williams. How could I have survived graduate school without you all? Thanks also goes to Henry Perry and JoCarole Mobley for their assistance with my research. Last, I especially want to thank my boyfriend, Jeffrey Boland, for his love, support, and ability to keep me calm when things did not turn out as planned. Jeff, you are my rock. To all of you, I sincerely appreciate the time and effort that you all have given to me and to my research. I am so lucky to be supported by such wonderful people.

ABSTRACT

Sliced tomatoes are used frequently in restaurants and fast-food establishments. Their limited shelf-life has caused scientists to study treatments to prolong their useful life. Previously, treatments for shelf-life extension have included mild heat treatment and modified atmosphere packaging. Although gaseous ozone has been shown to reduce spoilage microorganisms on produce, limited research has been performed regarding the oxidation potential of ozone on antioxidant compounds. Our objective was to evaluate the effect of gaseous ozone on lycopene and ascorbic acid in sliced tomatoes, as well as its effect on tomato color.

Two tomatoes of uniform size and color (USDA Stage 5) were cut into cross-sectional slices. Quart-size wide mouth sterilized Mason jars covered in aluminum foil were used as treatment vessels. Jars containing tomato slices and 1 mL aliquots of 24-h tomato spoilage culture were treated with ozonated air at treatment levels of 0, 90, 105, 120, and 135 min. Lycopene was extracted with solvent containing hexane:methanol:acetone, and absorbance of the samples was read in a spectrophotometer at 503 nm against a hexane blank. Lycopene content was calculated for each sample using a mathematical formula. A 2,6-dichloroindophenol titrimetric method was used for ascorbic acid determination. Color measurements were performed on ground tomato samples. All experiments were statistically analyzed using the mixed procedure (PROC MIXED) of SAS version 8.1 (SAS Institute, Cary, NC). Means were separated using Tukey's mean separation test. Significant differences were defined at $P < 0.05$.

No significant differences ($p>0.05$) in lycopene content were found between untreated tomato slices or slices treated for 90, 105, 120, or 135 minutes. The ascorbic acid content of sliced tomatoes treated with gaseous ozone for 135 minutes was significantly lower ($p < 0.05$) than untreated sliced tomatoes. No significant differences ($p>0.05$) were found in Hunter L, a, or b values between untreated tomato slices or slices treated for 135 minutes. Aerobic plate counts of spoilage microorganisms treated with gaseous ozone for times of 105, 120, and 135 minutes were statistically similar to each other, but all were significantly lower than untreated samples. Aerobic plate counts of samples treated for 90 minutes were not statistically different from either untreated samples or samples treated for 105, 120, or 135 minutes.

Results indicate that gaseous ozone treatment for a minimum of 105 minutes can be an effective means of decreasing spoilage microflora of sliced tomatoes. Lycopene and color measurements do not seem to be adversely affected, but ascorbic acid may be significantly reduced. Therefore, ozone treatments to reduce microbial loads may not be appropriate for fresh-cut produce items that are consumed to provide ascorbic acid in the diet.

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I. INTRODUCTION

Justification

Over the past few years, there has been an increase in consumer demand for produce that has been minimally processed, or "fresh-cut." However, the shelf-life of fresh-cut produce is limited. Gaseous ozone has been used to decrease microbial activity and extend the shelf-life of various types of produce, including strawberries, grapes, blackberries, carrots, broccoli, and cucumbers (Barth and others 1995; Liew and Prange 1994; Perez and others 1999; Sarig and others 1996; Skog and Chu 2001).

While ozone is effective at destroying microorganisms, it reacts with other organic compounds as well. In this manner, ozone may have a detrimental effect on produce quality. Skog and Chu (2001) observed desiccation of cucumbers stored in ozonized rooms, while Perez and others (1999) reported a reduction in aroma compounds of strawberries. Antioxidants, such as lycopene and ascorbic acid, also have the potential to be oxidized by ozone.

Sliced tomatoes are used frequently in restaurants and fast-food establishments. Their limited shelf-life has caused scientists to study treatments to prolong their useful life. Previously, treatments for shelf-life extension have included mild heat treatment, calcium chloride dips, modified atmosphere packaging, and ethylene treatments. Although gaseous ozone has been shown to reduce spoilage microorganisms on produce, limited research has been performed regarding the oxidation potential of ozone on

antioxidant compounds. Therefore, we investigated the effect of gaseous ozone on the content of the antioxidants lycopene and ascorbic acid in sliced tomatoes, due to their presence at high levels.

II. LITERATURE REVIEW

Tomatoes

Lycopersicum esculentum is the scientific name for the tomato (Gould 1992). Like potatoes and eggplants, they belong to the nightshade family of plants (IFPA 2002). Botanically, or based on its plant parts, the tomato is classified as a fruit. Technically, because it is pulpy and contains one or more seeds, it is classified as a berry (Gould 1992). Legally, however, the tomato is considered a vegetable.

Tomatoes are a valuable commodity. Among all vegetables produced in the United States and in other countries where they are grown, tomatoes rank second to potatoes in dollar value (Gould 1992). The United States Department of Agriculture ranks tomatoes fourth in consumption among fresh vegetables by farm weight. Consumption of tomatoes is showing a positive trend, with an increase from 15.5 to 17.3 pounds per capita per year between the years of 1990 and 2000 (USDA 2002).

Tomatoes account for nearly 9% of total sales in retail produce departments (California Tomato Commission 2002c). Recent consumer research that focused on tomato buying habits indicated that over 98% of consumers purchase tomatoes. The attribute that consumers considered most important was appearance, followed by color, variety, and price (California Tomato Commission 2002a). More than half of the total crop of field tomatoes is used in foodservice (California Tomato Commission 2002b).

There are dozens of varieties of tomatoes in a wide range of sizes, shapes, and colors. One of the most commonly marketed varieties is the *Beefsteak* tomato, which is

large in size, bright red in color and slightly elliptical in shape. Other varieties include globe, plum, cherry, green, yellow pear, and currant tomatoes (IFPA 2002).

The tomato fruit consists of skin, pericarp, and locular contents. Locular cavities contain parenchyma cells, which are jelly-like and surround the seeds of the tomato (Shi and Maguer 2000). Tomatoes usually contain 7.0 to 8.5% total solids. Of this, about 1% is skins and seeds. The percentage of solids in tomatoes varies widely due to many factors, including variety, soil type, and amount of rainfall during the growing and harvesting season (Gould 1992).

A variety of types of acids are present in tomatoes. Citric acid is generally considered to be the most prevalent, followed by malic acid (Gould 1992). Pectin is another constituent of tomatoes and is formed between the microscopic cells of the fleshy red tissues of tomatoes. Pectin holds these tissues together, therefore pectinase enzymes which break down pectin can affect the textural integrity of tomatoes (Gould 1992).

Tomatoes make a significant contribution to the nutritional status of humans. The concentration and availability of several nutrients as well as tomatoes widespread consumption gives tomatoes this significance. One small red-ripe tomato is reported to contain about 40% of the adult RDA for vitamin C, also known as ascorbic acid. Tomatoes also are considered a good source of vitamin A that is present in the carotene form. In addition, tomatoes contain small amounts of the B vitamins thiamin, niacin, and riboflavin, as well as small amounts of iron and potassium (USDA 2002).

It has been suggested that tomatoes have anticancer benefits. Giovannucci and others (1995) found that the intake of tomato products, which include the compound

lycopene, was inversely associated with the development of prostate cancer. Franceschi and others (1994) found a reduced risk of digestive tract cancer with high intake of tomato products.

Some scientists have disputed the anticancer benefits of tomatoes. They argue that tomato products may represent an overall healthier eating pattern, such as a Mediterranean diet (Giovannucci 2002). However, in a study of epidemiological data by Giovannucci and others (2002), it was reported that, overall, data suggests an association between consumption of tomatoes and tomato products and a decreased risk of prostate cancer. Researchers theorize that lycopene, an antioxidant found in high quantities in tomatoes, may be responsible for this proposed effect.

Color is an important quality attribute of food products. In the case of tomatoes, the degree of quality of color is considered to be a practical representation of total quality. Several factors influence the composition and color of tomatoes. These include heredity, cultivation, soil and plant nutrition, maturity, and handling practices (Gould 1992). The characteristic red color of tomatoes is the result of the carotenoid pigment lycopene. Because tomato products have a tendency to discolor with reduced amounts of lycopene, it is important to prevent the destruction of this pigment. Factors that may destroy lycopene include the presence of metallic ions and the presence of oxygen (Gould 1992).

One method for the measurement of tomato color is the HunterLab Color and Color Difference Meter. This is a tristimulus colorimeter that measures color on three scales by using three filters. This gives three readings: *L*, which measures visual lightness, *a*, which measures where the color is on a red-green scale, and *b*, which

measures where the color is on a blue-yellow scale (Gould 1992). This method of color measurement is objective and thus is useful in conducting tomato research.

One practice that can reduce the nutritional quality of tomatoes is cutting or slicing of the tissue of the fruit. Tomatoes are sometimes marketed in a state called "fresh-cut," in which plant tissues have been physically damaged by cutting (IFPA 2002). This may increase their susceptibility to nutrient loss. Some of the most commonly used varieties of fresh-cut tomatoes include round/globe, cherry, and roma (IFPA 2002).

Fresh-cut Produce

The International Fresh-cut Produce Association defines fresh-cut produce as "any fruit or vegetable or combination thereof that has been physically altered from its original form, but remains in a fresh state" (Lamikanra 2002). Fruits or vegetables are trimmed and/or peeled and/or cut into product that is 100% usable. These products are prepared in order to offer convenience, flavor, and high nutrition to consumers and retail food service operations (IFPA 2002).

The fresh-cut produce industry has been around since the 1940s. In the 1970s, there was a great increase in production due to foodservice demands. Restaurants saw an opportunity to save on labor costs by switching to the more convenient fresh-cut produce, and the industry responded. In the 1980s, North America had a great increase in its number of restaurants, and salad bars became quite popular with consumers. Women started to work outside the home in larger numbers, which led families to seek time-saving, prepackaged produce items (Lamikanra 2002).

In the late 1980s, the plastics industry responded to the fresh-cut industry's need for better packaging materials. This, along with other factors such as producers demanding higher-quality produce and engineering technologies such as air-drying techniques has allowed for better quality of fresh-cut produce (Lamikanra 2002). However, problems with quality still surface today.

The quality of fresh-cut produce is judged by several factors, including appearance, texture, flavor, and nutritive value. There are many factors that can influence one or more of these quality attributes. One of these is physical damage during either harvesting or handling. Since fresh-cut produce has a lack of protective skin and plant tissues are damaged and exposed during cutting, it is especially vulnerable to this type of quality loss (Watada and Qi 1999).

There are many physiological reactions that occur when plant tissues are cut. Ethylene production is stimulated in some produce when tissues are injured, which can cause undesirable effects such as softening. Increases in respiration can occur in some produce. This may be due to changes in mitochondrial structure. Membrane deterioration of plant tissue can also occur. This results in decompartmentation of both cellular structure and organization, as well as the loss of normal cellular function. Additionally, secondary metabolite accumulation may occur, which results in an increase in enzymatic activity and oxidation reactions. Physical damage to fresh-cut produce can also affect quality attributes such as water loss or ascorbic acid content (Lamikanra 2002).

An increased susceptibility to microbial spoilage may also occur. Increased microbial populations may be associated with increased respiration rates in fresh-cut

produce, resulting in a shortened shelf-life. Tissue decay is also associated with microbial spoilage, specifically by aerobic and lactic acid bacteria. Some microorganisms produce enzymes that degrade pectin, which can lead to the softening and breakdown of plant tissue (Lamikanra 2002).

There are a variety of treatments that have been used to extend the shelf-life of fresh-cut produce. These treatments have different effects. The use of organic acids, such as citric acid, may reduce enzymatic browning reactions in some fresh-cut produce products. Reduced O₂ or elevated CO₂ atmospheres (up to 10%) may reduce ascorbic acid losses (Lamikanra 2002). One promising treatment is gaseous ozone. Ozone can oxidize and thus remove wound-induced ethylene because both compounds are gases and mix readily with each other (Wills and others 1998).

Ozone

Ozone (O₃) is a molecule composed of three oxygen atoms. It is an unstable gas that decomposes into oxygen (O₂). As this decomposition reaction occurs, the extra oxygen atom splits off from the molecule and is present as a free oxygen atom. These free atoms are toxic to bacteria and can oxidize organic compounds. Ozone is produced synthetically by exposing molecules of oxygen to a source of high energy, such as electrical discharge or ultraviolet radiation (Chester 1998).

The most common type of ozone generation system is based on corona discharge. The principle of this is a low-current electrical discharge occurring over a gas-filled gap at a voltage gradient. A corona cell consists of two metal electrodes, a gas-filled gap, and

a material that is dielectric. Oxygen-bearing gas flows through the discharge gap while high voltage is applied to the electrodes. This influx of electrical energy causes reactions to occur, including chemical reactions such as the production of triatomic oxygen, or ozone (Chester 1998).

The discovery of ozone dates back to 1840. F.C. Schonbein named this compound after the Greek word that means "to smell," due to its characteristic, pungent odor. Ozone has been used for disinfection of drinking water since the early 1900's. Developments for use of ozone in the agricultural, food, and chemical industries started in the 1950's (Horvath and Huttner 1985). There are many uses for ozone in the food industry. Ozone is used to increase crop yield, protect raw commodities during storage, and sanitize water used for washing produce, packaging materials, and equipment (Graham 1997).

Although ozone is a useful compound with many applications, it has certain characteristics that make it inappropriate in some situations. It is important to weigh advantages and disadvantages when deciding whether or not to treat a food product with ozone.

There are many advantages to using ozone over other sanitizing agents. Most importantly, it is the strongest oxidizing agent that is commercially available for the treatment of aqueous solutions and gaseous mixtures that are contaminated with oxidizable pollutants or microorganisms. Ozone decomposes into oxygen, an inert by-product. Combining ozone with other treatments can convert non-biodegradable organic materials into biodegradable materials (Chester 1998).

Along with the advantages of ozone use come disadvantages. First of all, there is a higher capital cost associated with ozone systems due to the fact that it must be generated on-site. Corona discharge, the most commonly used method for ozone generation, is electrically inefficient. Nearly 75% of the electrical power sent to one of these units is converted into unusable by-products such as light and heat. Additionally, since ozone being the most powerful oxidizing agent available, it has the potential to be the most dangerous to humans (Chester 1998).

The process of applying ozone is referred to as "ozonation." The Food and Drug Administration recognizes ozonation as Generally Recognized as Safe (GRAS). The FDA also recognizes ozone as an antimicrobial agent. Previously, ozone only had FDA approval for the treatment of bottled drinking water, and for its use in meat-aging coolers in amounts up to 0.1 ppm. In June 2001, the FDA approved the use of ozone for the treatment, storage, and processing of foods (21CFR173.368). In countries such as France, Germany, United Kingdom, Scandinavia, Japan, and the Netherlands, ozone has been used for a number of years in applications such as storage of meat, cheese, fruit, and other food products (Graham 1997).

Ozone and Produce

The recommended method of removing microorganisms from produce is by the use of chlorinated water. However, chlorine has disadvantages. Chlorine can leave behind a chemical salt residue. Additionally, gaseous chlorine is quite expensive and is banned in some areas.

In recent years, ozone has been used in the produce industry. There are several applications in which ozone can be used, including the sterilization of process water, fruit and vegetable washing, and modified atmosphere storage of produce.

Ozonated water has been shown to reduce microbial counts on the surface of produce, and is particularly effective against *Escherichia coli*, which a foodborne pathogen of great concern to the produce industry (Liangji 1999). Research conducted by Garcia and others (2001) showed that a combination of ozone and chlorine significantly extended the shelf-life of lettuce as compared to chlorine treatments alone. Although ozonated water has shown promise as a treatment for some types of produce, the quality of other types of produce could be harmed by the use of water. Whole fruits such as strawberries and sliced produce such as tomatoes can suffer from tissue damage if they come in contact with water.

Ozone has also shown promise as a retardant of mold and bacteria in cold storage of produce (Liangji 1999). Gaseous ozone has been used to decrease microbial activity and extend the shelf-life of various types of produce. Strawberries, grapes, blackberries, carrots, broccoli, and cucumbers have all been treated with varying levels of efficacy (Barth and others 1995, Liew and Prange 1994, Perez and others 1999, Sarig and others 1996, Skog and Chu 2001).

In a study by Sarig and others (1996), the effect of gaseous ozone on post-harvest decay of table grapes was studied. The recommended method for control of post-harvest grape decay is the use of SO₂ fumigation. Sulfur compounds are a common allergen and are banned in certain products and restricted in others. In exploring alternative methods

of fumigation, ozone was studied due to its potent antimicrobial properties. Since ozone may induce the resistance of plants to pathogenic microorganisms by way of causing them to elicit phytoalexins, which are naturally present in grapes, this treatment was of particular interest to this group of researchers.

Three cultivars of table grapes (*Vitis vinifera*) were inoculated with *Rhizopus stolonifer* and treated with gaseous ozone at a rate of 8 mg min⁻¹ at an air flow of 500 ml min⁻¹. Ozone significantly reduced the population of microorganisms on all three cultivars. Ozone also stimulated the production of resveratrol, a phytoalexin, in the grapes. Firmness of the grapes was not adversely affected by ozone treatment.

As compared to SO₂ fumigation, gaseous ozone controlled decay just as effectively. As an added benefit, the quality and freshness of the ozone-treated grapes were superior to the ones treated with SO₂. Thus, it was decided that a short-term postharvest exposure of grapes to ozone might be a satisfactory alternative to SO₂ fumigation.

Blackberries are susceptible to fungal decay. Preharvest fungicides are typically used to control this. However, these compounds are under review in many countries due to the possible health risks associated with their use. Researchers are looking for alternatives to fungicides that could control decay and improve the shelf-life of blackberries. Barth and others (1995) studied the effect of gaseous ozone storage on fungal growth in blackberries. These researchers also wanted to assess quality attributes after ozone exposure. Therefore, anthocyanin content and color retention were also examined.

Results of the fungal analysis showed that while 20% of control fruits had visible signs of fungal growth and decay, no observable fungal decay was present on ozone-treated fruits. No significant differences in total anthocyanin content were observed among treatments over a 12 day storage period. Additionally, no difference was observed in the retention of color among treatments during the 12 day storage period. The researchers concluded that ozone is a likely alternative to fungicides for the control of postharvest rot in small fruits.

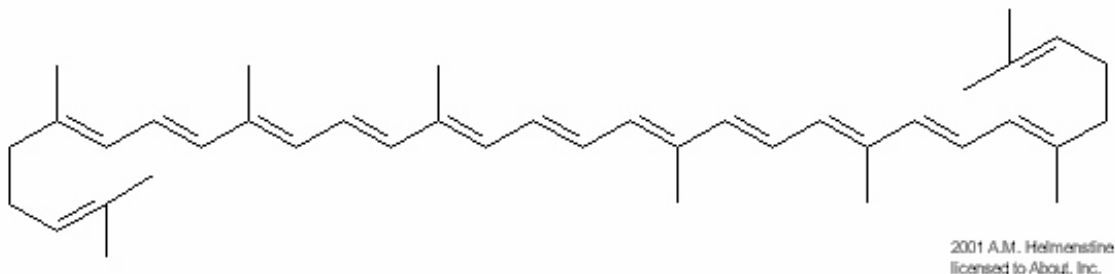
Skog and Chu (2001) studied the effect of gaseous ozone on the qualities of several fruits and vegetables in cold storage. The produce that they investigated included broccoli, cucumbers, mushrooms, apples, and pears. The three vegetables were found to have adverse quality effects due to ozone treatment, including color changes such as browning. However, the broccoli and cucumbers showed minimal effects and had better overall quality than untreated samples. Apples and pears tolerated the ozone treatments with no observable adverse effects.

Carrots are prone to postharvest diseases, including watery soft rot by *Botrytis cinera* and gray mold by *Sclerotinia sclerotiorum*. Liew and Prange (1994) conducted research on the effect of ozone on postharvest diseases and physiology of carrots. Carrots were inoculated with *B. cinera* and *S. sclerotiorum* and treated with gaseous ozone at concentrations of 0, 7.5, 15, 30, and 60 l liter⁻¹. They found that although gaseous ozone reduced daily growth rates of both fungi at the highest concentration, it also had detrimental effects on the carrots. Respiration rate, electrolyte leakage, and total color differences increased with ozone concentration. Ozone did not affect carrot weight loss.

The researchers suggested that the efficacy of ozone as a disinfectant be individually assessed for each commodity at its ideal storage temperature. Overall, it was concluded that an ozone supply of 15 l liter⁻¹ for 8 hours a day at 2°C for carrots during storage could provide some protection against postharvest diseases with a minimum amount of physical and physiological damage.

Lycopene

The structure of lycopene (about.com 2002) is show below.



Lycopene

Molecular Weight: 536.89

Molecular Formula: C₄₀H₅₆

Lycopene is a chemical compound that is classified as a carotenoid. Carotenoids are pigments that are synthesized by plants and microorganisms. The color of carotenoids is the result of a system of conjugated double bonds. These double bonds can occur in either cis or trans form. Carotenoids present in food are more commonly in the trans form, with only a small percentage in the cis form. Lycopene is the most abundant carotenoid present in ripe tomatoes, composing about 80-90 % of color pigments (Shi and Maguer 2000). The amount of lycopene in fresh tomatoes varies, depending on variety,

maturity, and environmental conditions under which the tomatoes matured (Shi and Maguer 2000). On average, tomatoes contain between 3 to 5 mg lycopene per 100 g raw material (Shi and Maguer 2000). Some varieties of deep-red color contain as much as 15 mg per 100 g, while yellow varieties only contain about 0.5 mg per 100 g (Shi and Maguer 2000).

It has been reported that a greater concentration of carotenes exists in the stem end than in the blossom end of tomatoes. It has also been reported that carotene concentrations are higher in tomatoes in the summer (June to August) and lower in winter (October to March). Tomatoes grown in greenhouses as well as ones that are picked green and ripened in storage have been reported to have less carotenes than vine-ripened ones (Shi and Maguer 2000). Additionally, research by D'Sousa and others (1992) indicates that the skin and pericarp layers of tomatoes are rich in lycopene. This may indicate that most of the lycopene may be found attached to the insoluble fiber portion of the fruit.

An the cellular level, lycopene is found in the chloroplasts of tomatoes. In the early stages of tomato development, green chlorophyll is the dominant pigment present in the chloroplasts. As the fruit ripens, chlorophyll degrades and tissue color changes from green to white. Degradation of chlorophyll induces the synthesis of lycopene which causes tissue color to change from white to red (Shi and Maguer 2000).

Lycopene has gained quite a bit of attention due to its proposed health benefits. Giovannucci and others (1995) found that the intake of tomato products, which include the compound lycopene, was inversely associated with the development of

prostate cancer. Franceschi and others (1994) found a reduced risk of digestive tract cancer with high intake of tomato products. Kohlmeier and others (1997) found that higher amounts of lycopene in adipose tissue was associated with a lower risk of cardiovascular disease, while Kristenson and others (1997) found that low serum lycopene levels were associated with increased mortality from coronary heart disease.

The determination of lycopene in tomato products can be done either by physical or chemical means. Physical determination involves the use of color evaluations. Colorimeters are used to obtain chromaticity values which estimate color intensities. Since this type of method is based on external measurements, it is nondestructive and is reported as being closely related to visual perceptions of tomatoes. This method could be used in the tomato processing industry for a quick, on-line measurement of color quality (Shi and Maguer 2000).

Chemical methods of lycopene determination require the extraction of the pigment from tomato samples. Extraction of lycopene from tomato products can be a difficult task. Since lycopene has been reported as being sensitive to oxygen, light, and acids, care must be taken to avoid exposing tomato samples to these while analysis is performed. Also, one must carefully choose an extraction method because there is currently not a standardized method for lycopene analysis. Lycopene is only soluble in solvents such as chloroform, hexane, and petroleum ether, so one of these must be a constituent of the extracting solution (Shi and Maguer (2000). Most chemical methods of lycopene analysis use the solvents hexane and acetone for extracting lycopene. Bartlett method and reference Additionally, it can be difficult to homogenize tomato samples.

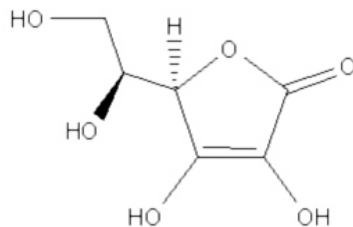
The two types of chemical methods of lycopene determination are spectrophotometric and chromatographic. Chromatographic methods allow for separation of lycopene, including its cis-trans isomers. There are several types of chromatographic methods, including thin-layer, paper, and high-performance-liquid chromatography (HPLC). Reversed phase HPLC methods can allow for the partial separation and detection of cis-trans isomers (Shi and Maguer 2000). Although HPLC methods allow for better separation of lycopene and its isomers, it may not always be necessary to obtain such a high degree of specificity for the detection of lycopene.

Spectrophotometric methods have been traditionally used for the determination of lycopene from tomato, and these methods are still considered acceptable for routine lycopene analysis (Shi and Maguer 2000). Either a sample of pure lycopene to construct a standard curve or a mathematical formula is needed for spectrophotometric determination of lycopene. When performing extractions for subsequent spectrophotometric measurements, it is important to obey Beer's law, which is the relationship between the absorbance of a solution and the concentration of the absorbing species (Penner 1998). Linear curves are expected for analyses that obey Beer's law (Penner 1998).

Ascorbic Acid

While tomatoes are a good source of the carotenoid lycopene, they also are an excellent dietary source of ascorbic acid, which is otherwise known as vitamin C. In fact, one small red-ripe tomato is reported to contain about 40% of the adult RDA of this

vitamin (Gould 1992). Ascorbic acid is an important vitamin in human health. Ascorbic acid has many important roles in the body. It can prevent the development of the disease scurvy, is a cofactor in the synthesis of collagen, can increase the absorption of nonheme iron, and acts as an antioxidant (Whitney and Rolfes 2002). Its antioxidant properties have been attributed to reducing the risk of several diseases, including arteriosclerosis, diseases of the cardiovascular system, and certain forms of cancer (Harris 1996). The structure of ascorbic acid is as follows (Roche 2002).



Since it is reported that more than 90% of the ascorbic acid in human diets comes from fruits and vegetables, it is important to prevent losses of this compound from produce. Various preharvest and postharvest factors can influence the ascorbic acid content of produce. Preharvest factors include both climactic conditions and cultural practices (Lee and Kader 2000).

Climactic conditions include such things as light and average temperature. For instance, fruit on the outside of a plant may develop more ascorbic acid than fruit that is on the inside of a plant and therefore shaded from light. Also, fruit grown in cooler temperatures may have higher amounts of ascorbic acid than fruit grown in hotter climates (Lee and Kader 2000).

Cultural practices include the use of nitrogen-containing fertilizers and irrigation practices. In some types of produce, higher levels of nitrogen in fertilizers can reduce their ascorbic acid content. Also, less frequent irrigation may increase levels of ascorbic acid. The production of ascorbic acid by plants may be a strategy of protection against drought injury. Therefore, produce grown under conditions of low nitrogen and less frequent irrigation may have higher levels of ascorbic acid (Lee and Kader 2000).

There are several postharvest factors that may affect the ascorbic acid contents of produce. Physical damage to fresh-cut produce can affect ascorbic acid content (Lamikanra 2002). Temperature management during storage is important in extending the shelf-life of produce as well as maintaining the quality of the products. Citrus fruits, for instance, have been found to lose ascorbic acid if stored at high temperatures. However, acidic fruits seem to lose less than vegetables because ascorbic acid is more stable under acidic conditions (Lee and Kader 2000). Also, temperatures that are too cold can affect ascorbic acid content. Chilling injury has been found to induce losses of ascorbic acid in crops that are chilling sensitive (Lee and Kader 2000).

Various methods of analysis have been reported for the determination of ascorbic acid, including spectrophotometry, fluorometry, titration, electrophoresis, and high-performance liquid chromatography (HPLC). The Association of Official Analytical Chemists Official Method of Analysis for ascorbic acid is the 2,6-dichloroindophenol titrimetric method. The principle of this method is that ascorbic acid reduces 2,6-dichloroindophenol, an oxidation-reduction dye, to a colorless solution. At the titration end point, excess reduced dye appears rose pink in acidic solutions. A buffered

solution of HPO_3 -HOAc is used to maintain the proper acidity for the reaction and also to avoid the oxidation of ascorbic acid at higher pH (AOAC 1990).

II. MATERIALS AND METHODS

Tomatoes

One twenty-pound case of Stage 5 (light red) ripeness tomatoes was obtained from Neel's Produce (Knoxville, TN). All tomatoes used for sliced tomatoes in the lycopene, ascorbic acid and color experiments were from the same case and were kept under refrigeration until needed. Two tomatoes of uniform size and color were chosen for all 5 treatments. Three replications were performed.

Preparation of Tomato Slices

Tomatoes were cut into cross-sectional slices of 7 mm thickness with a Rival® Electric Food Slicer (The Rival Company, Kansas City, MO) under reduced light. Top and bottom slices were discarded, and five middle slices were used for each replication. Each treatment used one slice each from each tomato. In order to control variability within tomatoes, slices were numbered by their horizontal positioning from the stem end. Slices were grouped so that two of the treatments used slice 1 from one tomato and slice 5 from another tomato, two treatments used slice 2 from one tomato and slice 4 from another tomato, and one treatment used slice 3 from both tomatoes. Slices were placed in a vertical position on holders so as to allow gaseous ozone to circulate throughout the treatment vessel. All slices of tomatoes were kept under refrigeration at 4°C until they received ozonation treatment within 8 hours of slicing.

Preparation of Commercial Tomato Juice

Twelve 12 fl oz. cans of Campbell's tomato juice, containing ascorbic acid at levels of 15 mg/8 fl oz, were purchased from a local grocery store. To ensure homogeneity, cans were mixed together in a 4 L plastic vessel on a magnetic stir plate and stir bar. The juice was placed in a foil-covered 2.8 L Erlenmeyer flask and kept in the refrigerator at 4°C until needed.

Preparation of Inoculum

Tomatoes were placed in a plastic zip-top bag and placed under refrigerated conditions for several days. One mL aliquots of supernate were taken from the bottom of the bag and added to 9 mL tryptic soy broth (TSB, Difco Laboratories). Tubes were incubated for 48 h at 35°C. A new 24-h culture was prepared for each treatment block.

Treatment Vessels

Quart-size wide mouth sterilized Mason jars were used as treatment vessels. Jars were covered in heavy-duty aluminum foil so as to block out light that could oxidize lycopene or ascorbic acid. Two holes were drilled in a Mason jar lid. One hole accommodated the tubing used to pump in gaseous ozone, and a smaller hole allowed excess ozone to escape from the jar. The tubing allowed the ozone gas to enter the jar approximately 2 cm from the bottom.

Ozone

Gaseous ozone was created using an Activated Ozone Generator (Golden Buffalo, CA), and a Tetratec Deep Water aquarium air pump. This system was designed to produce 0.9 g ozone/h at a flow rate of 2.4 L/min. In order to double the concentration of ozone generated by the system, the two exit ports of the ozone generator were connected together using Tygon tubing and a t-shaped adaptor. This increased the concentration of ozone produced by the system to approximately 1.8 g ozone/h.

Application of Gaseous Ozone

Jars containing tomato slices and 1 mL aliquots of 24-h culture were treated with ozonated air at treatment levels of 0, 90, 105, 120, and 135 min. Treatment times were randomized with Microsoft Excel 2000 (Microsoft Corporation) random number generation. All studies were performed under an exhaust hood and at ambient temperature ($22 \pm 2^\circ\text{C}$).

Sample Preparation

After treatment, tomato slices were passed through an Oster® Heavy Duty Food Grinder (Sunbeam-Oster Company, Inc., Schaumburg, IL) three times. The larger of the two blades supplied with the food grinder was used in the grinding process.

Lycopene Extraction

Lycopene analysis was performed with a modified version of a method developed at the University of California at Davis (Barrett 2002). Approximately 1.5 g of ground tomato was weighed in triplicate. Samples were placed in 50 mL polypropylene centrifuge tubes (Corning Incorporated, Corning, NY) with 40 mL of solvent containing hexane:methanol:acetone (Fisher Scientific, Atlanta, GA) at a ratio of 8:3:3. Samples were blended with a Vortex-Genie (Scientific Industries, Bohemia, NY) and placed in a light-free cardboard box for 1 h.

Lycopene Analysis

After extraction, the quantity in mL of the upper hexane layer was measured. Duplicate 3.5 mL samples of the hexane layer were placed in disposable 4.5 mL UV cuvettes (Fisher Scientific, Atlanta, GA). Samples were placed in a Unicam UV1 Spectrophotometer (Unicam UV-Visible Spectrometry, Cambridge, UK), where they rested for 5 min in order to allow air bubbles to dissipate. Absorbance of the samples was read at 503 nm against a hexane blank. Lycopene content was calculated for each sample using a formula developed by Barrett (2002). Formula used for calculation of lycopene levels is as follows:

$$(\text{g lycopene} / \text{g fresh wt} = (\text{A}_{503} \times 537 \times 22) / (\text{wt} \times 172)).$$

A₅₀₃ = absorbance at 503 nm,

537 = molecular weight of lycopene (g/mole),

22 = hexane layer (ml),

wt = sample weight,

172 = extinction coefficient of lycopene in hexane.

Ascorbic Acid Analysis

The Association of Official Analytical Chemists' Official Method of Analysis 967.21 for ascorbic acid determination in vitamin preparations and juices (2,6-dichloroindophenol titrimetric method) was used (AOAC 1990). Samples of 25 g of ground tomato or tomato juice were combined with 25 mL of metaphosphoric acid-acetic acid buffer solution. Samples were vacuum-filtered using a Buchner funnel and Whatman® grade 4 filter paper (Whatman, Maidstone, England). The volume of filtrate collected was recorded.

In the first replication of the experiment, the total volume of filtrate was used for titration. In order to reduce the amount of indophenol required for titration, further analyses used 15 mL of filtrate and 15 mL of buffer solution. A standard curve was prepared at 1mg/mL using Fisher L-Ascorbic Acid, 99 + % (A.C.S., Fisher Scientific, Atlanta, GA). The amount of ascorbic acid was calculated using the regression line from the standard curve. Ascorbic acid was recorded in mg/100 g.

Color Analysis

Ground tomato samples were placed in small plastic cups. Color measurements were made using a HunterLab MiniScan™ XE Plus colorimeter (Hunter Associates Laboratory, Inc., Reston, VA). The instrument was standardized against white, black, and pink tiles. L, a, and b values were recorded for each sample.

Microbiological Analysis

After ozonation treatment, 100 mL of 0.1% peptone was added to jars containing tomato culture. 10^{-4} to 10^{-7} dilutions of the culture were surface-plated onto standard methods agar (SMA, Difco Laboratories). Plates were incubated for 24 h at 35°C and observed for colony growth.

Statistical Analysis

Lycopene analysis and ascorbic acid analysis of tomato juice experiments were performed in triplicate. Experimental design of lycopene analysis in tomato slices was a randomized block design with replication and sampling. Ascorbic acid analysis and color analysis of tomato slices used a completely randomized design with replication. Ascorbic acid analysis of tomato juice used a completely randomized block design with replication and sampling. Microbiological analysis of tomato culture used a completely randomized design with replication. All experiments were statistically analyzed using the mixed procedure (PROC MIXED) of SAS version 8.1 (SAS Institute, Cary, NC). Means were separated using Tukey's mean separation test. Significant differences were defined at $P < 0.05$.

IV. RESULTS AND DISCUSSION

Ozone generated in air at 0.9 g/h, then doubled in concentration to approximately 1.8 g/h, was allowed to flow over 1.0 mL of peptone containing 10⁹ inoculated tomato spoilage microorganisms at 2.4 L/min until greater than 1 log reduction of the microorganisms occurred. It required more than 100 min for this to occur.

Tomato Spoilage Microorganisms

In preliminary experiments, various treatment times of gaseous ozone were tested to determine approximate times needed for significant reduction of tomato spoilage microorganisms. [Figure A-1](#) shows aerobic plate counts of tomato spoilage microorganisms treated with gaseous ozone for 0 to 120 minutes. Preliminary data showed treatments of gaseous ozone for less than 120 minutes to be ineffective at reducing aerobic plate counts of tomato spoilage microorganisms. There was a significant reduction in microbial counts (3.0 log) from 90 to 120 minutes of treatment. Therefore, treatment times of 0, 90, 105, 120, and 135 minutes were selected to determine at what point ozone had a significant effect on spoilage microorganisms in the treatment jar with sliced tomatoes. [Figure A-2](#) shows aerobic plate counts of tomato spoilage microorganisms treated with gaseous ozone for 0 to 135 minutes. Samples treated for 105, 120, and 135 minutes were statistically similar to each other, but all were significantly lower (more than a 2 log decrease) than untreated samples ($p < 0.0001$). Aerobic plate counts of samples treated for 90 minutes were not statistically different

from either untreated samples or samples treated for 105, 120, or 135 minutes. The ozone treatments of 105 min and greater were therefore adequate to cause a significant decrease in microorganisms that would typically be found on fresh tomatoes. This decrease is similar to what was found on fresh-cut salads treated in a commercial processing operation (Garcia, 2001).

Lycopene Extraction

An extraction method was developed for these experiments because currently there is not a standardized method for lycopene analysis. Several challenges were encountered in developing this methodology. Since lycopene is reported as being sensitive to oxygen, light, and acids, great care must be taken to avoid exposing tomato samples to these stressors while analyzing for this compound (Shi and Maguer 2000).

It was found to be rather difficult to achieve adequate homogenization of tomato samples without causing excessive foaming, which could oxidize lycopene. Several instruments were used, including a blender, homogenizer, stomacher, and food grinder. The food grinder was found to give the best results without causing excessive foaming. Tomato samples were pushed through the food grinder three times in order to push the tomato skin that got caught behind the blades through the machine.

When performing extractions for subsequent spectrophotometric measurements, it is important to obey Beer's law, which is the relationship between the absorbance of a solution and the concentration of the absorbing species (Penner 1998). Linear curves are expected for analyses that obey Beer's law. This was achieved by determining a good

ratio of tomato:solvent. An adequate quantity of ground fresh tomatoes was determined to be less than 2.0 g of sample in 40 mL of the hexane:methanol:acetone solvent. Sample sizes of tomato ranging from 1.0 to 4.0 g were compared as to see at what point the extracting solvent reached a plateau. Samples of less than 1 g were not tested because of two reasons. First, they are impractical to weigh. Secondly, samples this small would not provide an accurate representation of the ground tomatoes due to the presence of large particles and seeds. Table 1 shows a large jump in absorbance from 1.5 to 2 g sample and relatively steady readings from 2 to 4 g sample. This indicates that at a sample size of 2 g, the extracting solvent was beginning to become saturated with components in the tomato sample. Therefore, it was decided that 1.5 g was of adequate size to ensure sample homogeneity, indicated by a lower standard deviation among samples, without overloading the extracting solvent. It was also determined that this quantity of sample was adequate to get similar results from subsamples of material analyzed from the same ground tomato sample.

Table 1. Effect of tomato sample size on absorbance readings of extracted lycopene.

Sample size (g)	Absorbance (503 nm)
1.0	0.601 ± 0.238 ^a
1.5	0.620 ± 0.096 ^a
2.0	0.882 ± 0.126 ^b
3.0	0.927 ± 0.030 ^c
4.0	0.879 ± 0.042 ^c

^an=6; ^bn=5; ^cn=3.

One issue that occurred during extraction of lycopene was that some samples did not seem to have lycopene fully extracted from them. Occasionally, a piece of tomato in the interior portion of the sample would be protected by the rest of the sample and would still be red in color after the extraction time had elapsed. A suggestion for improvement of the extraction method would be to periodically vortex samples during the extraction period. This may help to ensure that all tomato pieces in the sample have adequate contact with extracting solvent, thus improving the accuracy of the results.

Lycopene Content of Sliced Tomatoes

[Figure A-3](#) shows the effect of ozone treatment on lycopene content. No significant differences ($p > 0.05$) in lycopene content were found between untreated tomato slices or slices treated for 90, 105, 120, or 135 minutes. This is in agreement with Giovanelli and others (2001), who found that the carotenoid content and antioxidant activity of the lipophilic fraction of tomatoes were not significantly reduced by processing techniques. These techniques included drying tomato halves, as well as the production of tomato pulp, paste, and puree.

This is also in agreement with Barth and others (1995), who found no significant differences in total anthocyanin content of blackberries among ozonation and control treatments over a 12 day storage period. Lycopene is considered to be more of an antioxidant than the anthocyanins.

Ascorbic Acid Content of Sliced Tomatoes

[Figure A-4](#) shows the effect of ozone on the ascorbic acid content of sliced tomatoes. Due to material restraints, only the two extreme treatments, 0 and 135 minutes, could be analyzed for ascorbic acid content. The ascorbic acid content of sliced tomatoes treated with gaseous ozone for 135 minutes was significantly lower ($p < 0.0001$) than untreated sliced tomatoes. This is in agreement with Giovanelli and others (2001), who found that the ascorbic acid content and antioxidant activity of the hydrophilic fraction of tomatoes were significantly reduced by processing techniques.

Since the tomatoes used in these experiments were cut into cross-sectional slices, a greater surface area of the water-containing portion of the fruit was exposed to ozone as compared to the lipid-containing portion. This may account for the significant reductions found in ascorbic acid content.

Ascorbic Acid Content of Commercial Tomato Juice

Since experiments examining the effect of gaseous ozone on ascorbic acid content of sliced tomatoes could not be performed at all treatment levels, commercial tomato juice was treated to estimate the effects of gaseous ozone on a tomato matrix. [Figure A-5](#) shows the effect of ozone on ascorbic acid content of commercial tomato juice. Tomato juice treated with gaseous ozone for times of 90, 105, 120, and 135 minutes were statistically similar to each other, but all were significantly lower than untreated samples ($p < 0.0001$).

In comparing the two analyses of ascorbic acid in tomato products, there were

different methodologies used for treatment of commercial tomato juice and treatment of sliced tomatoes. Commercial tomato juice had gaseous ozone bubbled through it, while sliced tomatoes were placed in a container and surrounded by gaseous ozone. Bubbling the gas through the tomato juice gave the ozone greater surface contact, thus subjecting the tomato juice to greater oxidative stress than the sliced tomatoes.

Differences Between Lycopene and Ascorbic Acid

Lycopene and ascorbic acid are both antioxidant compounds. Ascorbic acid is a polar, water-soluble antioxidant (Whitney 2002). It is present in the water-containing portion of fruits and vegetables. Lycopene is a nonpolar, lipid-soluble antioxidant (Shi and Maguer 2000). Research by D'Sousa and others (1992) indicates that the skin and pericarp layers of tomatoes, which are the lipid-containing portions, are rich in lycopene. As previously mentioned, the tomatoes used in these experiments were cut into cross-sectional slices, thus giving greater ozone exposure to the water-containing portion of the fruit as compared to the lipid-containing portion. This may account for the significant reductions found in ascorbic acid content while lycopene content was not found to be significantly reduced.

Another factor that could have caused differences in lycopene and ascorbic acid contents of ozonated sliced tomatoes is the chemical structure of the antioxidants. The structure of ascorbic acid is fairly uncomplicated.

Lycopene is a polyene hydrocarbon, an acyclic open-chain unsaturated carotenoid with 13 double bonds, 11 of which are conjugated (Shi and Maguer 2000). This extended

system of conjugated double bonds gives lycopene its unique color and antioxidant activities (Shi and Maguer 2000). Perhaps the conjugated double bond system of lycopene somehow protected it from oxidation by gaseous ozone.

Physical damage to fresh-cut produce caused by slicing can also cause water loss, which could in turn cause a loss of ascorbic acid content (Lamikanra 2002). Additionally, physical damage may cause secondary metabolite accumulation to occur, which may result in an increase in enzymatic activity and oxidation reactions (Lamikanra 2002). These secondary metabolites might only target water-soluble antioxidants, which would cause the destruction of ascorbic acid and not lycopene. Enzymes such as ascorbate oxidase, which oxidizes ascorbic acid to dehydroascorbic acid, is normally found in plants bound to cell walls (Lee and Kader 2000). Physical damage done to the cell walls of the tomatoes by slicing may have released this enzyme which oxidized the ascorbic acid present in the fruit tissue.

Findings are in agreement with Giovanelli and others (2001), who found that the carotenoid content and antioxidant activity of the lipophilic fraction of tomatoes were not significantly reduced by processing techniques, while significant differences were found in ascorbic acid contents and antioxidant activity of the hydrophilic fraction. These researchers theorized that hydrophilic antioxidants are more sensitive to oxidative stress, which contributes to changes in hydrophilic antioxidant activity of the final processed tomato products.

Color Analysis of Tomato Slices

Table 2 shows Hunter color values of ozonated versus control tomato slices. No significant differences ($p>0.05$) were found in Hunter L, a, or b values between untreated tomato slices or slices treated for 135 minutes.

This is in agreement with Barth and others (1995). These researchers observed no significant differences in the retention of color in blackberries among ozonation and control treatments during a 12 day storage period. However, this data disagreed with Liew and Prange (1994), who found that total color differences in carrots increased with ozone concentration. These differences might be expected, however, because the carotenoids in carrots are primarily in the form of β -carotene, which is much more susceptible to oxidation than lycopene. The pigments in blackberries are primarily in the form of anthocyanins, which, like lycopene, are not as susceptible to oxidation changes.

Lycopene is the most abundant carotenoid present in ripe tomatoes, composing about 80-90 % of color pigments (Shi and Maguer 2000). Theoretically, a reduction in this pigment should cause differences in color readings. Analysis of sliced tomatoes showed no significant reduction of color or lycopene by gaseous ozone. Therefore, results of the color analysis support results of the lycopene analysis in these experiments.

Table 2. Effect of gaseous ozone treatment on Hunter color values of sliced tomatoes^a.

Treatment time	L	a	b
0	36.46	19.62	14.18
135	34.96	19.83	14.09

^an=3.

This argument is supported by findings by Arias and others (2000). In this study, lycopene content, measured by HPLC, was correlated with L^* , a^* , b^* color readings.

Applications of This Research

Applications of this research include using gaseous ozone in modified atmosphere packaging of fresh-cut tomatoes. Results indicate that gaseous ozone treatment could reduce the natural spoilage microorganisms of tomatoes, and thus may extend the marketability of sliced tomatoes.

Future Research

While this research answered several questions as to the application of gaseous ozone to sliced tomatoes, many questions still remain. Future research should be conducted to study the effect of gaseous ozone on enzyme activity of produce tissue. Polymer films and other packaging materials need to be tested for resistance to ozone treatment before ozone could be used in modified atmosphere packaging for sliced tomatoes. The effect of ozone on ethylene production in produce tissues should be investigated. Shelf-life studies should be performed to see if gaseous ozone actually extends the useful life of sliced tomatoes. Sensory analysis of ozonated tomatoes should be performed, as ozone may affect flavor components or texture attributes. Additionally, analysis of lycopene in tomato juice should be performed to determine whether the stability of lycopene is related to its chemical structure or to its physical location in the tomato fruit.

V. CONCLUSIONS

Results indicate that gaseous ozone treatment for a minimum of 105 minutes can be an effective means of decreasing spoilage microflora of sliced tomatoes. Ozonation treatments that are effective in decreasing spoilage microflora of sliced tomatoes may not adversely affect lycopene and color measurements. However, ascorbic acid in tomato slices and tomato juice may be significantly reduced.

Fresh-cut produce containing high amounts of ascorbic acid should not be preserved with ozone gas if it is to be consumed primarily for its ascorbic acid content. However, sliced tomatoes are not eaten primarily for their ascorbic acid content. Their main purpose is for visual appeal of food items. Therefore, the significant reduction in ascorbic acid content by ozone treatment of sliced tomatoes would not be detrimental from a nutritional standpoint.

A significant reduction in spoilage microflora indicates that marketability of the sliced tomatoes can be extended, while the retention of color in the ozone-treated tomato slices is advantageous from a visual standpoint. Furthermore, ozone-treated tomato slices could still be marketed as a good source of lycopene, an antioxidant that has received a large amount of positive press in recent years.

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APPENDIX

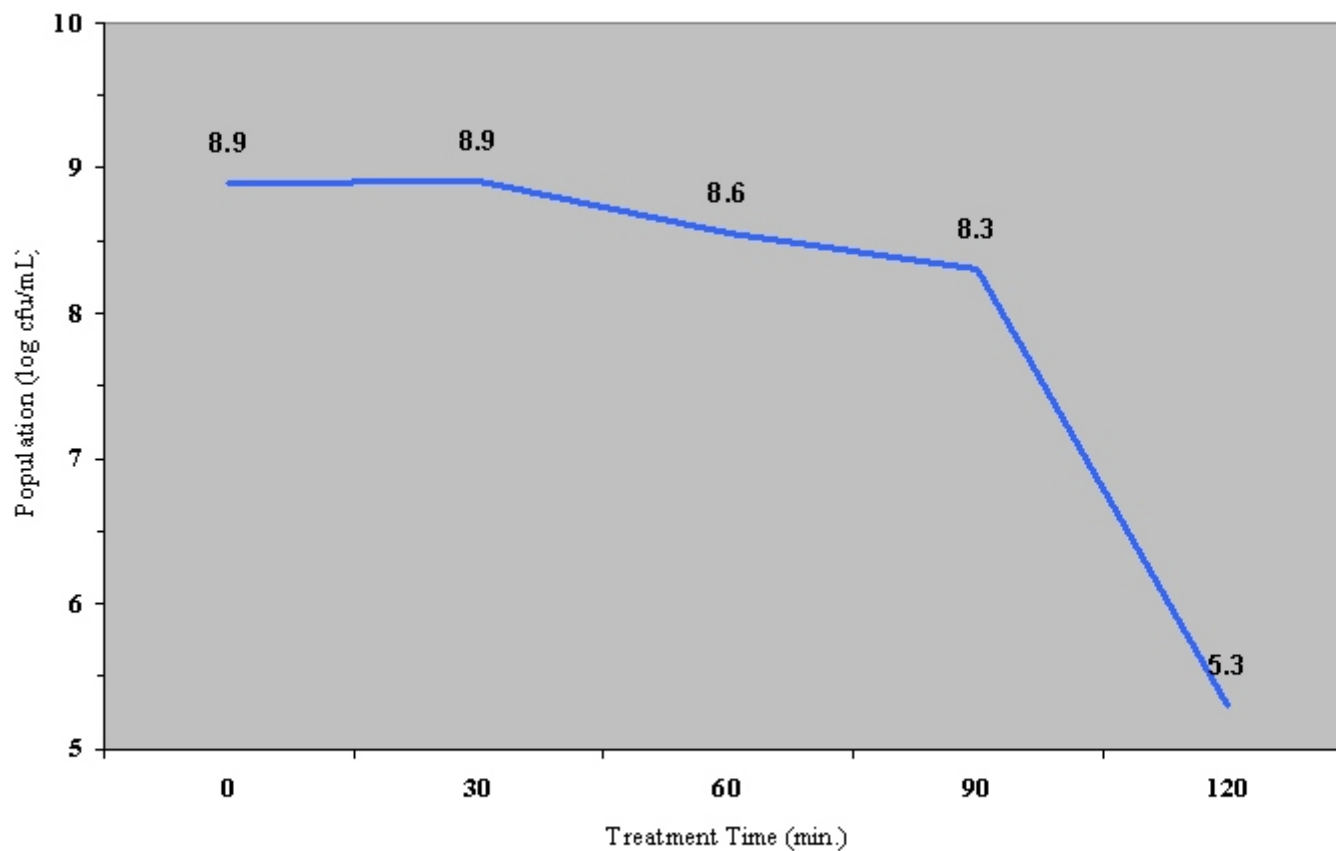


Figure A-1. Preliminary data of the effect of ozone treatment (appx. 1.8 g/h) on spoilage microflora of tomatoes.

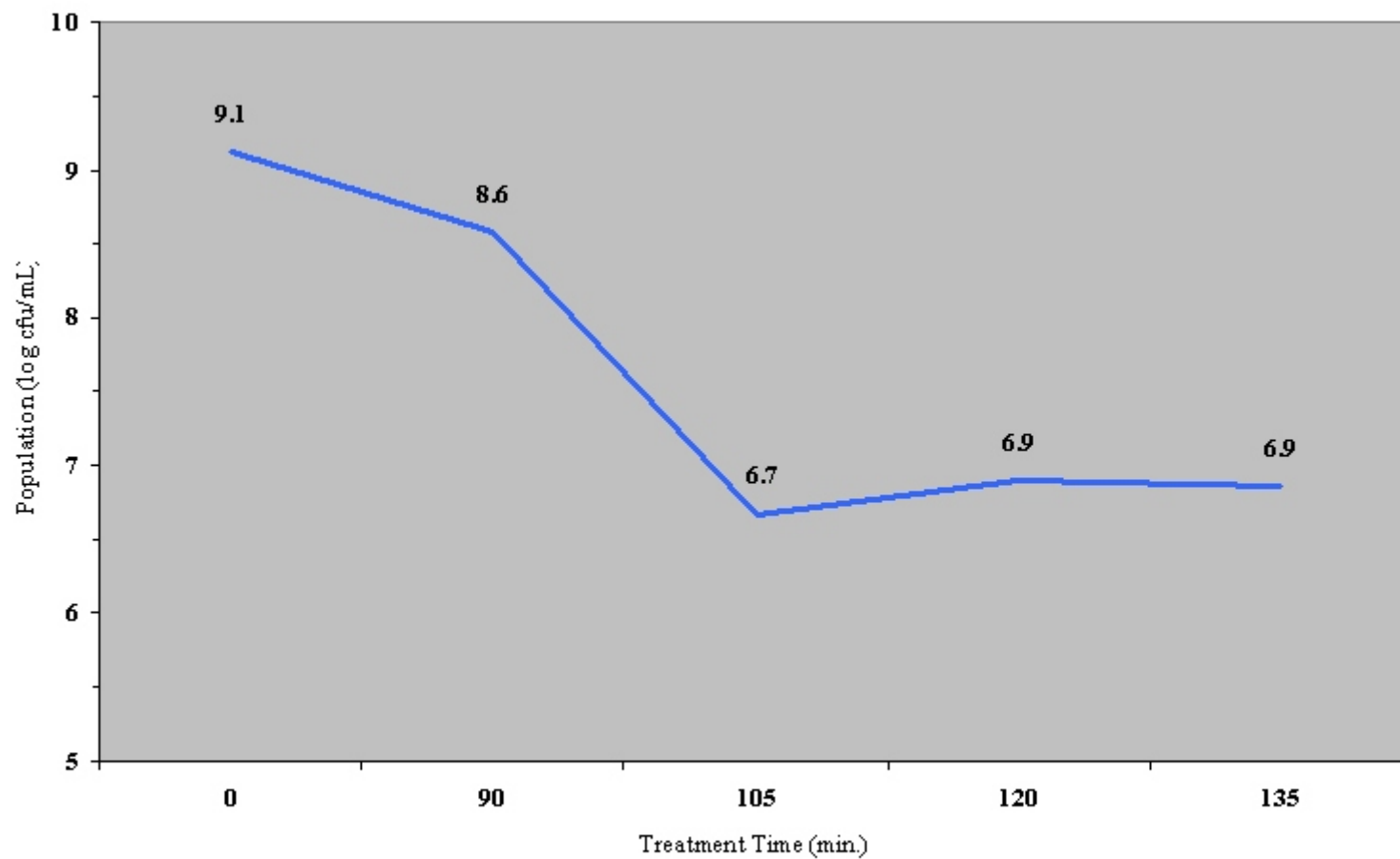


Figure A-2. Effect of ozone treatment (appx. 1.8g/h) on spoilage microflora of tomatoes.

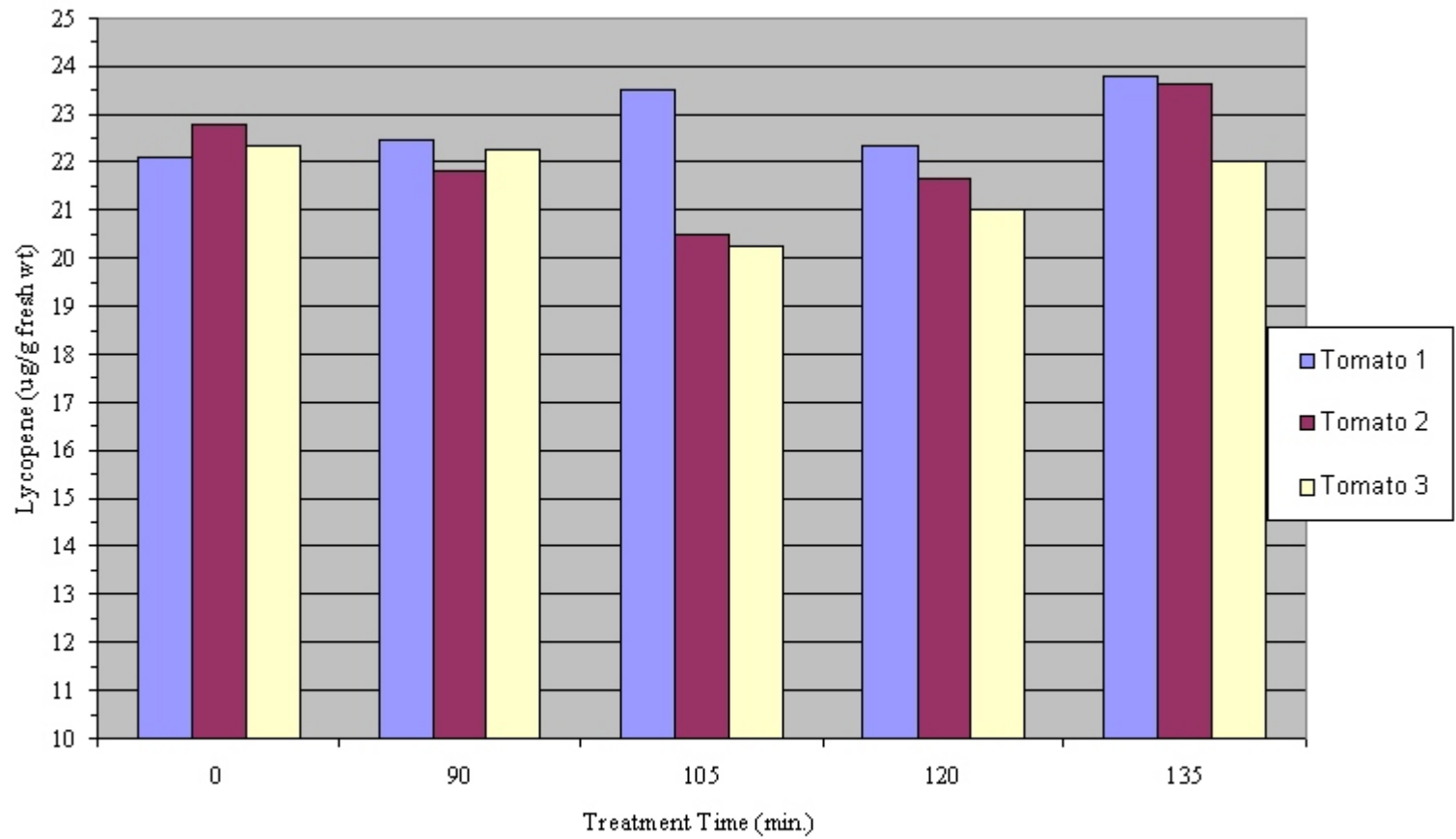


Figure A-3. Effect of gaseous ozone treatment on lycopene content of sliced tomatoes.

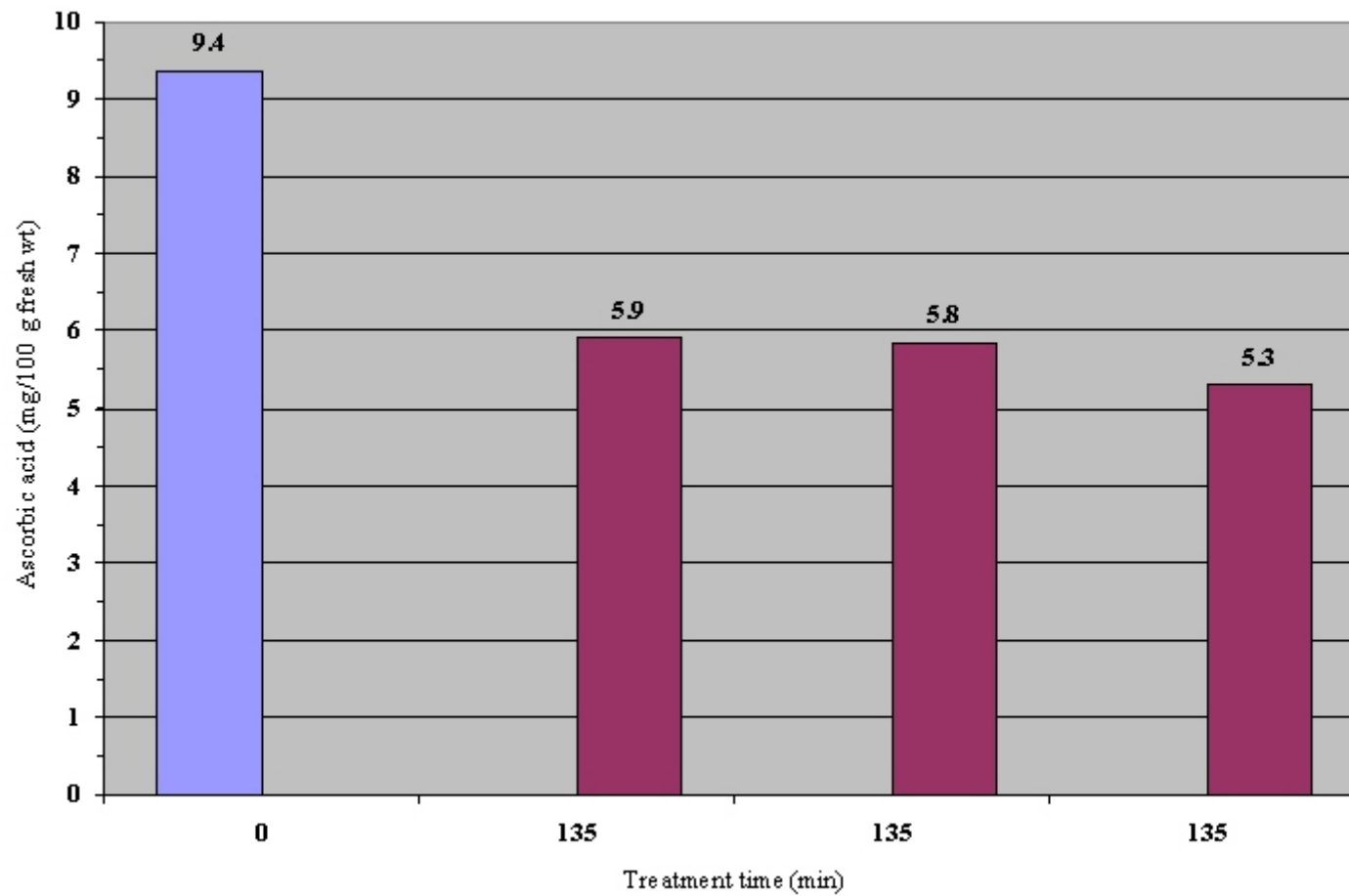


Figure A-4. Effect of gaseous ozone on ascorbic acid content of sliced tomatoes.

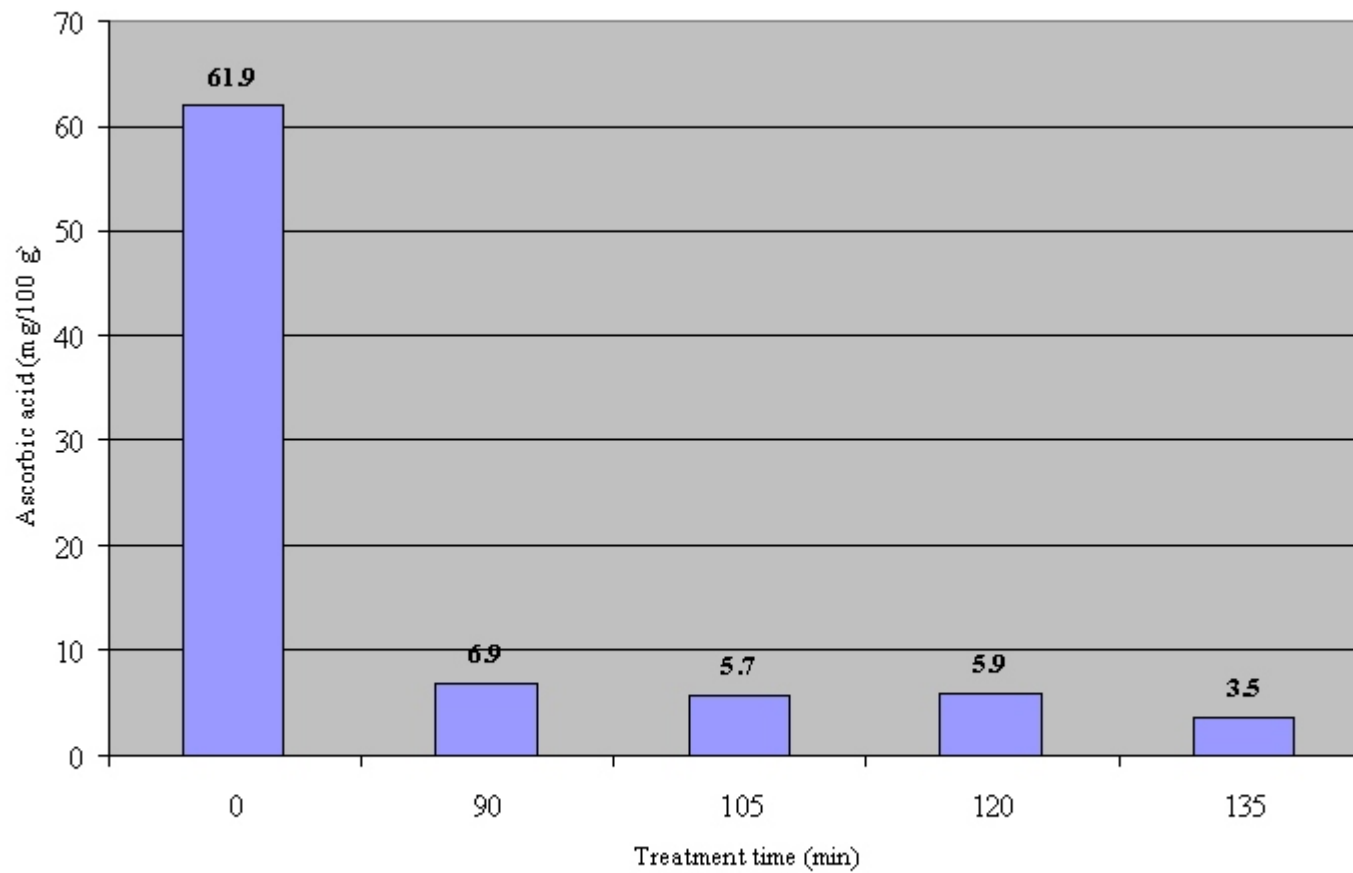


Figure A-5. Effect of ozone treatment (appx. 1.8 g/h) on ascorbic acid content of tomato juice.

VITA

Sarah Catherine Malone was born in Chattanooga, Tennessee in 1977 to Larry and Bette Malone. She graduated from Central High School in June of 1995. Sarah then attended Carson Newman College and the University of Tennessee at Knoxville, where she obtained a Bachelor of Science degree in Human Ecology with a concentration in Nutrition in May of 2000. The following fall, Sarah entered the University of Tennessee at Knoxville Department of Food Science and Technology's Master of Science program. Her main focus was in the area of fresh-cut fruit and vegetable processing, under the direction of Dr. John Mount. Sarah graduated with a degree of Master of Science in May of 2003. She is currently employed with Nestle USA, Prepared Foods Division, in Gaffney, South Carolina, as a Food Technologist. Sarah is Foodservice Brand Leader in the department of Product Development/Value Analysis.