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The Effects of Chlorine Dioxide Gas on The Sensory Properties of Whole Tomatoes

Christopher Gottilla
Clemson University, CGOTTIL@g.clemson.edu

THE EFFECTS OF CHLORINE DIOXIDE GAS ON THE SENSORY PROPERTIES OF WHOLE TOMATOES

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Christopher Gottilla
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Dr. Kay Cooksey, Committee Chair
Dr. Aaron Brody
Dr. Patrick Gerard

ABSTRACT

This experiment sought to measure the changes in sensory quality that occur in tomatoes as the result of chlorine dioxide gas treatment. Tasti-Lee tomatoes (VFFF Hybrid #5755) were treated with two different levels of chlorine dioxide gas (10mg and 50mg of ClO_2-forming material per kg of tomato) utilizing sachets. Testing compared the treated tomatoes and an untreated control group. These tests included flat-plate compression for firmness, sensory evaluation using a difference from control test, color analysis of the skin and flesh using a colorimeter, and enumeration of aerobic microbes and yeasts and molds. Flat-plate compression of whole tomatoes revealed no significant ($p < 0.05$) difference in firmness between treated and untreated tomatoes at the beginning (day 0) and end (day 17) of the experiment. Likewise, sensory analysis of cut tomatoes yielded no significant differences between treated and control tomatoes on day 0 or day 17. The colorimeter analysis showed a significant ($p < 0.05$) decrease in color index and increase in L* on day 0 as a result of the treatments. Color index also increased significantly in the two treatment groups from day 0 to 17, but it decreased for the control tomatoes. However, the sensory panel did not detect the initial or prolonged changes in the treated tomatoes. The 50mg/kg ClO_2 treatment resulted in a 1 Log CFU/mL reduction in aerobic microbes on day 0, compared to the control group. This reduction in aerobic microbes was not residual, as the treatment and control groups had similar aerobic counts on day 7 and day 14. Overall, no practically significant negative effects (bleaching, phytoxicity, etc.) were initially measured in the tomatoes as a result of the chlorine dioxide treatments. Significant differences in rates of senescence between the

treated tomatoes and the control tomatoes were not observed, giving no measurable indication that tomato shelf-life was extended as a result of the treatments.

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CHAPTER ONE

INTRODUCTION

The tomato is among the most widely consumed produce in the world (Sinesio et al., 2010). This is due not only to its availability in regions all over the world, but also to the fact that it is present both in processed products, such as ketchup, and fresh foods, such as salad (Valpuesta, 2002). In the United States, in the fruit and vegetable category, it is second only to potatoes in quantity of production (Sargent and Moretti). Additionally, tomatoes are known to be of important nutritional value. They are a rich source of antioxidants, Vitamin A, and Vitamin C (Sinesio et al., 2010; Heuvelink, 2005). In fact, one tomato has approximately 25% of the amounts of Vitamin A and Vitamin C needed by the average adult per day (Valpuesta, 2002). These factors, in conjunction with the recommendation to eat five servings of fruits and vegetables per day, make the tomato an important part of proper nutrition for people all over the world (Bari et al., 2002).

Biologically, the tomato is considered to be a fruit. It is also climacteric, which means that tomatoes form ethylene during the ripening process, and the ethylene produced acts as a feedback chemical to further advance the stage of ripening (Seymour et al., 2013). Tomatoes are roughly 93-95% water and 5-7% solids, with carbohydrates (mainly glucose and fructose) accounting for half of overall dry matter (Rosca and Rosca, 2013; Heuvelink, 2005). Tomatoes tend to have, compared to other fruits and vegetables, relatively high resistance to bacteria and fungi because of their acidic nature (Wade and Beuchat, 2003).

Tomato Harvesting

Harvested tomatoes can be used for sale in the fresh market (75%) or for processing (25%) (Arazuri et al., 2010). The stage at which tomatoes are harvested depends mainly on their intended use. Fresh market tomatoes will normally be harvested at the mature-green stage, allowing time for transport before they fully ripen. Further transport distances and times will normally call for slightly earlier harvesting so that consumers purchase their tomatoes when they are close to fully ripe. Tomatoes used for processing are harvested when fully ripe (at least 90% red), and they are immediately shipped to plants or other processing locations from the field.

Tomato harvesting primarily involves three steps before distribution: separation from the plant, sorting, and treatment. Sometimes visual inspection for defects will be carried out before any of these processes take place. Tomatoes can be separated from the plants to which they are attached by hand, but today this process is almost always accomplished by mechanical means. After the entire plant is removed from the ground, it is placed into a shaker (Heuvelink, 2005). The two primary types of shakers, according to Arazuri et al. (2010) are belt shakers and rotary shakers. On a belt shaker, several belts holding the plants vibrate, shaking tomatoes free from the larger plant. Then, the tomatoes fall to an additional series of belts for further sorting. The rotary shaker involves a similar process. A cylindrical rotor containing rings above with equidistant sticks vibrates the plant, allowing the tomatoes to fall onto other belts below.

Sorting can be done by hand in some operations to avoid damage, but it is usually carried out by a series of conveyer belts with different sized holes or by diverging rollers,

which allow tomatoes to fall into bins according to size. Spring-loaded pans can also be used to sort by weight. Visual inspection for defects may take place at this step. After sorting into bins, the tomatoes are then transported to their final destinations (Heuvelink, 2005).

How the tomatoes are physically handled and chemically treated, both directly after harvesting and after packaging, is vitally important to the final quality of the tomatoes. Tomatoes should be picked at a time of day when it is not overly hot and must be shielded from the sun after harvesting to prevent sun damage. After they are isolated from tomato plants, tomatoes are normally placed into chlorinated water for disinfection. The water should be several degrees warmer than the tomatoes to prevent cooling of internal gases and subsequent entry of water into the stem scars. Ethylene can be used for mature-green fruit to initiate ripening. It can be applied in aqueous form while still on the plant (e.g., with aqueous ethrel). However, this treatment is generally applied after packaging with a treatment level of 100-150ppm (Heuvelink, 2005).

During the ripening process, an appropriate relative humidity, air content and temperature should be maintained. To minimize evaporation during ripening and heat production from respiration, a relative humidity of 85-95% is suggested. Air contents should contain below 2% CO_2, otherwise the ripening action of ethylene may be inhibited. Temperature should be kept constant to prevent shriveling from water loss and surface condensation on the tomatoes, which can lead to undesirable microbial growth. The best storage temperature depends on how ripe the tomatoes are. Mature-green tomatoes are optimally stored at 20-25°C. Ripening fruit should be stored at 10-12°C,

and fully ripe fruit should be kept at 8-10°C. With increasing temperature (up to 30°C), softening will occur more quickly as a result of increased production of ethylene. Prolonged temperatures above 30°C can substantially lower carotenoid and lycopene pigment production, though temperatures between 35°C and 40°C can delay ripening for several days without adversely affecting sensory properties (Heuvelink, 2005).

Shelf-Life

The shelf-life of a tomato can vary greatly depending on numerous factors, such as strain on the fruit or plant, maturity at harvest, and storage conditions. As a very general average, the postharvest shelf-life of a whole tomato can be estimated at about three to four weeks (Zambre et al., 2010). Shelf-life is decreased by any type of minimal processing, such as dicing or slicing, because of cell damage during processing and the lack of a complete protective skin covering for the tomato flesh (Hakim et al., 2003). With certain forms of additional protection, such as modified atmosphere packaging and refrigeration, the high-quality shelf-life of sliced tomatoes can be estimated at about two weeks from the time of slicing (Sargent and Moretti). The extension of tomato shelf life is an important concern in today's world, as approximately 20% or more of all fruits are thrown away before they are eaten (Seymour et al., 2013).

Changes During Ripening

As a tomato ripens and senesces, it undergoes characteristic changes. Chemically, chlorophyll, polysaccharides and starches tend to degrade, and carotenoids are synthesized. Carotenoids are formed in both the chromoplast and chloroplast of the plant cell by a variation of the isoprenoid pathway. These reactions initially form lycopene, and carotenes are formed through subsequent reactions (Heuvelink, 2005). This increase in carotenoids (coupled with the decrease in the green pigment chlorophyll) is the biggest contributor to the rich red color normally present in ripe tomatoes (Zhang and McCarthy, 2012). Other compositional changes, such as in calories, proteins and fat, are slight (Heuvelink, 2005).

As a tomato ripens, its rate of respiration accelerates (Heuvelink, 2005). This is of important interest to researchers, since respiration intensity is directly correlated with shelf-life (Jacxsens et al., 2001). Ethylene, produced by tomatoes as they ripen, acts in a feedback system for tomatoes by further accelerating ripening and the production of ethylene. In tomatoes, ethylene is formed by a methionine to S-adenosylmethionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC) pathway. The amount of ethylene produced by a tomato can increase to between ten and one hundred times as it ripens (Heuvelink, 2005).

Another major change that tomatoes undergo during the ripening process is softening. This characteristic change is especially important in the assessment of tomato shelf-life. Softening in tomatoes is primarily caused by a decrease in cell wall rigidity

and adhesion (Chaïb et. al, 2007). This is primarily caused by the breakdown of pectin and hemicellulose polymers in cell walls. The mechanisms of softening are not fully understood, but it is known that cell wall hydrolytic enzymes contribute heavily to decreases in tissue firmness. According to Heuvelink (2005), the major classes of these enzymes include polygalacturonases, pectinases, pectinmethylesterases and carboxymethylcellulases. In an extended discussion of tomato textural changes, Valpuesta (2002) cites four main compounds as responsible for textural changes in tomatoes: pectin methylesterases, expansins, β1, 4 endoglucanases, and galactosidases. Pectin methylesterases affect both susceptibility to pectinases as well as cell wall structure. Expansins break hydrogen bonds that exist between hemicellulose and cellulose polymers. β1, 4 endoglucanases sever bonds within hemicellulose. Galactosidases break down galactose-containing compounds that are stored within the cell wall.

Flavor and aroma changes in tomatoes are also extremely complex. Approximately 400 aroma compounds have been identified in tomatoes, about 30 of which are important to flavor (Sinesio et al., 2010). These compounds include sugars, acids, amino acids, and a number of volatiles, including aldehyde, ketone and alcohol volatiles (Sinesio et al., 2010; Valpuesta, 2002). Lipoxygenases are important enzymes in the formation hexanal aldehyde and alcohols, which are significant contributors to both characteristic aroma and flavor in tomatoes (Valpuesta, 2002). In general, fruit firmness is negatively correlated with fruit odor and overall flavor (Sinesio et al., 2010).

Attributes to Measure Ripening

As any survey of the tomato-quality literature will show, there are a variety of objective methods to track the ripening process of tomatoes. These methods vary greatly in both their complexity and accuracy. Three common chemical analyses include pH-testing, brix, and the amounts of specific vitamins contained, such as Vitamin C (Artés et al.,1999; Fernández-Ruiz et al., 2010; Heuvelink, 2005). Generally, Vitamin C levels and pH increase during ripening. Brix also decreases over time through the process of respiration, in which carbohydrates and organic acids are oxidized to carbon dioxide and water (Heuvelink, 2005).

Probably the two most common methods of tracking ripening, both of which were done in this experiment, are color and texture analysis. As lycopene increases and chlorophyll decreases, most strains of tomato go from green, to pink (30-60% red), to red (60% or more red) (Hakim et al., 2003). Firmness decreases with ripening and is inversely proportional to the ripeness of the fruit (Lien et al., 2009).

Ripening Stages Based On Color

Although there are different ways to classify the different tomato ripening stages, in the United States, the six ripening stages defined by the USDA are the most popularly used. These six stages are: green, breaker, turning, pink, light red, and red (Camelo and Gómez, 2004). Approximate colorimeter values for each of these stages (except for

turning) can be seen below. For a full discussion of colorimeter values, see the chapter titled, "Colorimeter Testing."

Stage of Development	Ripening Stage	L*	a*	b*	Chroma	Hue angle
Mature-green	1	62.7	-16.0	34.4	37.9	115.0
Breaker	2	55.8	-3.5	33.0	33.2	83.9
Pink	4	49.6	16.6	30.9	35.0	61.8
Light Red	5	46.2	24.3	27.0	36.3	48.0
Red-ripe	6	41.8	26.4	23.1	35.1	41.3

Table 1-1: Colorimeter Values at Various Stages of Ripening (Heuvelink, 2005)

In the green stage, the surface of the tomato is completely green. This may include various shades of green. In the breaker stage, some of the area of the tomato (under 10%) changes from green to tannish-yellow, red, or pink. In the turning stage, 10-30% of the surface has changed from green to one of the previously mentioned colors. During the pink stage, 30-60% of the surface of the tomato is covered with pink or red color. When 60-90% of the tomato is covered with a pinkish-red or red color, it is considered to be in the light red stage. Finally, at the peak of tomato ripeness, the tomato reaches the red stage, during which the surface is covered with at least 90% red color (Sargent and Moretti).

Figure 1-1: Ripening Stages According To The USDA (Dumke, 2010)

Purchasing Considerations

The main factors that a consumer considers in assessing the quality of a tomato are color and appearance, texture, aroma and flavor (Ahmed et al., 2013). Visual appearance is especially important, since it is the first thing the consumer assesses. Although subsequent purchases may be based on other factors, like texture, appearance is usually the basis of first purchases (Rosca and Rosca, 2013). For most types of tomatoes, consumers want to see a shiny and uniform color and no defects, such as shriveling or rotting (Sargent and Moretti). Other important visual factors include the presence or absence of decay and size (Sinesio et al., 2010). Generally, firmer tomatoes are more likely to sell (Sirisomboon et al., 2012). One consumer study showed that most people do not like soft or mealy texture in their tomatoes (Seymour et al., 2013). Tomato flavor and aroma are very complex to analyze, as over four hundred differentiated perceivable

compounds having been identified in the tomato fruit (Sinesio et al., 2010). However, it can be generally said that tomatoes are expected to have an aroma and flavor that are sweet rather than sour. Tomatoes that lack aroma are also a turnoff for consumers (Seymour et al., 2013).

Experiment Objectives

There are two primary objectives of this research. First, this experiment seeks to measure any initial sensory differences caused by chlorine dioxide gas treatments on tomatoes. Sensory alterations that may occur as a result of chlorine dioxide gas are described in the chapter titled, "Chlorine Dioxide." Second, rates of senescence in tomatoes treated by chlorine dioxide are compared to rates in untreated tomatoes.

CHAPTER TWO

OTHER METHODS OF PRESERVATION

In exploring a specific means of tomato preservation, it is important to be aware of the other means that exist. Currently, there are numerous methods employed and suggested to preserve tomatoes. Some of these are still in research stages and have not yet seen any commercial use. This section will explore the most commonly used methods of preservation, and some uncommon methods of tomato preservation with noteworthy and interesting experimental results will be reviewed.

Chlorinated Wash

Washing with chlorinated water is a common method of cleaning produce, including tomatoes (Bari et al., 2002). Normally, the water is prepared with small concentrations of either calcium hypochlorite or sodium hypochlorite, which act to disinfect the surface of the produce. Hong and Gross (1998) reported that concentrations of 0.105 to 1.05% sodium hypochlorite are normally used. This method is simple, but its current form has many disadvantages. Although the washing can remove dirt on the tomato's surface, it cannot be used in acceptable concentrations to kill all surface pathogens (Bari et al., 2002). These chemicals can also produce carcinogens, such as chloramines and trihalomethanes. For this reason, chlorinated wash has been banned in several countries throughout the world, including Germany, the Netherlands, Switzerland

and Belgium (Ahmed et al., 2012). Already, higher concentrations are needed than may be safe, since the common use of chlorinated wash has led to microbial resistance development (Leverentz et al., 2001). Unlike other means of preservation, washing has little residual effect, allowing for easy contamination after the produce has been sanitized (Hong and Gross, 1998). Finally, dipping in chlorinated wash may cause the surface color of the tomato to become tainted. This was confirmed by Workneh et al. (2012), who noticed adverse effects in color after dipping whole tomatoes in chlorinated wash for twenty minutes.

Modified Atmosphere Packaging (MAP)

Modified Atmosphere Packaging (MAP) is one of the most used systems to preserve tomatoes, especially for the storage of sliced tomatoes. It basically consists of a reduction of oxygen and a controlled amount of carbon dioxide within the package, achieved by gas flushing or scavenging (Batu and Thompson, 1998; Aday and Caner, 2011). Maximum preservation benefits are achieved for tomatoes when oxygen levels are approximately 3-5% of the atmosphere. Levels of carbon dioxide varied in the literature, but generally, 5% or less was suggested or used ("Postharvest physiology of tomatoes," 1978; Hakim et al., 2003). For example, in an experiment with sliced tomatoes, Hakim, et al. (2003) used 2.5% oxygen and 5% carbon dioxide. Using specified gas mixtures and a package with controlled permeability slow the respiration of the produce, reducing the rate of senescence and ripening (Aday and Caner, 2011).

Through the use of MAP, surface browning, ethylene biosynthesis, and microbial growth are all reduced (Artés et al., 1999). Additionally, it reduces water loss from the fruit by creating a water-saturated atmosphere (Batu and Thompson, 1998). This is especially of concern in tomatoes, which are composed largely of water. MAP can be cheaper than other forms of preservation, especially refrigeration (Gong and Corey, 1994).

MAP packaging for tomatoes also has some disadvantages. Since it affects fruit metabolism, it can also affect the formation of flavor compounds, and in turn, can result in a loss of flavor (Boukobza and Taylor, 2002). When gas conditions are not carefully controlled, tomatoes can be negatively affected. Oxygen levels below 2% and above 5% can cause browning and color changes, as well as an increase in fermentable volatiles, such as ethanol (Hakim et al., 2003). When oxygen levels are too low, harmful microbes, such as *Clostridium botulinum*, may flourish. MAP alone is often not sufficient to produce desired preservation effects and extended shelf-life, so it may need to be coupled with other preservation methods, such as temperature control (Siripatrawan and Assatarakul, 2009). It is also claimed by some researchers, such as Artés et al. (1999), that sufficient fruit firmness is not retained with MAP. However, Hakim et al. (2003), in their experiment with sliced tomatoes, reported that firmness increased with the aid of MAP.

Ethylene Scavengers

As previously explained, tomatoes are climacteric and so use the production of ethylene to initiate a feedback system. As a tomato releases more ethylene into the atmosphere, this ethylene further advances ripening (Seymour et al., 2013). Since this feedback mechanism exists, one way to slow the ripening process is to use ethylene scavengers, which absorb ethylene. These scavengers are normally used in sachets within the package, since they are not supposed to come into direct contact with food (García-García et al., 2013). A few common chemicals used in these sachets include potassium permanganate, carbon, and zeolite (García-García et al., 2013; Aday and Caner, 2011). One particularly helpful experiment, done by García- García et al. (2013) with cherry tomatoes, saw promising results when cardboard trays lined with PLA (which absorbs ethylene) and wrapped in LDPE were coupled with the ethylene scavengers potassium permanganate and carbon. These tomatoes retained firmness after thirty days at 19.5 to 20.5 °C and 53% to 57% RH.

Refrigeration

Another method employed to preserve tomatoes is refrigeration. This method, especially in conjunction with modified atmosphere packaging (MAP), can be effective in slowing senescence and microbial growth, thus extending tomato shelf-life. One of the major reasons this method is not used as much as may be expected is the high cost of refrigeration in comparison to other systems (Gong and Corey, 1994). In addition to

being costly, cold temperatures can negatively affect the formation of important flavor and aroma volatiles (Boukobza and Taylor, 2002).

A danger also exists in potential chilling injury to the tomatoes. Susceptibility to chilling injury can vary with time of harvest, degree of ripeness, cultivar, and length of exposure to colder temperatures (Hong and Gross, 1998; Reddy et al., 2000). In part, this may explain the slight differences in the literature of the reported temperature needed to cause chilling injury. According to Siripatrawan and Assatarakul (2009), temperatures below 10°C for two weeks and below 5°C for six to eight days can cause chilling injury. Gong and Corey (1994) as well as Hong and Gross (1998) report that chilling injury can occur as high as 12 °C. Some common symptoms of chilling injury include: uneven ripening, failure to ripen fully, water-soaked areas, surface-pitting, and increased degradation due to microbes (Hong and Gross, 1998; Siripatrawan and Assatarakul, 2009).

Less Common Methods of Preservation

This section will give a brief review of experimental preservation methods that exist in the literature but are not currently widely used for commercial purposes. Ahmed et al. (2013) used whey permeate from cheese as a chemical for preserving sliced tomatoes and compared it to tomatoes preserved with 3% chlorine wash. The permeate consists of mainly water, lactose, proteins and minerals. Different extracts from permeate were used, including permeate concentrate obtained by evaporation, delactosed

permeate obtained by crystal removal from permeate concentrate, and delactosed concentrate produced by further evaporation of the delactosed permeate. After treating whole tomatoes, the tomatoes were sliced and placed on polypropylene trays with absorbent paper and covered with polypropylene bags. The whey permeate seemed to yield promising results, with whey permeate-treated tomatoes being 22% firmer than those treated with chlorine at the end of 21 days. This result may be due in part to whey's calcium content, which serves to decrease cell wall breakdown in the tomato.

Several other chemicals that have been used in liquid solution to preserve tomatoes include sodium, calcium, and anolyte. Sodium does have the ability to extend the shelf-life of produce, but sodium in solution, among other problems, normally causes a depletion in calcium, requiring a calcium supplement for the produce (Atta-Aly et al., 1998). Calcium dips are able to reduce the activity of enzymes that degrade the tomato cell wall, which helps to maintain a firmer fruit (Artés et al., 1999). Anolyte in solution is able to produce many of the desirable results of chlorine wash with less exposure time (Workneh et al., 2012).

Methyl jasmonate in a vaporized state, coupled with modified atmosphere packaging, had tremendously successful results in one experiment by Siripatrawan and Assatarakul (2009), resulting in a shelf- life of approximately nine weeks for whole tomatoes. Methyl jasmonate and modified atmosphere packaging were separately able to produce shelf-lives of about six weeks.

Two other miscellaneous methods of tomato preservation, which are worthy of mention, are the use of hyperbaric pressure and the use of ozone. Liplap et al. (2013), in citing Goyette et al., noted that elevated pressures (0.3 to 0.9 MPa) were able to reduce respiration rates, enhance lycopene content, and help maintain the freshness of tomatoes. Another experiment by Zambre et al. (2010) used various temperatures (15°C, 25°C and 35°C) and ozone levels ranging 20-50 ppm for ten minutes. Ozone was found to have an oxidizing effect and to delay the onset of ripening.

CHAPTER THREE

COLORIMETER TESTING

Colorimeter testing is one of the most (if not the most) popular ways for tracking changes in tomato maturity. One major reason for this is that ripeness stage can be easily correlated with color. It is also quick, objective, and does not damage the tomato (Fernández-Ruiz et al., 2010). In the next sections, the basic information resulting from a colorimeter test, different ways of interpreting results, and examples of colorimeter methods used in experiments in the literature will be discussed.

Colorimeter Values

A colorimeter test yields three basic values: L*, a* and b*. The L* value represents the degree of lightness or darkness in the sample, on a scale of 0 to 100 (Fernández-Ruiz et al., 2010). A reading of 0 represents a completely black color, while a reading of 100 indicates a completely white sample (Wold et al., 2004). The second measurement, a*, represents the degree of greenness or redness (Fernández-Ruiz et al., 2010). Negative numbers indicate a higher degree of green color in the sample, and negative a* samples can be said to have no red color (Wold et al., 2004). A positive a* value reveals that red color is present, with higher values indicating more pronounced red color (Batu and Thompson, 1998). The third parameter, b* measures the degree of blueness to yellowness (Fernández-Ruiz et al., 2010). Negative b* values indicate that the object being tested is completely blue and contains no yellow, with lower values indicating stronger degrees of blue color. Positive b* values result when a sample

contains yellow, with stronger yellow colors yielding higher b* values (Batu and Thompson, 1998).

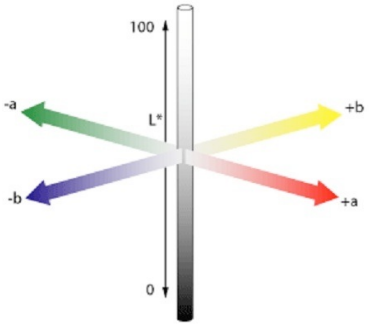

Figure 3-1: Colorimeter Colorspace (Kvidera, 2010)

In the literature, virtually every experiment involving a colorimeter and tomatoes used multiple readings for each sample. The primary reason for this is the heterogeneous nature of tomatoes. Tomatoes, for example, tend to be most green at the stem end and least green at the flowering end (Heuvelink, 2005). Colorimeter readings around the radial axis of the tomato can vary as well. Therefore, an average of multiple colorimeter readings may be necessary to create a representative colorimeter value for the tomato. The number of measured points per sample varied in the literature. For example, one experiment by Pék and Helyes (2010) used three points, a second experiment by García-García et al. (2013) picked five points, and a third experiment by Zambre et al. (2010)

picked ten points. It can also help the consistency of colorimeter results to select only tomatoes that are at about the same stage of ripening, which can be done visually (Zambre et al., 2010).

The results of a colorimeter test are rarely expressed in simple L*, a* or b* values. Rather, combinations and calculations involving these numbers are used. There seems to be disagreement in the literature about which combinations are best to use to quantify the color of tomatoes. Possibly the most agreed upon colorimeter testing fact is that a* is extremely important. There is in fact a linear relationship between a* and ripening, making it an important parameter in determining the rate and stage of ripening (Heuvelink, 2005). Most tomatoes begin with a chlorophyll to carotenoid ratio of about 10/1, which accounts for their original green color. This ratio falls to 1/1 at the breaker stage. Eventually, as the tomato becomes fully ripe, more lycopene is synthesized and all chlorophyll degrades, resulting in a red tomato (Heuvelink, 2005).

The importance of b* and L* in assessing the ripening of tomatoes is not as clear. Beta-carotene, an orange pigment present in tomatoes, can be measured using b* (Heuvelink, 2005). Also, in experiments in which it is relevant, b* can be a measure of discoloration due to chilling injury (Artés et al., 1999). In one experiment, Ahmed et al. (2012) indicated that b* decreased as ripening advanced. However, other results express that b* changes little with ripening, or that changes in b* are not even correlated with changes in a* (Camelo and Gómez, 2004; Wold et al., 2004). L* values tend to decrease over time, since tomatoes normally darken as they ripen (Camelo and Gómez, 2004;

Ahmed et al., 2013). This darkening is related to an increase in lycopene and physical deterioration during senescence.

Although other ratios and calculations involving L*, a* and b* may be used, two of the most common expressions of color are hue (arctan b*/a*) and chroma $(a^{*2} + b^{*2})^{1/2}$. In the next section, summaries of colorimeter procedures and results from the literature will be given.

Examples from the Literature

In their evaluation of tomato color indexes, Camelo and Gómez (2004) took an average of four colorimeter measurements per tomato, with one measurement at the distal area and four around the equatorial zone. As their tomatoes ripened, they observed a decrease in L*, indicating a darkening as the tomatoes matured from pink to red. They also noted that b* values did not change significantly during the ripening process. They indicated that their different methods of expressing color, including hue $[\tan^{-1} (b^*/a^*)^2]$, chroma $[(a^{*2} + b^{*2})^{1/2}]$, color index $[2000 \times a^*/L^* \times (a^{*2}+b^{*2})^{1/2}]$, color difference with true red $([(L^*-50)^2 + (a^*-60)^2 + b^{*2}]^{1/2})$, a*/b* and $(a^*/b^*)^2$, essentially expressed the same results.

In an experiment by Ahmed, Martin-Diana et al. (2012), whey permeate was used as an alternative method of preserving fresh-cut tomatoes. Hue (arctan b*/a*) and chroma $[(a^{*2} + b^{*2})^{1/2}]$ were found, and they took twenty to thirty measurements per treatment

per day, with apparently only one reading per sliced tomato. Overall, they found that while a* increased with ripening, b*, hue, and chroma decreased.

Color variations among green, post-harvest red and vine-ripe tomatoes were assessed by Wold et al. (2004). Two color measurements were taken per tomato around the equatorial area, and color was expressed as a*/b* and hue (arctan $(b^*/a^*)^2 * 3.14$). One major finding was that in vine-ripened tomatoes, both lycopene and b-carotene concentrations increased linearly as the tomatoes matured, as expressed by a*/b*.

García-García et al. (2013) conducted an experiment testing the ability of active packaging (cardboard trays lined with PLA and wrapped in LDPE) to preserve cherry tomatoes. On each tomato, five colorimeter readings were taken at equidistant points. To track maturation, a*/b* values were used. They found that for the first two weeks of experimentation, a*/b* increased greatly. However, over the following two weeks, a*/b* values decreased because of an increase in b* values.

CHAPTER FOUR

SENSORY ANALYSIS

Hedonic Scales

Hedonic scales use scores within a numerical range to evaluate sensory properties. In the literature, hedonic scales for sensory evaluation of tomatoes are extremely common. Bari et al. (2002), in their experiment testing the effectiveness of electrolyzed acid water against *Escherichia coli* O157:H7 on tomato surfaces, used a five-point sensory hedonic scale. Their rankings included: unacceptable (1), limited quality for consumption (2), medium (3), good (4), and very good (5). Workneh et al. (2012), in an experiment involving many different forms of preservation, used a 9-point hedonic scale for visual analysis. Their analysis specifically took into account color, shininess, surface defects, signs of mold growth, and dehydration. Their ratings included: unusable (1), unsaleable (3), fair (5), good (7), and excellent (9). Ahmed et al. (2013) used a similar 1-9 hedonic scale evaluating aroma, appearance, texture and overall quality. Their experiment looked at the potential use of whey permeate to preserve tomatoes. Two different scales were used for appearance and aroma by Domínguez et al. (2012). They included three types of tomatoes (Raf, Amadeo and Nereida) and tested the effects of fungicides on these cultivars. A visual scale of 1-9 was utilized with: poor, inedible (1), fair, limit of usability (3), good, limit of marketability (5), very good (7), and excellent (9). Their aroma scale ranged from 1-5: lack of characteristic aroma (1), moderate (3), and full characteristic (5).

Other Common Sensory Methods

Since this primary research does not use the sensory methods that are most common for tomato testing in the literature, a brief survey of other common sensory methods will be given here. Many of these methods, in real-life sensory situations, may undergo modifications because of experimental limitations or unique desired outcomes.

An unstructured line scale is a frequently used method to measure the amount of a perceived stimulus present in a sample. A line is drawn, usually 15cm or 6in in length, with opposite attributes listed at both ends of the line. For example, to the left of the line the attribute "sour" could be placed, and to the right of the line "sweet" could be placed. Subjects mark on the line to indicate which attribute they perceive and to what degree they perceive it (Meilgaard et al., 2007). Stolzenbach et al. (2011) used unstructured line scales in their analysis of twenty-one different Danish honeys, looking at a number of characteristics pertaining to appearance, texture, mouthfeel and taste. For example, they had a line scale for amount of brown color, ranging from "none" to "extremely."

A triangle test is used to determine if a difference exists between two products. Normally, participants are given a set of three samples, two of one kind and one of another. The participants are then asked to identify which sample is different than the others. This test is specifically useful for evaluating whether or not a change in a product (ingredients, processing, storage, etc.) can be detected. It is also helpful for determining if a difference exists between samples when there are not easily identifiable and specific attributes to compare (Meilgaard et al., 2007). In one experiment in the literature by

Radovich et al. (2004), this method was used to test for apparent sensory differences caused by various cabbage irrigation methods and times of irrigation. Subjects were given three samples, two of one irrigation method and one of another, and asked to identify the odd sample. One limitation of this test is that it cannot determine preference, only difference. As the authors noted, "major production factors (e.g., irrigation) that contribute to cabbage that consumers prefer remain to be determined."

The duo-trio test is somewhat similar to the triangle test. Participants are given a marked reference sample and two blind samples. They are then asked to identify which sample matches the reference. Although it yields similar results to the triangle test, it is somewhat less statistically powerful, since there is a 1 in 2 chance of guessing correctly (rather than a 1 in 3 with the triangle test) (Meilgaard et al., 2007). Kolanowski et al. (2007) used this method to determine the effectiveness of microencapsulation in protecting fish oil from spoiling. Subjects were given two coded samples, one encapsulated and one not encapsulated, and an encapsulated reference sample. Participants were asked to smell the samples and match the reference to a coded sample. This test, performed multiple times, allowed the researchers to determine the point at which non-encapsulated fish oil began to have an off odor.

The "A"-"not A" test is utilized when samples involve peculiarities, complex stimuli or strong lingering flavors. This test is also used when one of the products being tested is a familiar or common standard to which all other products must be measured (marked "A"). Subjects may be given anywhere between one and ten samples. They are then asked to identify each sample as "A" or "not A" (Meilgaard, et al., 2007). This

method was used by Nachtsheim and Schlich (2013) to compare fat perception levels in milk/cream and high fat emulsions. One modification in this experiment was the fact that participants also marked how sure they were about their decisions.

In a two-out-of-five test, participants are given five blind samples and asked to identify which two belong to one (same) sample source and which three belong to the other. This test is particularly effective because the chance of guessing correctly is only 1 in 10. However, this can be a difficult test to conduct because of sensory fatigue by participants and memory effects (e.g., it might be hard to "remember" what sample one tasted like by the time you get to sample five) (Meilgaard et al., 2007). Musetti and Fava (2012) used this method to determine if treating apple slices with hexanal vapor creates a perceptible difference. They used different two-out-of-five tests for odor, color and flavor. Participants were given either two control and three treated slices or three control and two treated slices and asked to identify which two samples were of the same treatment.

A difference-from-control test can be used to indicate whether or not a difference exists between one or more products and a control. It is often used with particularly heterogeneous products, such as meats or baked goods. In this test, subjects are given a marked control and a set of samples, and they are asked to indicate on a scale (e.g., 0-10) how much each sample differs from the control. They are informed beforehand that among the samples will be blind controls. One particular advantage of this test is that it yields results which allow the comparison of in-batch differences to across-batch

differences (Meilgaard et al., 2007). This method was used by Lunardello et al. (2012) to determine sensory differences created by the addition of different hydrocolloids (carrageenan, xanthan gum and alginate) to yogurt. Overall differences were determined by participants, ranging from identical to the control (1) to extremely different from the control (9).

CHAPTER FIVE

MICROBIAL ANALYSIS

Many procedures exist for recovering microbes from tomatoes and produce in general. Physical methods for extracting microbes include stomaching, blending, homogenizing, rubbing, shaking and macerating (Burnett and Beuchat, 2001). Some medium fluids used in the recoveries include peptone water, various buffers, enrichment broths, and beef extracts (Lukasik et al., 2001). A variety of different types of plates and petrifilms exist to isolate both specific types of microbes, such as yeasts or mesophiles, and specific microbes, such as *Listeria monocytogenes*.

In many experiments involving the microbial analysis of tomatoes, some kind of homogenization method is used. For example, in an experiment by García-García et al. (2013) using cardboard trays lined with PLA in conjunction with LDPE wrap to preserve cherry tomatoes, 25g of tomato were taken per sample and homogenized in 250mL of saline. Plate count agar was used to enumerate both mesophilic microflora and yeasts and molds. Multiple dilutions were created, and microbial counts were reported as CFU/g. Similarly, in their analysis of the microbial qualities of two different types of tomatoes, Carmen et al. (2013) stomached 25g of tomato sample for two minutes with buffered peptone water. Then, 1mL of homogenized sample was added to 20mL of plate count agar and gently mixed. After incubation, they reported the total aerobic mesophilic organisms in log CFU/g. Ahmed et al. (2013) did sampling on multiple days (0, 7, 14 and 21) in their testing of whey permeate as an alternative preservation method to

chlorine. They blended 25g of tomato sample with 225mL of peptone saline stomacher. Plating was done both on plate count agar (for aerobic plate counts) and potato dextrose agar (for yeast and mold plate counts). Results were reported in log CFU/g.

Another alternative and less difficult method than homogenizing is the use of physical rubbing or agitation to remove microbes. Such a method was used by Franklin, et al. (2004) in an experiment testing the ability of nisin incorporated into film to inhibit *Listeria monocytogenes* on hot dogs. First, films were coated with methylcellulose and hydroxypropyl methylcellulose solution, with various amounts of nisin added to the films (156, 2,500, 7,500, and 10,000 IU/mL). Then, they inoculated the hotdogs in their packages with 1mL of solution, containing approximately 5 log CFU/mL of *Listeria monocytogenes*. Each package was aseptically opened, and 9mL of 0.1% peptone was added. After massaging the package by hand for two minutes, the peptone water was transferred to a beaker. Serial dilutions were created, and results were given as CFU per package.

CHAPTER SIX

TEXTURE ANALYSIS

Texture is a common parameter tested to assess the ripening stage or progress of produce. In general, texture is a key property of both fruits and vegetables (Chen and Opara, 2013). As such, proper texture attributes, including firmness, mealiness, juiciness, and crispiness are vital to the consumer perception of tomatoes (Sinesio et al., 2010). Although all of these attributes can be helpful in statistical analysis, most studies focus on only one textural aspect (Chaïb et al., 2007).

The texture of fruits and vegetables is derived from turgor pressure, the composition of plant cell walls, and the lamella that hold individual cells together (Sinesio et al., 2010). Firmness, a particularly important aspect of texture, is negatively proportional to the ripeness of the fruit, decreasing as ripening progresses (Lien et al., 2009). Textural changes also correlate well with sensory analyses, such as odor and overall flavor (Rosca and Rosca, 2013; Sinesio et al., 2010).

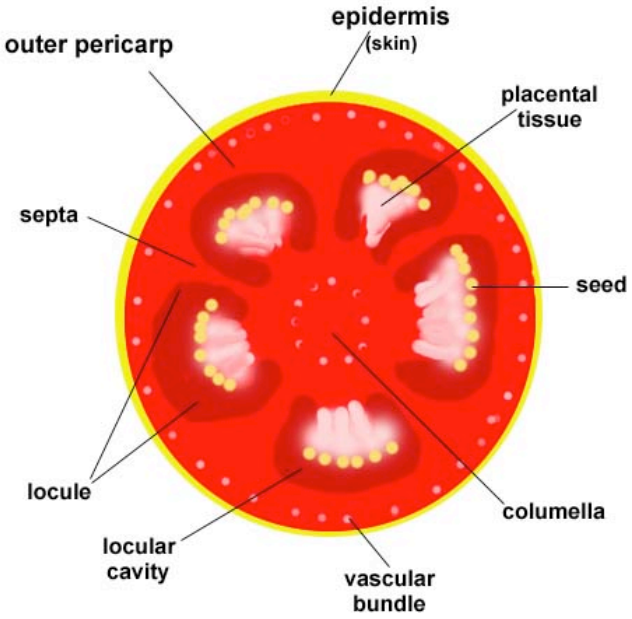

Figure 5-1: Diagram of a Tomato (GardenWeb, 2009)

The following sections will explore more specifically the kinds of texture analysis tests commonly used with tomatoes and which parameters can be obtained from texture profile analysis. Various procedures and methods from experiments in the literature will also be surveyed.

Common Methods

According to Biswas et al. (2014), common ways of testing tomato firmness include acoustic stiffness sensors, flat plate compression, and puncture tests. In a similar vein, Rosca and Rosca (2013) name the penetration test, compression test, and the Warner-Bratzler shear test as the most common mechanical testing methods. To this list,

Valente et al. (2011) add deformation.

Penetration and compression are both accepted as reliable testing methods, and they are by far the most common methods of texture analysis (Lien et al., 2009). The penetration test can be useful with both whole and sliced tomatoes in assessing degree of ripeness and skin strength (Rosca and Rosca, 2013). The puncture strength of a tomato is determined primarily by the strength of the skin, the intactness of the pericarp, the number of locule walls, and total turgor (Biswas et al., 2014). However, the flat-plate compression method may be preferable with whole tomatoes, since it tends to yield more consistent results (Rosca and Rosca, 2013). This may be in part because it more accurately represents the strength of the entire tomato rather than picking small isolated points, as in the puncture test. Although some have argued that relaxation parameters should be obtained in addition to compression or puncture, this is rejected in practice in most texture analysis experiments, and the biological meaning of relaxation values is uncertain (Lana et al., 2005). In addition to the relevant parameters to the puncture strength of a tomato, the strength of a tomato during flat-plate compression is determined by the rigidity of the cell wall, cell density, and cell-to-cell adhesion (Biswas et al., 2014).

Parameters Obtainable by Texture Analysis

According to Rosca and Rosca (2013), there are three basic types of results that can be attained through texture analysis: properties that are familiar to mechanical

engineers, empirical results, and imitative results. Properties that are familiar to mechanical engineers include parameters such as Poisson's Ratio, maximum penetration force, shear modulus, and Young's Modulus (Chen and Opara, 2013). Empirical results involve less complex and easily attainable values through procedures such as puncturing, compression, or extruding. Imitative results relate to quantifiable characteristics and feelings that would be experienced as a person consumes the product (Rosca and Rosca, 2013). There are a multitude of possibilities in this realm, but just some of the parameters that can be tested for include springiness (how well a sample returns to its original form after being compressed), cohesiveness (how much a sample can be compressed before rupturing), hardness (the maximum force required to compress the sample), and chewiness (Chen and Opara, 2013). Additionally, Chaïb et al. (2007) put forward firmness, mealiness, meltiness, juiciness and crispness as being particularly important to fruit texture.

In general, all texture profiles involve a combination of force, time/distance, and amount of deformation. Which of these variables are measured may change depending on what results are desired (Chen and Opara, 2013). For example, the amount of force to compress 5mm could be determined, or how many millimeters a sample compresses under 10N of force could be obtained. According to Rosca and Rosca (2013), empirical and imitative data tend to correlate well with sensory judgments.

Difficulties with Texture Analysis

Although texture analysis is certainly useful in obtaining experimental data, it is not without its drawbacks. In general, it can be said that, "Instrumental texture analysis methods remain a challenging area for the entire fresh fruits and vegetable arena" (Rosca and Rosca, 2013). Since so many methods exist for texture analysis, is can be difficult to compare results using different instruments (Chen and Opara, 2013). For example, flat-plate compression results may be difficult to compare with puncture tests performed with probes. For tomatoes and other produce, which are heterogeneous by nature, obtaining consistent results can be a real challenge. According to Rosca and Rosca (2013), "The main problem of texture analysis is that the shape and consistency of foodstuffs deviates very much, and the reproducible results require a careful preparation of specimens and the testing method." Such being the case, multiple measurements are often desired per fruit. However, in some instances, this may not be feasible. For example, flat-plate compression tests on whole tomatoes are destructive to the tomato, so if a second compression test were to be run on the same tomato, the results would differ drastically from the first. Although more measurements can be taken per tomato if the tomato has been sliced, sliced tomatoes usually vary more in firmness tests than whole tomatoes (Lana, et al., 2007). Additionally, some aspects, such as juiciness, mealiness, and skin toughness, are difficult to obtain with texture analysis and may require additional sensory testing to be performed (Chaïb et al., 2007).

Examples from the Literature

In testing for whole tomato texture, Sirisomboon et al. (2012) used both flat-plate compression and puncture testing. Using a 150mm diameter flat plate and a speed of 10mm/min with 19-24 tomatoes, a double-cycle load relaxation test was run. From this test, many different parameters were obtained, including: deformation at max force, deformation ratio, initial firmness, average firmness, modulus of elasticity, degree of elasticity, energy absorption, and relaxation ratio. It was discovered that average firmness, initial firmness, and modulus of elasticity reduced significantly as ripening progressed. Also, the authors believed based on their results that degree of elasticity is more accurate than relaxation ratio as an overall indicator of firmness. Using the same number of samples and the same test speed, a 2mm stainless steel plunger with a flat end was used for puncture testing. The parameters obtained from this testing included: rupture force, deformation at rupture point, toughness, initial firmness, average firmness, deformation at the bioyield point, bioyield force, apparent modulus of elasticity, and force of penetration in the flesh. Rupture force was found to correlate well with other parameters and to be a good overall representative value for tomato firmness.

Similarly, Biswas et al. (2014) used both flat-plate compression and puncture testing in an experiment assessing the effects of low-temperature storage on tomatoes. For both types of tests, fifteen tomatoes per storage temperature (2.5° C, 6°C and 20°C) were tested on days 0, 13, and 27. Flat-plate compression tests were done with a 50.85mm cylindrical probe. With a test speed of 1mm per second, the maximum force

required to compress 2mm was measured. Whole-fruit puncture was carried out with a 3.7mm diameter flat end cylindrical probe and a test speed of 10mm per second. The maximum force for the probe to penetrate 15mm into the tomato was recorded. Finally, to measure the firmness of the pericarp, additional firmness tests were run using 15mm thick tomato slices. The same 3.7mm diameter flat end cylindrical probe was used. Each slice was punctured in two separate places, and the maximum force required to penetrate 4mm was recorded. This data allowed the experimenters to quantify the differences between chill-induced softening (at 2.5° C and 6°C) and ripening-induced softening (at 20°C). Further, the researchers found that although both 2.5°C and 6°C caused softening due to turgor loss, storage at 2.5°C also caused softening due to loss in tissue integrity.

Domínguez et al. (2012) did a thorough analysis of the effects of fungicides on different tomato cultivars. The fungicides used included fenhexamid and pyraclostrobin plus boscalid. The three cultivars tested were Raf, Amadeo and Nereida. Texture analysis was done using two flat-plates (dimensions unspecified), and maximum deformation was acquired at 10N of force with a test speed of 25mm per minute. For each set of variables, there were three tomatoes tested per day. One interesting aspect of this experiment is that testing days varied by cultivar, depending on how many homogenous samples for each cultivar were obtained. All cultivars were tested on day 0. Raf was tested on day 10, Amadeo was tested on days 5 and 10, and Nereida was tested on days 7, 14 and 21. Overall, the experimenters found that the effects of the fungicides depended on both the type of fungicide used and the tomato cultivar.

In a fairly unique experiment, Errington et al. (1997) compared the compressions and relaxations of tomatoes compressed multiple times and only one time. In addition to a control group, they had two other types of tomatoes, labeled as PG-antisense and rin mutant fruit. The experiment lasted fourteen days, and five tomatoes were tested per type (repeat tomatoes and new tomatoes) each day. A 10cm diameter plate was used with a maximum force of 4N and a speed of 10mm per second. After compression was maintained, relaxation occurred for 10 seconds. Among other things in this experiment, N/mm were determined for compression. Interestingly, the tomatoes tested daily were firmer and retained more elasticity than those tested only once. The paper suggested that this might be due to biological changes resulting from wounds or water loss in the repeatedly tested tomatoes.

In an experiment with sliced tomatoes, Schouten et al. (2010) included a number of different variables, including temperature (4.0°C, 10.0°C and 20°C), cultivar, initial ripeness, and treatment (air and 1-MCP, ethylene, and air). For texture testing, only one 7mm slice was cut from each tomato. Between 32 and 40 slices were used for each combination of variables. At a speed of 5mm per minute, the maximum force required to compress a slice 1mm was measured. Measurements were either taken repeatedly on the same spot, or on eight different locations on the same tomato slice. The instrument, which contacted the tomato slices, was a 2.5mm flat tipped cylindrical probe. Using repeated measurements on the same spot produced unusable results due to the damage caused by each measurement. The main success of this experiment was the creation of calibrated models describing firmness as a function of storage temperature and cultivar.

Lana et al. (2007) examined the texture of sliced tomatoes at different stages of ripening and storage temperatures. The three stages analyzed were breaker (grade 3), pink (grades 5 and 6) and red (grade 9). The storage temperatures used were 2°C, 5°C, 8°C, 12°C and 16°C. For each set of variables on each testing day, five tomatoes were used, and three slices per tomato were tested. Using a 3.5 mm diameter flat faced cylindrical probe and a speed of 0.02mm per second, the force required to deform the slice 3mm was recorded. Four measurements were taken per slice. Their firmness data overall was very scattered. However, they did conclude that firmness did not vary much within ripening stages and that firmness of tomato slices decreased exponentially over time.

Although it is less common, texture analysis can also be performed on diced tomatoes. Such an experiment was performed by Lee et al. (1999). Their experiment included two different types of diced tomatoes (Halley 3155 and Heinz 8892), and the tomatoes were all processed by cold-filling, hot-filling, or aseptically. They ran a total of three instrumental sensory tests: a Kramer shear test, a back extrusion test, and texture profile analysis. The Kramer shear test and the back extrusion test were performed in triplicate, while twenty-five replicates were used for texture profile analysis per sample. In the Kramer shear test, 200g of sample were weighed and compressed with a 5-blade probe at 1mm per second. After 90% strain was obtained, firmness was taken as the area under the curve. In the back extrusion test, a 52mm internal diameter probe was used to push tomato sample contained within a cell from a height of 60mm to a height of 10mm, and firmness was taken as area under the curve. Finally, for texture profile analysis,

$1cm^2$ of sample was placed at the base of the instrument and compressed at 1mm per second until 75% strain was reached. Firmness was calculated as the area under the maximum peak of the curve. The experimenters found that cold-fill produced the firmest and crunchiest tomatoes. They were also able to correlate the Kramer shear test and the back extrusion test well with other sensory data obtained.

Nondestructive tests for tomato firmness do exist, but they are wrought with difficulties. In one experiment, Lien et al. (2009) used such a method. Dropping tomatoes from a non-destructive height (15mm), they measured both initial deformation and the amount of time to reacquire original shape. They also compared this test to both compression and penetration tests and found that these destructive methods gave much more accurate results overall.

CHAPTER SEVEN

CHLORINE DIOXIDE

Chlorine dioxide has the potential to act as a fungicide and an antibacterial agent in order to kill harmful microorganisms or preserve foods (Cardoso and Teixeira da Silva, 2012). Liquid chlorine dioxide was approved by the US EPA in 1967 as a disinfectant, but the gaseous form was not approved for use until 1988 (Valderrama et al., 2009). It can be used to sanitize during processing, during storage after harvest, or in a packaging system (Netramai et al., 2012). As to its mode of action, one paper notes, "The disinfection mechanisms, while not fully understood, appear to vary by type of microorganism" (Valderrama et al., 2009). However, it is understood that chlorine dioxide is a strong oxidizing agent and basically acts by transferring an electron and being reduced to a chlorine dioxide anion (Netramai et al., 2012). Chlorine dioxide has also been the focus of many food preservation research studies with products such as lettuce, blueberries, peppers, tomatoes, and cantaloupe.

Forming Gaseous Chlorine Dioxide

Until recently, gaseous chlorine dioxide was formed only in chambers. This process was technically difficult, expensive, and dangerous due to potential combustion (Wu and Kim, 2007). However, with the development of chlorine dioxide sachets, this all changed. Now, with sachets, two independently inert substances (in the form of powder) can be mixed to form chlorine dioxide by pouring both into the sachet and

mixing them. Within seconds, the two powders react to form gaseous chlorine dioxide. Although other reactions can be used to form chlorine dioxide gas, the main reaction used in sachets is as follows:

$$5NaClO_2 + 4H^+ \rightarrow 4ClO_2 + 2H2O + NaCl + 4Na^+$$

However, which types of residues are created and how much is produced can vary depending on multiple factors. These factors include, "Type of ClO_2-delivery system, ClO_2 concentration, treatment time and temperature, as well as, the type of food product" (Netramai et al., 2012). Therefore, before commercial use of chlorine dioxide gas in a system, it is vital to test it in the various conditions that the product will experience and with the actual product that will be sanitized.

Advantages of Chlorine Dioxide (ClO_2) Gas

Chlorine dioxide has been demonstrated to reduce pathogen populations in produce. For example, with chlorine dioxide gas treatments on tomatoes, Trinetta et al. (2013) successfully reduced microbial populations of *Alternaria alternata* and *S. vesicarium*, and Bhagat et al. (2010) saw decreases in *Salmonella* and *Listeria* populations. Pathogen reductions have been achieved by ClO_2 gas treatments in other produce, such as blueberries in an experiment by Sun et al. (2014) and in mungbean sprouts by Prodduk et al. (2014). The chemical has approximately two and a half times

the oxidizing capacity of chlorine, giving it maximum effectiveness against a wide range of microorganisms (Wu and Kim, 2007). As a gas, it has the potential to penetrate deeper into products and reach more remote crevices and cavities than aqueous sanitizers (Gómez-López et al., 2009; Trinetta et al., 2013).

Chlorine dioxide gas has also been successful in prolonging the shelf-life of various kinds of produce. As Guo et al. (2013) observed, "Numerous reports have demonstrated the beneficial effects of ClO_2 treatment on fruits and vegetables, such as blueberries, strawberries, fresh-cut cabbage, carrots, lettuce, mulberry fruit and tomatoes." Guo et al. (2014) demonstrated that cytochrome pathway respiration is slowed as a result of chlorine dioxide treatments. Also, they showed that ethylene biosynthesis is reduced by repression of *LeACS2*, *LeACS4*, and *LeACO1* genes, which are responsible for ethylene biosynthesis. Slowed respiration and reduced ethylene production result in a slowing of tomato senescence and a delay in fruit ripening.

Additionally, chlorine dioxide is not as prone to produce potentially harmful byproducts as chlorine. Its main byproducts are chlorite and chlorate ions, and the compound is specifically known to be inert in the presence of ammonia (e.g., it won't form chloramines) (Gómez-López, Rajkovic et al., 2009). As Netramai et al. (2012) noted, "So far, the identified chemical residues left, after ClO_2-treatments are ClO_2, chlorite (ClO_2^-) and chloride (Cl^-), with ClO_2^- as a major by-product." Chlorine dioxide is known to be stable in a wide range of pH levels (pH of 3-9), making it applicable for use in many potential products (Cardoso and Teixeira da Silva, 2012; Gómez-López et al., 2009).

Finally, although chlorine dioxide gas used to be difficult to produce, with the advent of sachets, it is now simpler and less expensive. Before the use of sachets, chlorine dioxide had to be produced in firmly sealed chambers with expensive machinery, and many handling devices were necessary to prevent explosions and to deliver proper amounts of chlorine dioxide for sanitation (Wu and Rioux, 2010). But now, with the use of sachets, chlorine dioxide production and use is "less expensive, [less] inconvenient, and does not require as much technical expertise" (Wu and Kim, 2007).

Disadvantages of Chlorine Dioxide Gas

Although chlorine dioxide gas has much potential as a sanitizing agent and a preservative, it does have drawbacks. One paper reported that its oxidizing nature could potentially lower the nutritional qualities of the foods it comes into contact with (Gómez-López et al., 2009). But the main problem is that chlorine dioxide can alter the sensory properties of food products. These effects vary by food product and strength of treatment and include issues such as browning, bleaching, whitening, phytotoxicity, and reduction of positive sensory qualities in general (Aday and Caner, 2011; Gómez-López, Rajkovic et al., 2009; Guo et al., 2013; Mahovic et al. 2007). As noted by Trinetta et al. (2013), "ClO_2 gas has had a bleaching effect on green leaves, strawberry caps, and lettuce leaves." However, discoloration has not occurred in every (or necessarily most) experiments involving chlorine dioxide gas. One experiment with strawberries is a

notable example (Aday and Caner, 2011). Also, in some experiments, such as one performed with green peppers by Jin-hua et al. (2007), lower levels of chlorine dioxide did not alter sensory perception. Additionally, gaseous chlorine dioxide has tended to have less negative effects on sensory properties than aqueous chlorine dioxide (Trinetta, et al., 2013).

Experiments Involving Chlorine Dioxide

Wu and Rioux (2010) tested the effects of chlorine dioxide gas on potatoes. Chlorine dioxide gas was generated using sachets that contained both sodium chlorite and activating acids. The parameters that were varied included weight of materials used in the reaction (2, 3 and 4g), times of exposure to chlorine dioxide (2.5 and 5 hours), and concentrations of chlorine dioxide gas (16, 20, 24, 30, 32, and 40 mg/L). Some samples were inoculated with *Pseudomonas aeruginosa*. Over a period of 14 days, they tested for residuals left by the gas treatment, did microbial enumerations, and performed visual quality tests. The highest chlorine dioxide treatment for the longest time resulted in the greatest microbial reduction (4g of each material for 5 hours). Using a 9-point hedonic scale for daily visual analysis, no significant visual differences were discovered between the potatoes treated with 2g and 4g of material and the controls. However, strangely, the potatoes treated with 3g were slightly lighter in color than the controls.

Trinetta et al. (2011) analyzed the residues left on seven different types of produce by gaseous chlorine dioxide treatments. The products used included tomatoes,

oranges, apples, strawberries, lettuce, alfalfa sprouts, and cantaloupe. The produce was treated in a chamber by passing 2% chlorine gas through sodium cartridges (to create chlorine dioxide), and then adding nitrogen gas. The final concentration of chlorine dioxide was 100mg/L. The produce surfaces were washed, and the resulting solutions were analyzed on days 0, 1, and 14 for chloride, chlorite, chlorate, and free chlorine dioxide gas. Controls with no chlorine dioxide treatment were used for comparison. In their results, it was reported that apples, strawberries, lettuce, alfalfa sprouts, and cantaloupes had chlorine dioxide residue levels that were significantly higher than their control counterparts. Additionally, lettuce and sprouts had levels of chlorite residue that may not be safe in drinking water and experienced negative sensory alterations, including bleaching and browning. In the tomato samples, residues left by the treatment decreased over time, and on day 14 the residue levels were the same as the controls.

The effects of chlorine dioxide gas on green peppers were examined by Jin-hua et al. (2007). The peppers were treated with sachets producing various amounts of gas, including 0, 5, 10, 20, and 50 mg L^{-1} ClO_2. The experiment was run for 40 days, and all peppers were kept at a temperature of approximately 10°C. The 50 mg L^{-1} treatment had the greatest effect, with no visible rot on the peppers after 30 days. Concentrations of 20 mg L^{-1} and 50 mg L^{-1} significantly slowed the respiration of the produce. However, 10, 20 and 50 mg L^{-1} caused overall chlorophyll contents to be lowered, and 50 mg L^{-1} caused titratable acidity to be increased. Overall, the researchers concluded that chlorine dioxide gas significantly extended the shelf-life of the peppers.

Guo et al. (2013) analyzed the effects of chlorine dioxide gas on cut fruit. After slicing Hami melon fruit into 15-20mm cubes, 4.0g of sodium chlorate, 4.0g of oxalic acid and stabilizer were mixed into a sachet, with the aim of producing 60mg L^{-1} of gas. Both estimation equations and iodimetry were used to track the amount of gas produced. The fruit pieces were treated for 12 hours, and a fan was used to circulate the air. After treatment, the product was stored at 5°C and 95% RH for 19 days. Shelf-life was extended by the treatment from about 8 days (for the control) to 18 days. Weight loss was also reduced with treatment, probably due to less water loss as a result of less rotting. Finally, chlorine dioxide gas reduced respiration. The authors suggested a technical and specific reason for this phenomenon, saying, "It is presumed that ClO2 treatment might restrain the transfer of electrons in alternative respiration and cytochrome pathway respiration, which have [a] contributing role in the regulation of total respiration rate" (Guo et al., 2013).

Many past experiments involving chlorine dioxide gas have tested the effectiveness of the gas in inhibiting particular pathogens. One such experiment was performed by Trinetta et al. (2013). After isolating *Alternaria alternata* and *S. vesicarium* from infected tomatoes, they applied them to both petri dishes and punctured tomatoes. The petri dishes contained 15mL of solidified PDA and were inoculated with 10mm plugs containing the organism. The tomatoes were wounded with uniform punchers, and 50μL aliquots containing the microbes were applied to the punctures. Both the tomatoes and the petri dishes were treated with chlorine dioxide in a chamber. 2% chlorine gas was passed through three sodium cartridges to produce a treatment

approximately 10mg L^{-1} (carried by nitrogen). Air was circulated with a fan in the chamber. Exposure times to the gas varied, including 1, 3, 5, 7 and 10 minutes. Plates were incubated at 23°C for one week and tomatoes were stored at 25°C for 10 days. Both the petri dishes and tomatoes saw pathogen inhibition as a result of the treatment. One minute of treatment reduced spore count by 2.5 log spores mL^{-1} (for both organisms) and three minutes totally inhibited spores. For the punctured tomatoes, 5 minutes of treatment completely inactivated *S. vesicarium*, and 7 minutes of treatment fully inactivated *Alternaria alternata*. After only 5 days, the decay of the control tomatoes (without gas treatment) was significant.

Bhagat et al. (2010) tested the effects of chlorine dioxide on the reduction of *Salmonella* and *Listeria* populations and effects on tomato shelf-life. Tomato surfaces were inoculated with a cocktail consisting of either multiple strains of *Salmonella* or multiple strains of *Listeria*. After initial enumerations of microbial populations, concentrations of 0.1, 0.3, and 0.5 mg/L ClO_2 gas for 0-12 min at 22°C and 90% RH were used to treat tomatoes. They found the chlorine dioxide treatments to be effective in reducing theses pathogens. A 12 min treatment with 0.5 mg/L ClO_2 gas caused a 5 log reduction in both *Salmonella* and *Listeria*. Tomatoes for the shelf-life portion of the experiment were treated with 0.5 mg/L ClO_2 gas for 12 min at 22°C. By day 21, untreated tomatoes contained visible mold growth on the surface, but treated tomatoes did not. No difference in color was detected over a period of 28 days by either a trained panel or colorimeter testing.

CHAPTER EIGHT

MATERIALS AND METHODS

Treatment and Storage of Tomatoes

Approximately seven-hundred Tasti-Lee tomatoes (VFFF Hybrid #5755) were obtained from Flavor 1st Growers and Packers in Mills River, North Carolina. Tomatoes were transported in an air-conditioned vehicle. The tomatoes were treated with chlorine dioxide in 19L HDPE buckets, with each bucket being filled to approximately 5cm below the brim. The weights of the tomatoes contained in each of the nine buckets were recorded.

Figure 8-1: Tomato Treatment Setup

Two chemicals in the form of powder, Z-Series Research Fast Release Part A and Z-Series Research Fast Release Part B were obtained from ICA TriNova, Newnan, Georgia. Part A consisted of the clay mineral zeolite with sodium contained within the zeolite. Part B also consisted of zeolite, with an activator acid contained within the zeolite. When mixed within a sachet, these chemicals react to form gaseous chlorine dioxide. Of the nine buckets of tomatoes used in this experiment, three were treated with 50mg of Part A and Part B per kg of tomato, and three were treated with 10mg of Part A and Part B per kg of tomato. The additional three buckets, which did not receive chlorine dioxide treatment, were used as controls. For each treatment group, the two concentrations of chlorine dioxide were weighed, placed into 10cm by 12.5cm sachets, and shaken for approximately one minute in order to initiate the reaction (see Appendix A for the release curve of this mixture). All of the sachets were placed into their respective buckets with the perforated side facing upwards, towards the top of the bucket. The buckets were sealed with lids. Each of the lids had an 0_2 Cool battery powered fan taped to the inside in order to promote airflow and even distribution of the chlorine dioxide gas.

After treatment, the tomatoes were stored in 57cm by 69cm perforated polypropylene trays. The trays were sterilized before use. Tomatoes were stored in a single layer (so as to avoid bruising and premature ripening of bottom layers) in a room at ambient temperature (23°C).

Figure 8-2: Tomato Storage

Texture Analysis

Texture analysis was performed on days 0, 3, 7, 10, 14, and 17 of the experiment using a TA.XT.plus Texture Analyser. Before texture analysis, the weight of each tomato was recorded. After the machine was calibrated for force with a 2kg weight, individual whole tomatoes were placed on the instrument stage, with the stem facing upward. Compression tests were run with a 10cm diameter metal flat plate (ID: TA40) and the peak distance (mm) compressed with a force of 40N was recorded. Other parameters included a pre-test speed of 1mm/sec, a test speed of 2mm/sec, a post-test speed of 10mm/sec, and a trigger force of 0.049N. On each

test day, a total of 66 tomatoes were examined, with 22 being tested from each treatment group (50mg/kg and 10mg/kg) and the control group.

Microbial Analysis

0.1% peptone water was prepared and autoclaved at 120°C and 18-20psi for two hours. On each of the three test days (days 0, 7 and 14), a total of six tomatoes were used for microbial analysis (two from each treatment group and two from the control group). Each tomato was placed into a polyethylene Filtra-Bag with 225mL of 0.1% peptone water and massaged by hand for two minutes. Immediately afterwards, serial dilutions were made using an Eppendorf Reference pipette. For each tomato, a total of five dilutions were made (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}). This was accomplished by transferring 1mL of solution from each Filtra-Bag into a test tube containing 9mL of 0.1% peptone water. The test tubes were agitated for approximately sixty seconds using an Analog Vortex Mixer, and more diluted samples were made by adding 1mL of each agitated mixture to another test tube containing 9mL of 0.1% peptone water. The process was repeated for all dilutions.

Using an Eppendorf Reference pipette, the solutions were immediately plated in duplicate onto 3M Petrifilm Yeast and Mould Count Plates and 3M Petrifilm Aerobic Count Plates. On each petrifilm, the plastic cover was lifted, and 1mL of solution was placed into the middle of the grid. Then, the cover was dropped, and a plastic spreader was pressed against the droplet to create a circular spread of the fluid. The Aerobic Count

Plates were incubated for 48±3 hours at 32±1°C. The Yeast and Mould Count Plates were stored at room temperature (25°C) for 3-5 days. After incubation, results were analyzed with the aid of a Leica Quebec Darkfield Colony Counter, and results were recorded as colony forming units per mL of solution (CFU/mL).

Color Analysis

Color analysis was performed using a HunterLab MiniScan EZ colorimeter on days 0, 3, 7, 10, 14, and 17. Before each test day, the colorimeter was calibrated using a diagnostic standard (Serial No: MSEZ0844). On each test day, a total of 66 tomatoes were used for colorimeter analysis, with twenty-two tomatoes being selected at random from each of the two treatment groups and the control group. On each individual tomato, a total of sixteen colorimeter measurements were taken. First, eight measurements were taken on the outside of the tomato. This included four around the radial perimeter, two near the stem of the tomato, and two near the end opposite the stem. Each tomato was then cut symmetrically into fourths by cutting in half perpendicularly to the stem, and then cutting the resulting halves again perpendicularly to the stem. This resulted in four slices, each containing two inner sides. On each of these eight sides, one colorimeter measurement on the pericarp was recorded. All L*a*b* values produced by the colorimeter were copied into Microsoft Excel. Both external and internal values were copied and analyzed separately.

Sensory Analysis (Difference from Control Test)

A difference from control test was conducted on days 0, 3, 7, 10, 14, and 17 to evaluate perceivable differences between the treatments and control. Panel membership was comprised mainly of participants from Clemson University, including faculty and students. The number of participants per session varied from sixteen to twenty two.

Using a sterilized knife and cutting board, the tomatoes were cut into symmetrical eighths. This was performed by first cutting in half perpendicularly to the stem, cutting each resulting half into fourths perpendicularly to the stem, and then cutting all the resulting fourths into eighths perpendicularly to the stem. Each of these pieces was placed into a 5.5 oz. transparent polypropylene cup (produced by Platinum Crown) with a lid.

Each panelist was given a polypropylene tray overlaid with wax paper. On the tray was placed four samples, including one marked control ("C") slice, and three other slices with random three-digit codes. Included among these three were a tomato treated with 50mg/kg of chlorine dioxide, one treated with 10mg/kg of chlorine dioxide, and a blind control. The order of the samples was randomized to avoid any bias based on the presented order. Additionally, each panelist was given a toothpick and a napkin, and three score sheets for the difference from control test.

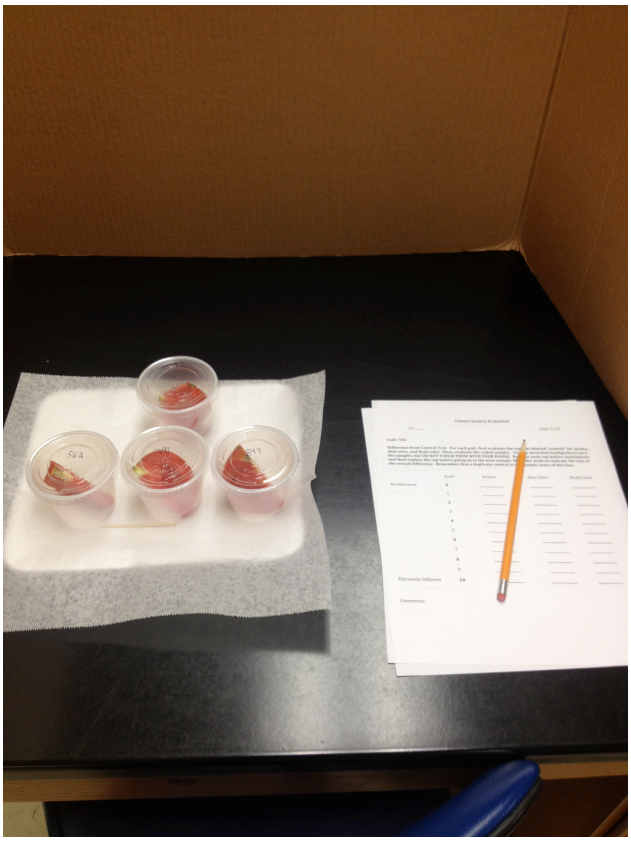

Figure 8-3: Sensory Panel Station

Panelists were instructed to evaluate, on three separate sheets, how different the numbered samples were from the control (e.g., control vs. sample 1, control vs. sample 2, and control vs. sample 3). On each sheet, three difference evaluations were made: aroma, flesh color, and skin color. Scores ranged from 0 (no difference) to 10 (extremely different). Panelists were not allowed to consume the tomatoes. Panelists were asked to use the toothpick rather than their hands to rotate the tomato in order to avoid any bias based on felt texture. See Appendix B for the full sensory ballot.

CHAPTER NINE

RESULTS AND DISCUSSION

Data analysis was performed using a GLM (general linear model) procedure in SAS. Analysis involved effect by testing day, treatment, and by treatment * day effect. Additionally, the least square means for each parameter were calculated and used in the data analysis.

Texture Analysis

It was originally expected that the chlorine dioxide treatments might have negative effects on texture. Mahovic et al. (2007) found that high ClO_2 concentrations of 88 mg ClO_2/2 h and 99 mg ClO_2/24 h produced phytotoxicity in the stem scars and wounds of tomatoes. However, the results of this experiment gave no indication that any adverse textural effects were caused by even the highest ClO_2 treatment as compared to the control group.

The tomatoes in the 50mg/kg treatment group, the 10mg/kg treatment group, and the control group became progressively softer, yielding larger compression distances on later days. Negative correlation between ripening and softening is well known and almost universally attested to by experimenters examining tomato textural changes. This softening over time is primarily caused by a decrease in cell wall rigidity and adhesion (Chaïb, et. al, 2007). Biswas et al. (2014) observed this trend for whole tomatoes held at

both high (20°C) and low (2.5° C and 6°C) temperatures. Errington et al. (1997), who compared whole tomatoes tested once and whole tomatoes tested repeatedly over time, also saw this trend. In that study, the wounds made in the tomatoes by repeated testing actually caused them to retain more firmness. Tomato slices are also known to soften over time. Lana et al., (2007) reported this decrease in firmness to be exponential.

There were two exceptions in this experiment: for the tomatoes in the 10mg/kg group, the average compression value decreased from 9.15mm on day 7 to 8.86mm on day 10, and the average compression of the tomatoes in the control group decreased slightly from 10.16mm on day 14 to 10.12mm on day 17. These exceptions were both relatively small and are most likely due to normal variations among the tomatoes.

The results obtained from texture analysis in this experiment showed no significant ($p < 0.05$) initial effects on the texture of the tomatoes from the chlorine dioxide treatment. However, there also seemed to be no reduction in rate of softening over time. As shown in Table 9-1, the control group of tomatoes with no chlorine dioxide treatment had an average compression of 7.00mm on day 0 and increased to 10.12mm on day 17. The tomatoes receiving a chlorine dioxide treatment of 10mg/kg began on day 0 with a mean compression of 6.08mm and increased to 10.47mm on day 17. The tomatoes that received the 50mg/kg chlorine dioxide treatment had a compression of 6.95mm on day 0 and 10.35mm on day 17. None of the groups of tomatoes had significantly different compression values, showing that all of the tomatoes softened at similar rates over time.

This is contrary to what is generally seen with other produce in the literature. For example, in one experiment by Zhong et al. (2006), 150 nl l^{-1} and 1000 nl l^{-1} of chlorine dioxide significantly increased the firmness of Xiaobai apricots over a period of 7-9 days, compared to untreated fruit. Additionally, Sun et al. (2014) found that chlorine dioxide gas treatment caused blueberries to retain greater firmness over a period of 9 days at both 10°C and 20°C storage. These differences between the literature examples and the current experiment are likely due to the fact that chlorine dioxide has different effects on different kinds of produce. Alternate treatment methods were also used in these other experiments (Sun et al. used chlorine dioxide pads, and Zhong et al. used a chlorine dioxide-producing chamber).

The data as a whole showed no significant differences in firmness between the tomatoes treated with chlorine dioxide and those that were not (both at the beginning and end of the experiment). This is clear evidence that no phytoxicity occurred as a result of the chlorine dioxide. Additionally, tomatoes treated with chlorine dioxide did not retain more firmness over time as compared to the tomatoes in the control group.

The primary difficulty with texture analysis of tomatoes, and produce in general, is heterogeneity (Rosca and Rosca, 2013). Tomatoes can be of different sizes, shapes, and at various stages of ripening. These effects were minimized as much as possible by selecting tomatoes that were similar in size and visually representative (in color) of the overall batch to which they belonged.

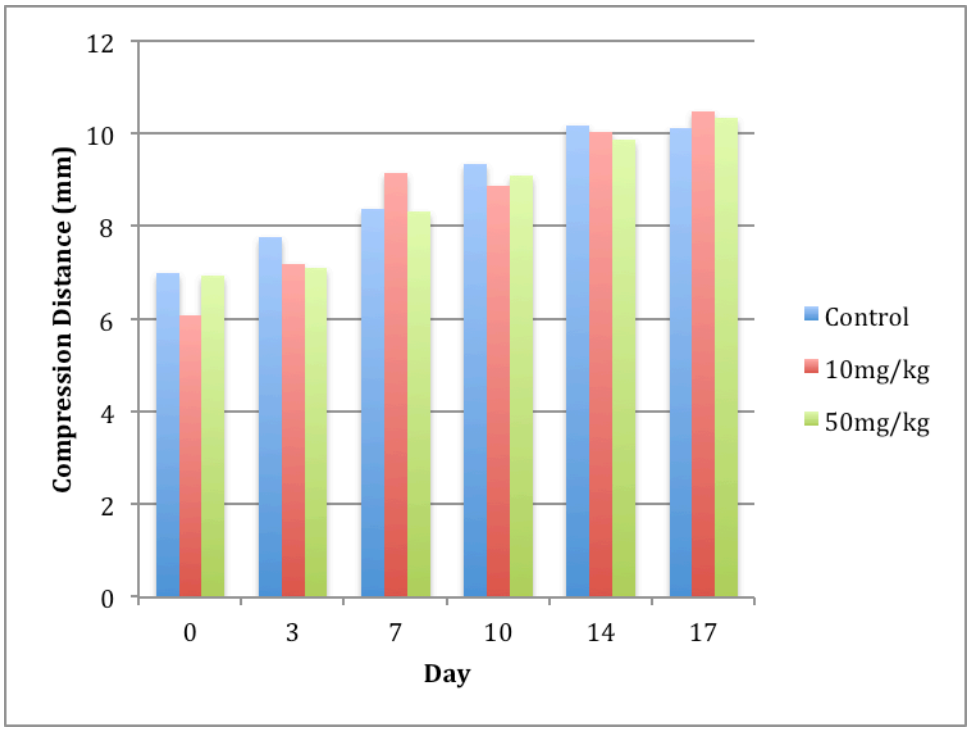

Figure 9-1: Average Tomato Compression Values

Day	Treatment	Compression (mm) ± St. Dev.
0	10mg/kg	6.080 ± 1.356
3	10mg/kg	7.185 ± 1.246
7	10mg/kg	9.146 ± 1.342
10	10mg/kg	8.864 ±1.619
14	10mg/kg	10.034 ± 1.513
17	10mg/kg	10.470 ± 2.083
0	50mg/kg	6.949 ± 1.510
3	50mg/kg	7.113 ± 1.234
7	50mg/kg	8.334 ± 1.661
10	50mg/kg	9.104 ± 1.102
14	50mg/kg	9.875 ± 1.633
17	50mg/kg	10.346 ± 1.827
0	Control	6.995 ± 1.507
3	Control	7.766 ± 1.552
7	Control	8.377 ± 1.223
10	Control	9.355 ± 1.336
14	Control	10.165 ± 1.177
17	Control	10.124 ±1.329

Table 9-1: Average Tomato Compression Values

Microbial Analysis

There was a significant microbial reduction ($p < 0.05$) in the aerobic plate counts on day 0 for the tomatoes treated with 50mg/kg of ClO_2 compared to the control tomatoes (5.91 Log CFU/mL of solution in the control group compared to 4.78 Log CFU/mL in the 50mg/kg treatment group). The reduction on day 0 from the control tomatoes to the 10mg/kg treatment tomatoes was also statistically significant. However, this reduction was less than 1 Log CFU/mL. After day 0, there was no significant difference in aerobic plate counts for all three groups of tomatoes as seen in Table 9-2.

Day	Treatment	Aerobic Plate Count Average Log CFU/mL ± St. Dev.	Yeast and Mold Count Average Log CFU/mL ± St. Dev.
0	Control	5.91 ± 0.00	2.66 ± 0.01
0	10mg/kg	5.01 ± 0.02	2.56 ± 0.08
0	50mg/kg	4.78 ± 0.16	2.74 ± 0.08
7	Control	5.08 ± 0.04	2.47 ± 0.08
7	10mg/kg	5.28 ± 0.02	2.20 ± 0.17
7	50mg/kg	5.02 ± 0.22	2.89 ± 0.05
14	Control	4.50 ± 0.17	2.62 ± 0.01
14	10mg/kg	5.10 ± 0.07	2.40 ± 0.17
14	50mg/kg	4.46 ± 0.31	2.91 ± 0.02

Table 9-2: Microbial Plate Counts

For the yeast and mold counts, every average microbial count for all three test days (days 0, 7 and 14) fell between 2 Log CFU/mL and 3 Log CFU/mL. This indicates

that there were no significant differences in yeast and mold counts between the tomatoes treated with chlorine dioxide and the control group.

Chlorine dioxide gas is known to have higher penetrating power than aqueous treatments (Gómez-López et al., 2009; Trinetta et al., 2013). However, the results of this experiment showed no residual effect in microbial reduction in tomatoes. This is consistent with an experiment conducted by Trinetta et al. (2011), who analyzed the residues left on seven different types of produce by gaseous chlorine dioxide treatments. In the tomato samples, residues left by the treatment decreased over time, and on day 14 the residue levels were the same as the control group. This was in contrast to apples, strawberries, lettuce, alfalfa sprouts, and cantaloupes, which had chlorine dioxide residue levels that were significantly higher than their control counterparts by day 14. Wu and Rioux (2010) found in an experiment treating potatoes with chlorine dioxide that after 14 days, chlorine dioxide residues were present in amounts less than 1mg/L. This indicated a dissipation of the chlorine dioxide over time from the product. Sun et al. (2014) found that chlorine dioxide gas treatment was far less effective in lowering microbial populations on blueberries after 9 days compared to 6 days. They attributed this to the volatility of ClO_2.

Furthermore, the rinse method used in this experiment is useful for recovering surface microbes, but it may not be the best option if a high recovery rate of microbes within the fruit is desired. Wang et al. (2012) showed that in tomatoes injected with salmonella, blending, stomaching and quartering (cutting the tomato into quarters at the stem and soaking it in solution) result in recovery rates superior to simply rinsing the

whole tomato. However, with surface inoculation of Salmonella, the rinse method recovered approximately the same rate of Salmonella as the other three methods.

Color Analysis

The data was analyzed by considering initial color values for tomatoes after treatment on day 0 and checking values at day 17 for any significant differences at the end of the experiment. Additionally, all the data were reviewed for any significant ($p < 0.05$) changes over time in one group compared to the others. For each of these categories, both outer (skin) colorimeter measurements and inner (flesh) measurements were analyzed. The three formulas used to analyze the data were ratio, hue, and color index. Ratio is defined as a*/b* and has been used by Camelo and Gómez (2004) in their evaluation of color indexes and by García-García et al. (2013) to measure color change in cherry tomatoes. Hue, arctan (b*/a*), was utilized by Ahmed et al. (2012). Color index [$2000 \times a^*/L^* \times (a^{*2}+b^{*2})^{1/2}$] has also been used by Camelo and Gómez (2004).

Exterior Colorimeter Analysis

For outside ratio (a*/b*), only the 10mg/kg and 50mg/kg treatment groups were different on day 0, with the control falling between the two. The 50mg/kg treatment had the highest ratio out of the three (0.75), and the 10mg/kg treatment group had the lowest (0.69). Likewise, hue values (arctan b*/a*) were only significantly different between the

10mg/kg group, which had a value of 0.60, and the 50mg/kg group, with a value of 0.64. The 50mg/kg group had both a higher a* value and a lower b* value than the 10mg/kg group (see Table 9-3).

There was no significant ($p < 0.05$) difference in a* on day 0 between the control tomatoes and the 50mg/kg treated tomatoes. This indicates that the chlorine dioxide treatments (especially the high treatment of 50mg/kg of tomato) did not cause a measurable loss in the red pigmentation of the tomatoes, measured by a*. In some produce, a lightening in color can be caused by loss in pigmentation due to chlorine dioxide treatment. This effect was reported by Jin-hua et al. (2007) in green peppers when they were subjected to 20 mg L^{-1} or higher of ClO_2.

On day 0, for color index taken on the skin ($2000 \times (a^*/L^*) \times (a^{*2} + b^{*2})^{1/2}$), the control had the highest value with 49,647 vs. 45,139 for the 10mg/kg group and 44,454 for the 50mg/kg group. The control group had a significantly lower ($p < 0.05$) L* value on day 0 than either of the other two groups, indicating that the tomatoes in the control group were darkest. This is important, because it shows that the chlorine dioxide treatments may have caused some minor bleaching. Such bleaching effects of chlorine dioxide have been seen before on various forms of produce, such as green leaves, strawberry caps, and lettuce leaves (Trinetta et al., 2013). Trinetta et al. (2011), who tested residues left by chlorine dioxide on various kinds of produce, reported that chlorine dioxide treatments caused both bleaching and browning in lettuce leaves and alfalfa sprouts. On day 17, there were no significant differences were found in exterior values between the three groups for any of the three equations.

Treatment	Day	L* Mean ± St. Dev.	a* Mean ± St. Dev.	b* Mean ± St. Dev.
Control	0	30.35 ± 1.55	20.95 ± 1.67	28.96 ± 2.08
10mg/kg	0	31.46 ± 1.88	19.97 ± 1.50	29.07 ± 2.63
50mg/kg	0	31.62 ± 2.43	20.33 ± 1.43	27.45 ± 2.44
Control	17	30.94 ± 1.86	21.78 ± 1.26	24.28 ± 1.86
10mg/kg	17	30.25 ± 1.11	22.77 ± 1.51	24.81 ± 2.37
50mg/kg	17	30.59 ± 1.39	22.39 ± 1.38	25.31 ± 1.82

Table 9-3: Outside Average L*a*b* Values

Day	Treatment	Ratio		Hue		Index	
0	50mg/kg	0.75	A	0.64	A	44,454	A
0	10mg/kg	0.69	B	0.60	B	45,139	A
0	Control	0.73	A	0.63	AB	49,647	B

Table 9-4: Significant Differences in Exterior Color Values

Interior Colorimeter Analysis

The only significant differences for inside (flesh) values taken on day 0 were ratio and hue for the control compared to the two ClO_2 treatment groups. The control tomatoes had the highest values for both parameters. For ratio, the control yielded an average value of 1.72, compared to values of 1.61 for the 10mg/kg group and 1.56 for the 50mg/kg treatment group. The control had an average hue value of 1.04, while the hue values for the 10mg/kg and the 50mg/kg treatment groups were 1.01 and 1.00, respectively. The control had an a* value that was nearly identical to the other two groups, but its b* value was slightly lower. However, this difference in b* was not statistically significant. Although there was a statistical difference in color shown by the hue and ratio equations, these differences were very slight. As evidenced by the sensory panel portion of this experiment (see following section on sensory analysis), simple observation of the tomatoes did not consistently identify such small distinctions in color.

On day 17, the control values for average inside ratio and inside hue were higher than the two chlorine dioxide treatment groups. Similarly to day 0, the control had an a* value that fell between those of the two treatments, but it had the lowest b* value. The b* discrepancies were only significantly different between the 10mg/kg group and the control group, and they are likely due to normal variations among tomatoes.

Treatment	Day	L* Mean ± St. Dev.	a* Mean ± St. Dev.	b* Mean ± St. Dev.
Control	0	32.13 ± 2.14	17.78 ± 2.14	10.36 ± 1.13
10mg/kg	0	32.91 ± 2.81	17.73 ± 1.50	11.08 ± 1.11
50mg/kg	0	33.99 ± 2.75	17.81 ± 1.46	11.54 ± 1.46
Control	17	31.44 ± 2.72	17.18 ± 1.86	12.62 ± 1.76
10mg/kg	17	32.19 ± 2.67	17.92 ± 2.41	14.08 ± 1.86
50mg/kg	17	32.81 ± 2.43	16.79 ± 1.77	13.45 ± 1.77

Table 9-5: Inside Average L*a*b* Values

Day	Treatment	Ratio		Hue	
0	50mg/kg	1.56	A	1.00	A
0	10mg/kg	1.61	A	1.01	A
0	Control	1.72	B	1.04	B
17	50mg/kg	1.26	A	0.90	A
17	10mg/kg	1.28	A	0.90	A
17	Control	1.37	B	0.94	B

Table 9-6: Significant Differences in Interior Color Values

Overall Changes

In addition to the previously described parameters for color, overall changes (from day 0 to day 17) for ratio, hue and color index were calculated and analyzed using SAS. Only the three outside (skin) calculations for ratio, hue, and index showed any significant ($p < 0.05$) difference between groups. For both ratio and hue, the tomatoes treated with 10mg/kg vs. the 50mg/kg chlorine dioxide and the 10mg/kg vs. the control tomatoes showed differences, but the tomatoes treated with 50mg/kg treated group vs. the control did not. This means that for both of these values (hue and ratio), the 50mg/kg treated group had values between the control and 10mg/kg treated groups. For all three groups, both hue and ratio increased from day 0 to day 17. Ahmed et al. (2012) also reported an increase in hue over time in their experiment using whey permeate as a preservative for tomatoes. García-García et al. (2013) reported a decrease in a*/b* in later stages of ripening. However, unlike this experiment, this decrease resulted from an increase in b* over time (in this experiment, all outside b* values decreased from day 0 to day 17; see Table 9-5).

Treatment	Day 0 Value	Day 17 Value	Overall Change
Control	0.73	0.89	0.16
10mg/kg	0.69	0.92	0.23
50mg/kg	0.75	0.89	0.14

Table 9-7: Change in Outside Ratio (a*/b*) From Day 0 to Day 17

Treatment	Day 0 Value	Day 17 Value	Overall Change
Control	0.63	0.73	0.1
10mg/kg	0.60	0.74	0.14
50mg/kg	0.64	0.73	0.09

Table 9-8: Change in Outside Hue (arctan b*/a*) From Day 0 to Day 17

The a* value for all three groups increased from day 0 to day 17, with a 0.83 increase for the control, a 2.80 increase for the 10mg/kg group, and an increase of 2.06 for the 50mg/kg treatment group. Only the a* changes for the 10mg/kg treated tomatoes and the 50mg/kg treated tomatoes were statistically ($p < 0.05$) significant. This pattern is evidence that senescence was not slowed by the treatments, since a* would be expected to increase faster as ripening rate increased (and ripening rate should decrease with increased treatment level). The change in b* over time was negative for all three groups, with a change of -4.68 for the control group, -4.26 for the 10mg/kg treatment group, and -2.14 for the 50mg/kg treatment group (all statistically significant). Although the rate of b* change did decrease with increasing treatment level (control > 10mg/kg > 50mg/kg), this pattern is not reflected in either the hue or ratio equations.

The only trend that appeared consistent and significant ($p < 0.05$) over time was change in the outside index from day 0 to day 17. Here, the changes were 5,889 and 5,229 increases for the 10mg/kg and 50mg/kg treatment groups, respectively, but the control decreased 3,362 from day 0 to day 17. This reflects the fact that the overall change in L* for the 10mg/kg and 50mg/kg groups were -1.21 and -1.03, respectively, but L* increased 0.59 in the control. Only the changes in L* for the 10mg/kg and 50mg/kg treatment groups were statistically significant. Therefore, the treated tomatoes

became slightly darker in color over time, but the control group actually became lighter (though this lightening in color was not statistically significant). Ahmed et al. (2013) also saw a decrease in L* over ten days with four different kinds of treatments, including chlorine. This darkening is related to an increase in lycopene and breakdown of cell wall structure during senescence (Camelo and Gómez, 2004).

Treatment	Day 0 Value	Day 17 Value	Overall Change
Control	49,647	46,285	-3,362
10mg/kg	45,139	51,028	5,889
50mg/kg	44,454	49,683	5,229

Table 9-9: Change in Outside Index ($2000 \times (a^*/L^*) \times (a^{*2} + b^{*2})^{1/2}$) From Day 0 to 17

Sensory Analysis (Difference from Control Test)

The difference from control test was conducted a total of six times during the experiment (days 0, 3, 7, 10, 14, 17). Participants were each given four samples of sliced tomato: a control (marked "C") and three samples labeled with three-digit numbers, including a blind control, a 10mg/kg treated tomato, and a 50mg/kg treated tomato. Overall, there was no clear pattern seen and no significant ($p < 0.05$) treatment * day interaction for any specific attribute (aroma, skin color, and flesh color) at the beginning and end of the experiment. This is of particular significance on day 0, on which no measurable negative effects were found when comparing the chlorine dioxide treatment groups (10mg/kg and 50mg/kg ClO_2) to the blind control. Some known negative side effects of chlorine dioxide gas on produce include initial browning, bleaching, and

whitening, but no such effects were seen in this experiment (Aday and Caner, 2011; Gómez-López, et al., 2009; Guo, et al., 2013). As noted by Trinetta et al. (2013), "ClO_2 gas has had a bleaching effect on green leaves, strawberry caps, and lettuce leaves." These effects are normally dependent on the level of chlorine dioxide treatment. For example, one experiment using chlorine dioxide sachets with green peppers found that treatments of 10, 20, and 50 mg L^{-1} ClO_2 lowered chlorophyll content and caused a decrease in characteristic color while 5 mg L^{-1} ClO_2 did not (Jin-hua et al., 2007).

So, although there was an objective difference in color changes between the two treatment groups and the control group (as measured by a colorimeter), it was minute enough that it could not be consistently discerned by panelists (see section on colorimeter results). This is similar to the results obtained by Wu and Rioux (2010). Using a nine-point hedonic scale for daily visual analysis, they found no significant visual difference between potatoes treated with 2g and 4g of ClO2-forming material and a control group. However, many other experiments in the literature report that chlorine dioxide gas can greatly reduce senescence and sensory quality loss over time. Trinetta et al. (2013) analyzed the residues left on seven different types of produce by gaseous chlorine dioxide treatments. They found that an exposure of only seven minutes to chlorine dioxide gas greatly decreased the rate of decay in inoculated tomatoes. In the previously mentioned experiment by Jin-hua et al. (2007) on green peppers, their results showed a significant slowing in respiration and decay rates with ClO_2 concentrations of 20 mg L^{-1} and

Treatment	Day	Aroma Mean ± St. Dev.	Skin Mean ± St. Dev.	Flesh Mean ± St. Dev.
Control	0	2.32 ± 2.24	2.47 ± 2.97	2.42 ± 2.61
10mg/kg	0	2.58 ± 2.41	1.58 ± 2.36	2.26 ± 2.66
50mg/kg	0	2.58 ± 2.63	3.00 ± 3.02	3.11 ± 2.87
Control	3	3.18 ± 2.82	1.77 ± 1.27	1.18 ± 1.22
10mg/kg	3	1.68 ± 2.21	1.36 ± 1.50	0.91 ± 1.31
50mg/kg	3	1.50 ± 1.37	1.45 ± 1.99	1.32 ± 1.09
Control	7	1.00 ± 1.17	1.29 ± 1.16	1.18 ± 1.19
10mg/kg	7	1.29 ± 1.45	1.00 ± 1.00	1.24 ± 1.64
50mg/kg	7	1.94 ± 1.56	0.88 ± 0.86	0.76 ± 0.75
Control	10	2.11 ± 2.02	1.68 ± 2.50	1.26 ± 2.05
10mg/kg	10	2.47 ± 2.48	2.05 ± 2.63	2.16 ± 2.29
50mg/kg	10	1.53 ± 1.54	1.63 ± 2.01	1.11 ± 1.45
Control	14	2.50 ± 2.65	1.67 ± 2.45	1.72 ± 2.47
10mg/kg	14	2.61 ± 2.60	2.22 ± 2.34	1.78 ± 2.49
50mg/kg	14	2.44 ± 2.20	1.67 ± 2.33	2.00 ± 2.33
Control	17	3.56 ± 2.78	2.75 ± 2.82	2.31 ± 2.41
10mg/kg	17	2.81 ± 2.43	2.13 ± 2.25	2.25 ± 2.21
50mg/kg	17	3.00 ± 2.73	3.00 ± 2.45	2.81 ± 2.81

50 mg L^{-1}. Guo et al. (2013) were able to decrease rotting and extend the shelf-life of sliced Hami melon fruit from 8 to 18 days with a treatment of 60mg L^{-1} of ClO_2 gas.

Table 9-10: Average Values For Difference From Control Test

Scale: 0 = no difference from control, 10 = extremely different

Key: For each category per day, the highest numbers are in gray, the lowest numbers are in red, and values that fell in the middle are in green.

Although not statistically significant, some of the comments given by participants may indicate that they perceived a difference in the samples. For example, one panelist (ID: 19) noted that the 50mg/kg treated tomato had a less intense smell and flesh color on

day 0. On day 17, only one participant (ID: 9) identified the control tomato as significantly riper than the tomatoes that received the 10mg/kg and 50mg/kg treatments.

CHAPTER TEN

CONCLUSION

The colorimeter results revealed that there was some initial lightening in color as a result of the treatments (as measured by L* and color index). The L* value of the control increased over the duration of the experiment (not statistically significant), while L* decreased for the two chlorine dioxide treated samples, showing progressive darkening over time.

The sensory data provided by the panelists indicated that no statistically significant difference could be perceived between the treated and untreated tomatoes on day 0. This lends support to the use of chlorine dioxide as a sterilant for tomatoes, since it can be used without perceivably altering sensory properties. Additionally, the chlorine dioxide treatments did have measureable effects on senescence (as measured by colorimeter analysis), but these effects were minor and not perceivable by sensory panelists on any of the testing days.

Compression distance changed across test days but not across treatments, indicating that chlorine dioxide did not have a measurable effect on firmness. The 50mg/kg treatment caused a 1 Log CFU/mL reduction compared to the control on day 0. This effect was not residual, though, as there were no reductions in microbial counts on days 7 and 14 as a result of the treatments. However, if another method of microbial recovery had been used that could recover more internal microbes (homogenizing, quartering, etc.), it is possible that a reduction would have been seen on days 7 and 14.

In a future experiment, it may be particularly helpful to compare methods of chlorine dioxide treatment, such as chamber treatment versus the treatment method used in this experiment. Also, it could be beneficial to compare ClO_2-forming chemicals from various producers to determine if the chemicals differ in their effects. These tests would allow for analysis based on treatment method and help determine if in fact there is a significant difference in initial sensory properties or shelf-life when alternate methods of ClO_2 application are used. Furthermore, since the effectiveness of chlorine dioxide gas is partially dependent on the moisture content of the environment during treatment, it would be useful to increase or vary the moisture content in the treatment environment and analyze how both initial sensory properties and preservation effectiveness are changed.

APPENDICES

Appendix A

Chlorine Dioxide Release Curve

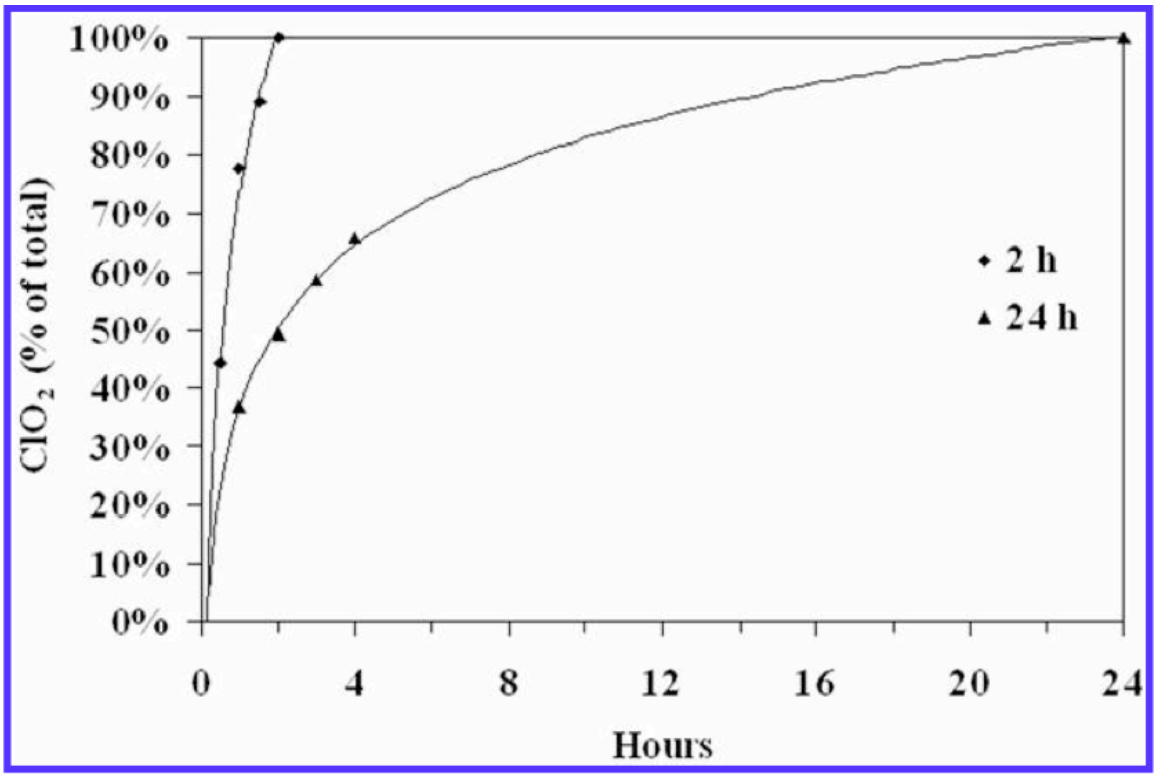

Figure A-1: The total percent of chlorine dioxide formed per time from dry matter for two termination times (Mahovic et al. 2007)

Appendix B

Difference from Control Test

Tomato Sensory Evaluation

I.D._____ Date_____

Code: _____

Difference-from-Control Test. For each pair, first evaluate the sample labeled "control" for aroma, skin color, and flesh color. Then, evaluate the coded sample. Use the provided toothpicks to turn the samples, but DO NOT TOUCH THEM WITH YOUR HANDS. Remove each cap before assessment, and then replace the cap before going on to the next sample. Mark the scale to indicate the size of the overall difference. Remember that a duplicate control is the sample some of the time.

	Scale	Aroma	Skin Color	Flesh Color
No difference	**0**	_____	_____	_____
	1	_____	_____	_____
	2	_____	_____	_____
	3	_____	_____	_____
	4	_____	_____	_____
	5	_____	_____	_____
	6	_____	_____	_____
	7	_____	_____	_____
	8	_____	_____	_____
	9	_____	_____	_____
Extremely Different	**10**	_____	_____	_____

Comments:

Figure B-1: Difference From Control Test Sensory Sheet

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